Introduction to TFF

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Main Agenda

- Biomanufacturing and Filtration
- Filtration Principles
- Various Types of Filters
- Molecular Weight Cut Off
- Normal Flow Versus Tangential Flow
- Units of TFF
- TFF Operations in Clarification, Filtration, Concentration, and Diafiltration
- Operation of TFF
- Hands - on Lab
  - TFF Operation of Minimate TFF systems (Pall Corp.)
  - CIP using 0.1 N NaOH
  - NWP and integrity test
  - Separation and Concentration of Two Dye Molecules (Acid Yellow, 0.3 kDa and Dextran Blue, 2000 kDa)

Training Objectives

- Understand the overview of biomanufacturing process.
- Identify the differences between upstream and downstream processes.
- Understand terms used in filtration operation; retentate, filtrate, permeate, desalting, buffer exchange, diafiltration, concentration, etc.
- Understand various types of filtration methods used in biomanufacturing
- Understand various types of filters used in filtration.
- Understand what Molecular Weight Cut Off (MWCO) means in terms of filtration operation.
- Understand the processes of clarification, filtration, diafiltration, and concentration.
- Understand the basic principles of filtration.
- Understand the difference in Normal Flow and Tangential Flow Filtration methods.
- Recognize the functional units of TFF operation.
- Apply CIP and storage methods for a large scale TFF system.
- Understand validation methods (flux/integrity test) used in TFF operation.
- Understand the main applications of TFF in large scale API manufacturing.
- Perform a bench scale TFF operation on separation and concentration to understand the important operation principles.
- Understand the critical parameters associated with large scale TFF operation.
- Perform basic trouble shooting methods in TFF operation.
- Ultimately reduce human performance deviations in manufacturing process.

Biomanufacturing & Filtration

Upstream Processing

Media Filtration

Liquid Prefilter

Sterilizing-grade Liquid Filter

Aeration

Air Prefilter

Sterilizing-grade Air Filter

Exhaust

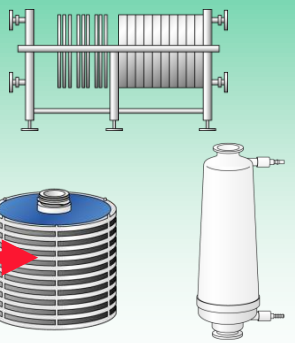
Air Prefilter

Sterilizing-grade Air Filter

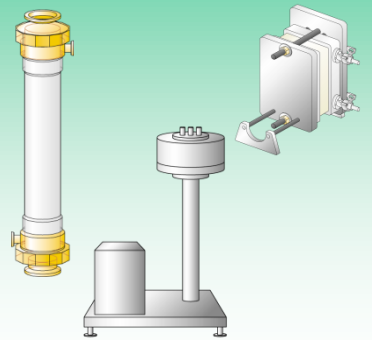
Bioreactor

Cell Harvest and Product Separation

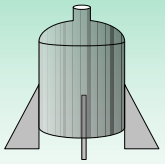
Buffer Filter



Depth Filtration



Tangential Flow Filtration



Centrifuge

Downstream Processing and Purification

Sterilizing-grade Liquid Filtration

Concentration Diafiltration

Buffer Filter

Capture Chromatography

Sterilizing-grade Liquid Filtration

Buffer Filter

Concentration Diafiltration

Buffer Filter

Purification Chromatography

Virus Removal Filtration

Polishing Chromatography

Buffer Filter

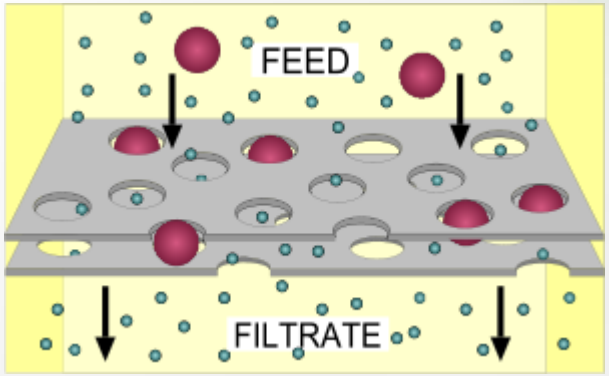
Concentration Diafiltration

Sterilizing-grade Liquid Filter

Formulation and Filling

Filtration

- ***Filtration separates particles based on size difference.***
- The fluid or particle that is smaller than the size of the pores in the filter passes through a filter (**filtrate or permeate**) while the bigger particles will be trapped by the filter (**retentate**).
- Ex) Coffee filter or air filter

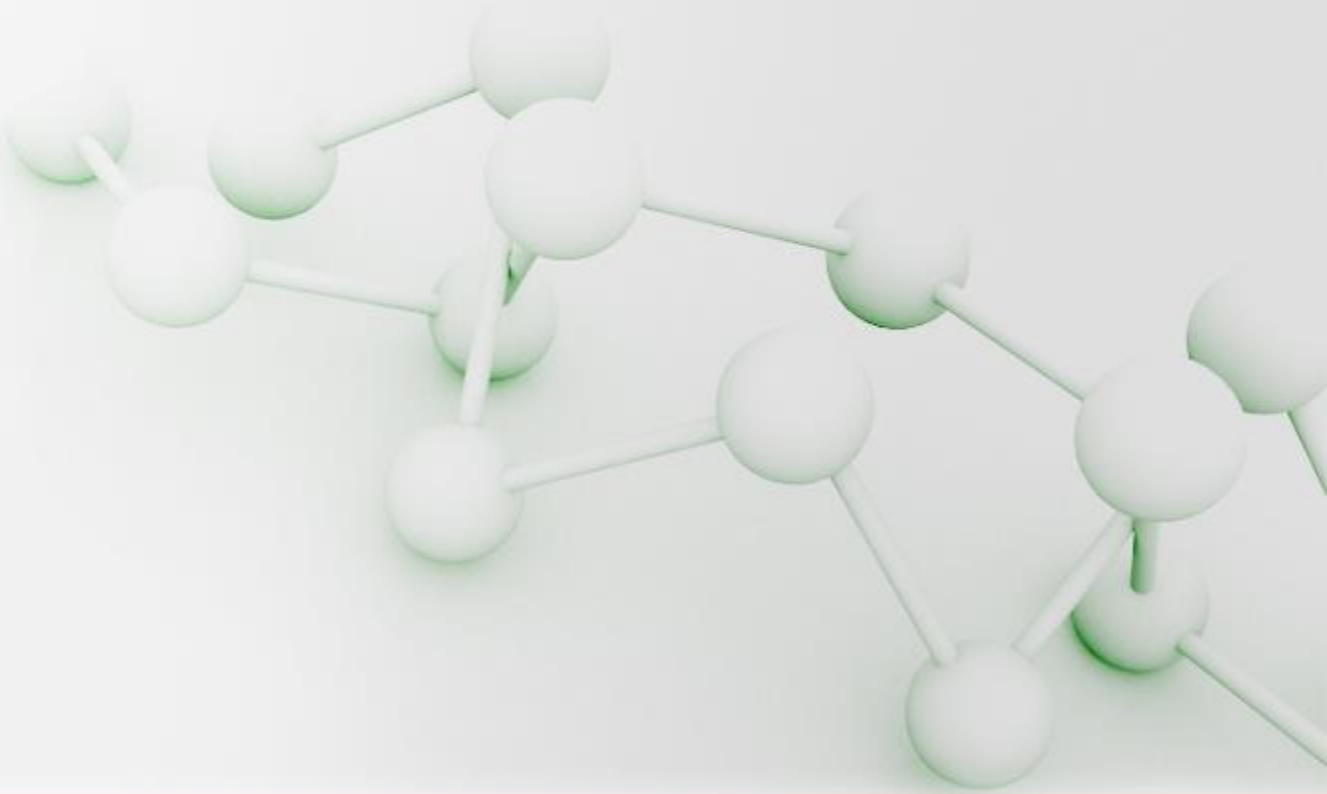


Commonly Used Terms

- **Feed:** The starting sample volume.
- **Feed Pressure:** The pressure (bars/psis) measured directly at the feed port (inlet) to the cassette holder. Bar = atmospheric pressure, Psi = pound per square inch
- **Fouling:** A build-up of retained or adsorbed species on the membrane surface resulting in decreased flux and possibly an increase in retention of permeable solutes.
- **Foulant:** The material causing fouling. The foulant may be the product or impurities (organic or inorganic) in the product or from other sources like water, buffers, etc.
- **Volumetric Flux:** The rate of volume flow across a unit area. Liters/second x area
- **Flow Rate (Filtrate Flow Rate):** The rate of sample flow through the membrane (rate of sample filtration), measured in volume/unit time.
- **Concentrate (Retentate):** The feed solution remaining above the membrane during or after concentration.

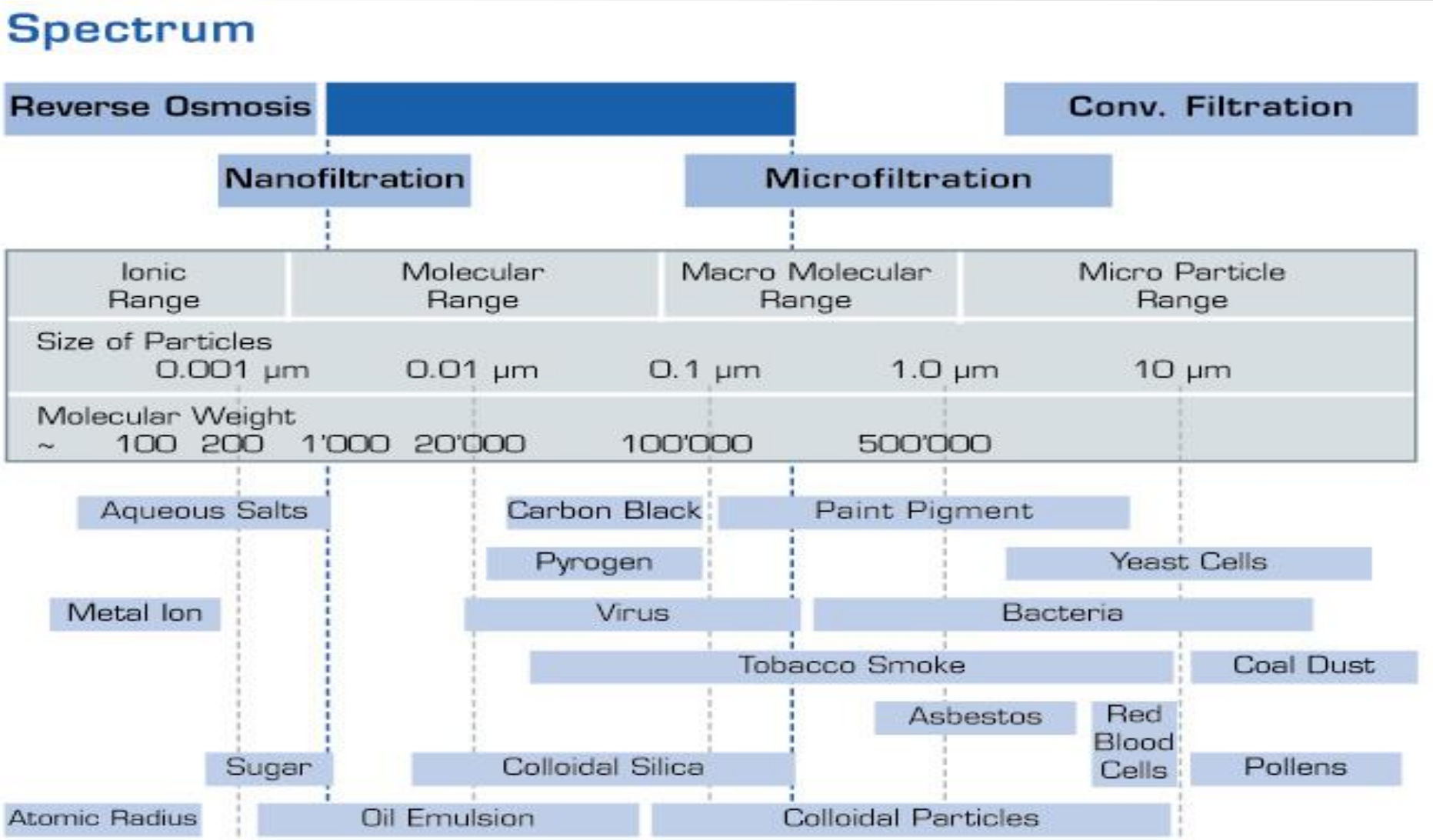
Commonly Used Terms

- **CIP (Clean in Place):** Act of using a chemical cleaning protocol to clean the membrane and membrane assembly free of any foulants.
- **kDa (kilodalton):** Relative atomic mass unit, e.g. H = 1 da, O = 16 da,



Various Filters

- Different filters based on their pore sizes: macro-, micro-, ultra-, and nano-filters



MWCO

The **Molecular Weight Cut Off (MWCO)** of a membrane or Nominal Molecular Weight Limit (NMWL), is defined by its ability to retain a given percentage of a solute of a defined molecular weight. Solute retention can vary due to molecular shape, structure, solute concentration, presence of other solutes and ionic conditions.

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

Filters Based On The Size Cut Off

- ***Macrofilters*** – separation of particles **10 µm or larger**. Filters are made out of glass fibers, sand, cloth (depth filters or general filters), and lab filter papers. Often used as pre-filters.

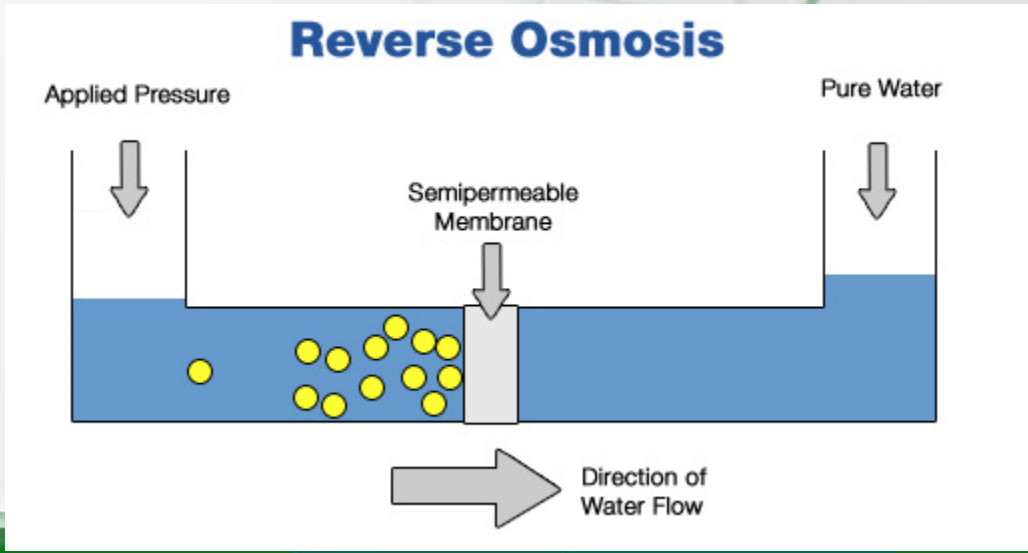
- ***Microfilters*** – separation of particles about **0.1 – 10 µm.** Particles that are same or larger than the pore size are 100% retained (bacteria and whole cells). Very often used to remove contaminating bacteria, fungi, and yeast from **heat sensitive solutions**.
  - 0.1 µm: removes Mycoplasma,
  - 0.22 µm: removes *E.coli*,
  - 0.65 µm: removes fungi and yeast,
  - 0.45 - 0.8 µm: removes general particles,
  - 1 – 2.5 or 5 µm: removes coarse particles

* **HEPA** (High Efficiency Particulate Air) filters can remove particles as small as 0.3 µm from air and used in biological safety hood and clean room.

Filters based on the size cut off

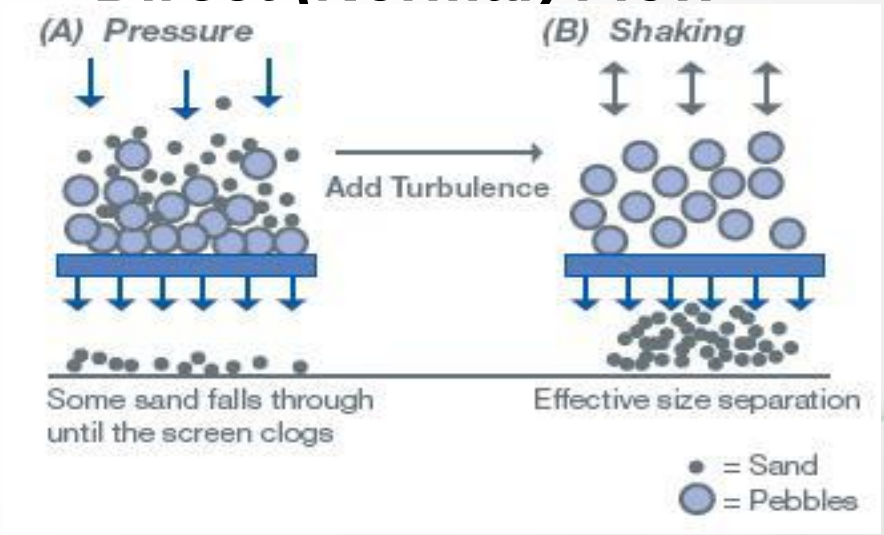
- ***Ultrafilters*** – separation of particles with molecular weight 1 – 1000 kDa and have pore diameters from 1 to 100 Å (angstrom=100 picometers). Used for fractionation, concentration and desalting.

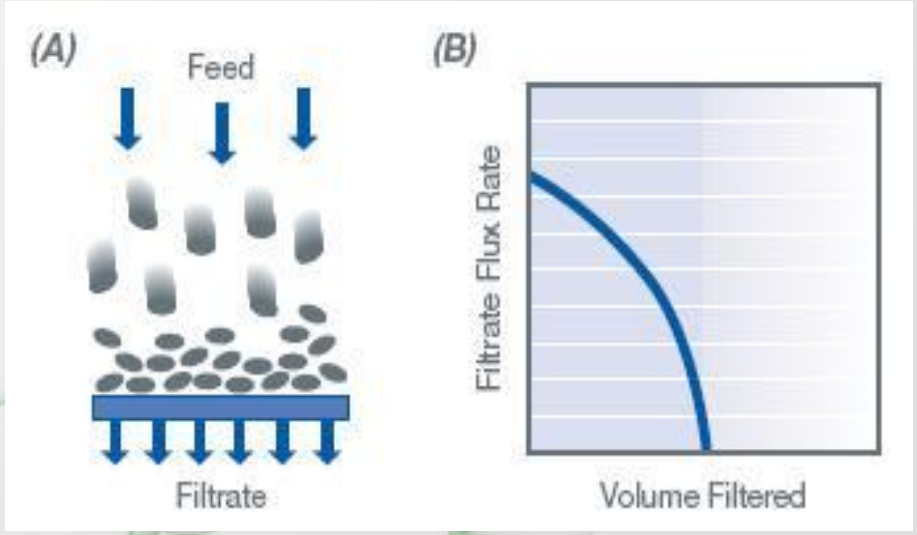
* **Reverse osmosis** – separates very low molecular weight materials (salts, viruses, microorganisms, pyrogens, etc.) from a liquid (water) that is under pressure flow using a special RO membrane that has very small pores or specific charge. It can retain smaller solutes than an ultrafiltration membrane. Often used in water purification.



Flow Direction in Filtration

- ***Direct (Normal) Flow***

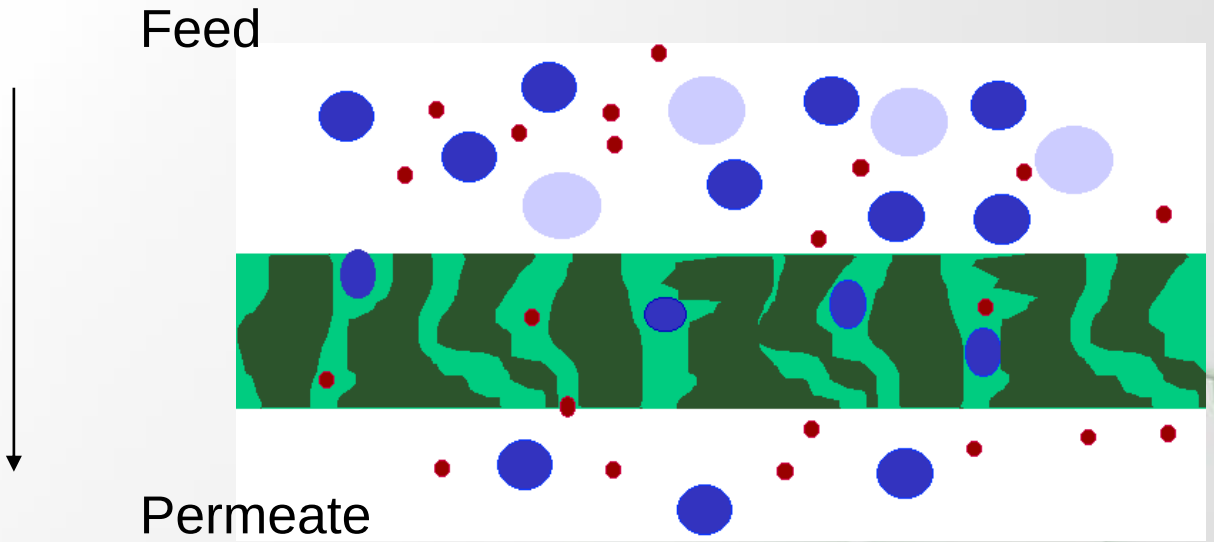




- Direct Flow Filtration (DFF), also known as ”dead-end” filtration, applies the feed stream perpendicular to the membrane face and attempts to pass 100% of the fluid through the membrane.
- *The feed is directed into the membrane. Molecules larger than the pores accumulate at the membrane surface to form a gel, which fouls the surface, blocking the flow of liquid through the membrane.* ***As the volume filtered increases, fouling increases and the flux rate decreases rapidly****.*

Flow Direction

- ***Direct (Normal) Flow Filtration***

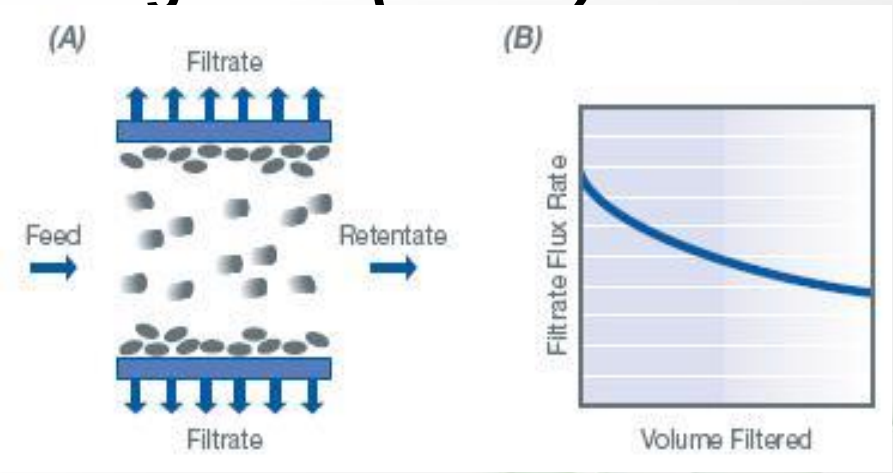


Internal plugging

Dramatic flux decline over time

Flow Direction in filtration

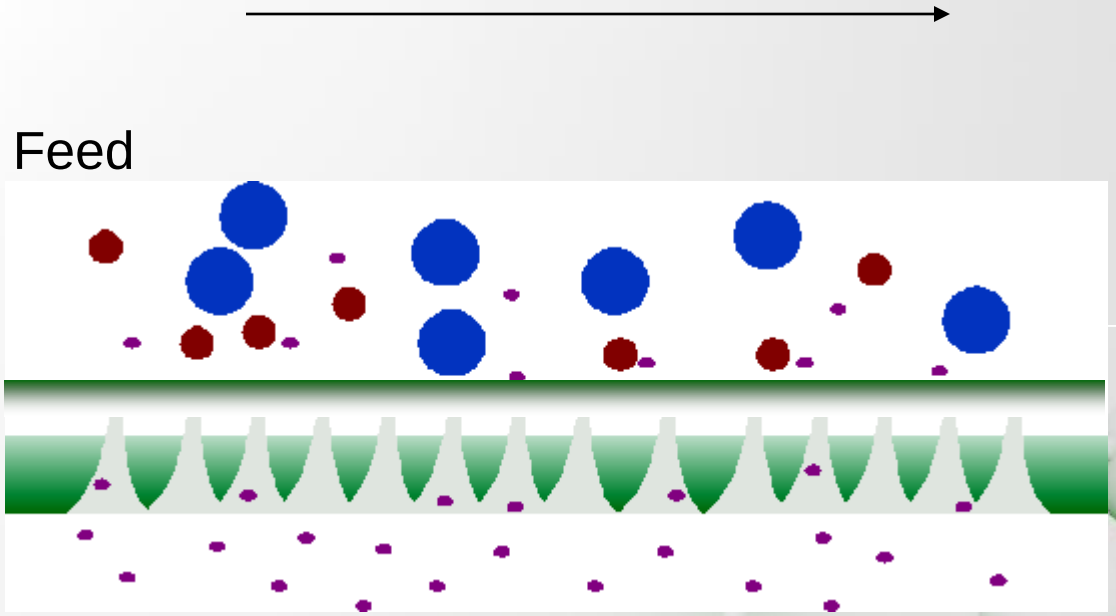
- ***Tangential (Cross) Flow***



- *Tangential Flow Filtration (TFF),* also known as *crossflow* filtration, is where ***the feed stream passes parallel to the membrane*** **face as one portion passes through the membrane (permeate) while the remainder (retentate) is recirculated back to the feed reservoir.**
- *Sample solution flows through the feed channel and along (tangent to) the surface of the membrane as well as through the membrane.* ***The crossflow prevents build up of molecules at the surface that can cause fouling. The TFF process prevents the rapid decline in flux rate seen in direct flow filtration allowing a greater volume to be processed per unit area of membrane surface.***

Flow Direction?

- ***Tangential Flow Filtration***

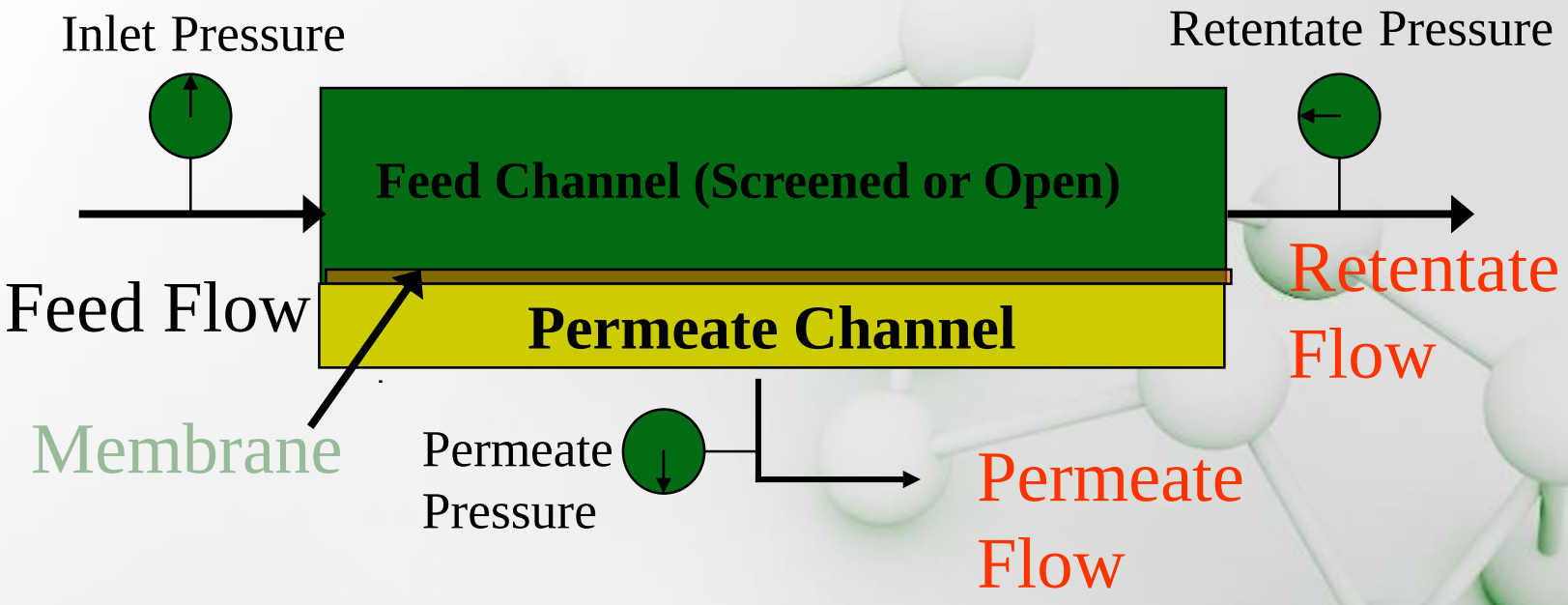


No internal plugging

Low pressure resistance

High flux performance

TFF Module

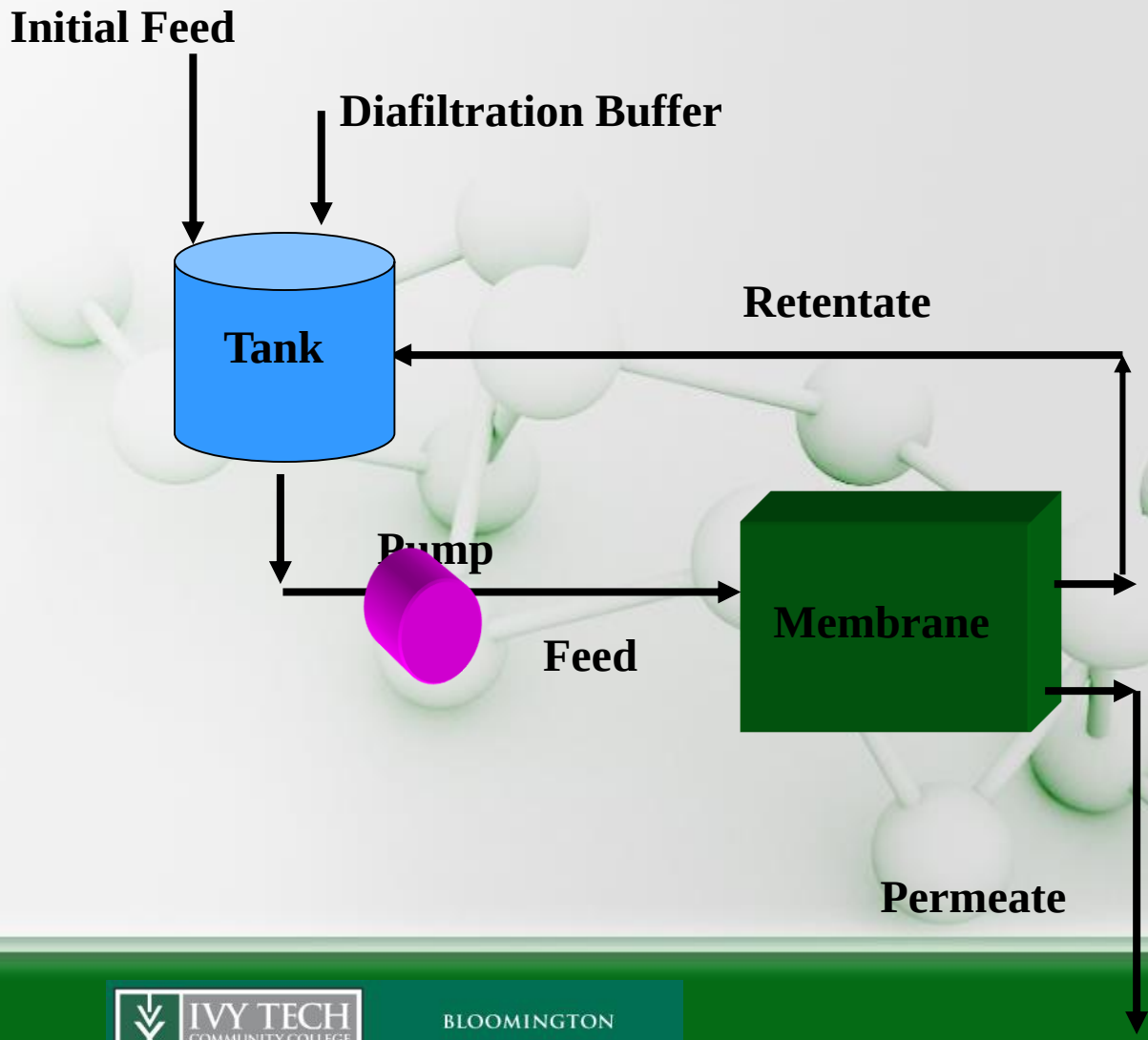

Inlet Pressure
Retentate Pressure
Feed Channel (Screened or Open)
Feed Flow
Permeate Channel
Retentate Flow
Membrane
Permeate Pressure
Permeate Flow

TFF Module

Basic TFF System

Basic Components:

- Membranes
- Pump
- Tank
- Piping



Concentration Using TFF

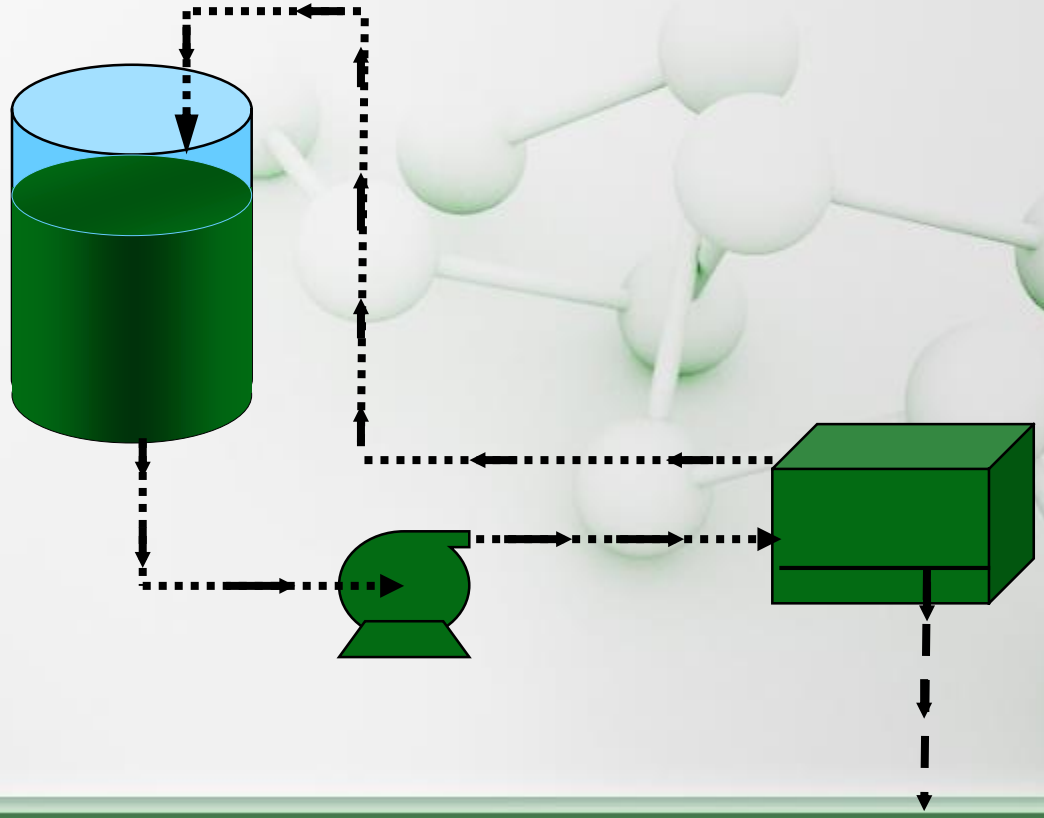
- **Concentration –** ***a process involves removing fluid from a solution while retaining the solute molecules. The concentration of the solute increases in direct proportion to the decrease in solution volume.***

- *A ultrafiltration membrane with a MWCO that is substantially lower than the molecular weight of the molecules to be retained is used.* A membrane with a MWCO that is 3 to 6 times lower than the molecular weight of the molecules to be retained. (e.g. ***150 kDa antibody is concentrated using a membrane with MWCO of 50 kDa***)

- The membrane is installed (or a disposable TFF capsule selected) and the TFF system is initialized (typically flushed with water and tested for water filtrate flow rate and integrity). Sample is added, a crossflow is established, feed and retentate pressures are set, then filtrate is collected. When the desired concentration is reached, the process is stopped and sample recovery or diafiltration may begin.

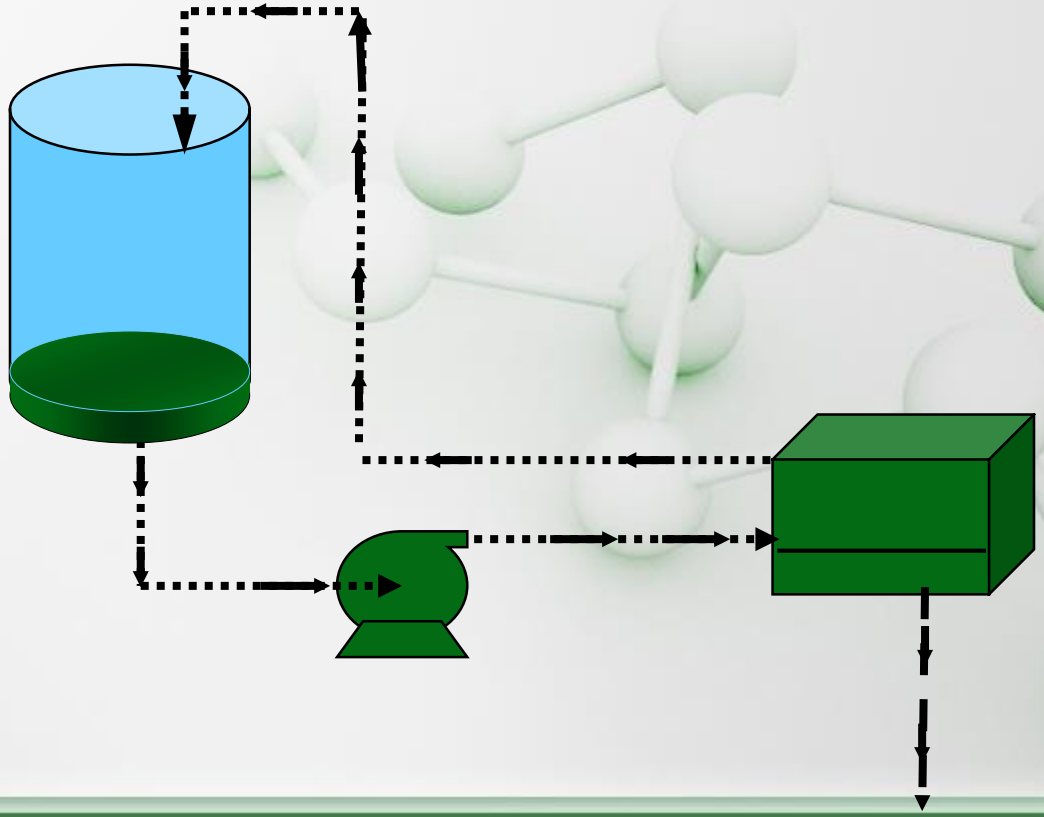
Concentration Using TFF

Concentration



Concentration using TFF

Concentration



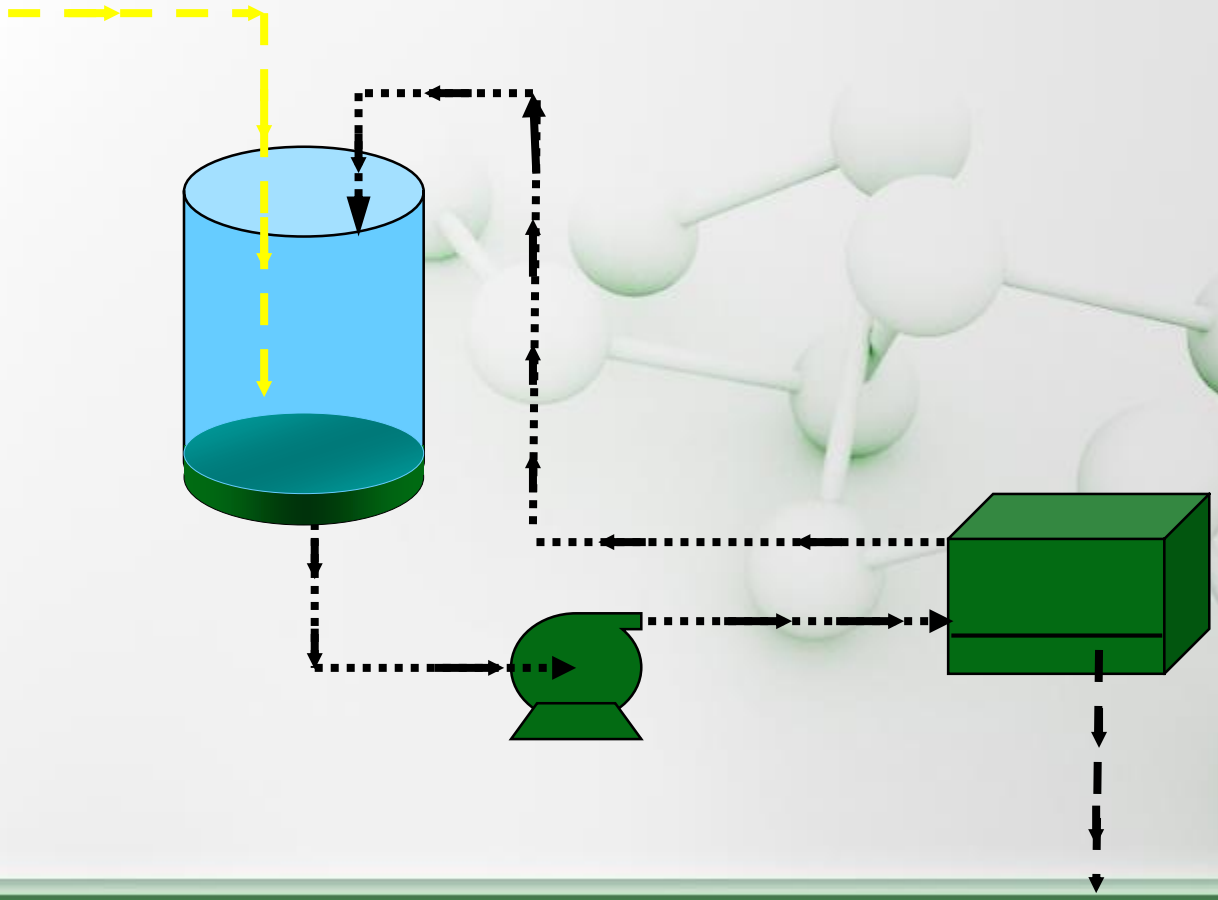
Diafiltration Using TFF

- **Diafiltration** – ***a process washes smaller molecules through a membrane and leaves larger molecules in the retentate without ultimately changing concentration.*** It can be used to remove salts or exchange buffers (e.g. removing salts from IEX elute fractions).

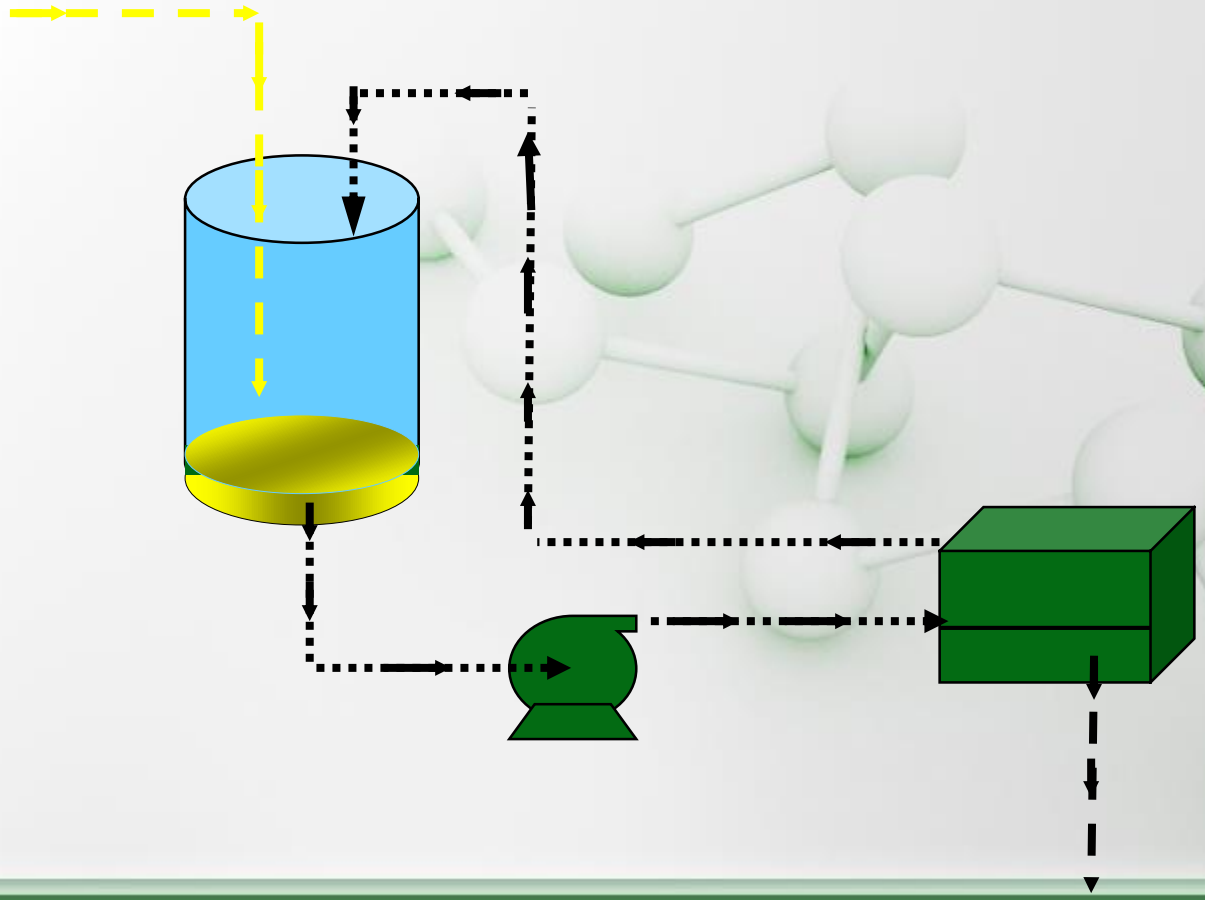
  - **Continuous diafiltration** - the diafiltration solution (water or buffer) is added to the sample feed reservoir at the same rate as filtrate is generated. The volume in the sample reservoir remains constant, but the small molecules (e.g. salts) that can freely permeate through the membrane are washed away.

  - **Discontinuous diafiltration** - the solution is first diluted and then concentrated back to the starting volume. This process is then repeated until the required concentration of small molecules (e.g. salts) remaining in the reservoir is reached.

Diafiltration using TFF



Diafiltration using TFF



Operation of TFF System

1. **Flush the TFF device to remove the storage agent**
2. **Clean in place (CIP)**
3. **Install a filter**
4. **Clean in place (CIP)**
5. **Flux system with water**
6. **Perform initial Normalized Water Permeability / Membrane Integrity tests to establish a baseline for the device performance.**
7. **Equilibrate system with the sample buffer (it helps remove air from the system, adjust system temperature and prevent possible precipitation or denaturation of biomolecules resulting from contact with flushing solution).**
8. **Process the Product (concentration and /or diafiltration, or fractionation).**
9. **Clean in place (CIP)**
10. **Perform NWP again to determine cleaning efficiency**
11. **Store TFF device.**

Flushing

- Flushing is done to remove storage or cleaning solution

- Flushing fluid (water) can be recirculated back to tank or directed to drain
  - ✓ Use clean water
  - ✓ Flushing directly to drain may be more efficient
  - ✓ Flushing with warm water may help

CIP

- Why Clean?
  - ✓ To remove product residue from the system: prevents cross-contamination of batches
  - ✓ To maintain low level of bioburden: kills bacteria, mold, viruses
  - ✓ To maintain low level of endotoxin
  - ✓ To restore membrane permeability: ensure reproducible filtration from run to run

CIP

- Cleaning Agents:
  - ✓ Must effectively remove any process material left in system
  - ✓ Must be compatible with materials of construction (membrane, module, system hardware)
  - ✓ Must be able to validate removal of cleaning agent from the system
  - ✓ Commonly used agents:
    NaOH at 0.1 - 1.0 N

CIP

- Cleaning Procedures:
  - ✓ Add cleaning (CIP) solution to tank:
    Be careful to mix thoroughly.
    If temperature of cleaning solution elevated (~40°C), cleaning will be more efficient.
  - ✓ Flush small volume of CIP solution to drain:
    Retentate and permeate directed to drain.
  - ✓ Operate system in total recycle mode:
    Retentate and permeate directed back to tank and recirculate for minimum of 30 minutes.
    The system can be held for a certain amount of time at recycle and flushed out.
  - ✓ Cleaning flows and pressures usually similar to processing flows and pressures.

Initial Normalized Water Permeability Test

- What is initial ***Normalized Water Permeability (NWP)*** Test?
  - ✓ *A measure of the ability of water to pass through the membrane*
  - ✓ Conduct initial NWP before using TFF

- Why is initial NWP needed?
  - ✓ ***It serves as a benchmark for the cleanliness of the membranes***

- How is NWP Measured?
  - ✓ Filter Pure Water Through the Membrane
  - ✓ Measure:
    - ➢ Permeate flow rate
    - ➢ Feed, retentate, and permeate pressures
    - ➢ Water temperature
  - ✓ Calculate the **Water Flux Normalized for area, pressure, and temperature**

NWP

- ***Normalized Water Permeability (NWP):***

NWP = (Water Permeate Flux Rate, LMH) x (Temp. Correction Factor at 20 ^{o}C)
(Transmembrane Pressure)

- **Permeate Flux (Filtrate Flux):**
  The rate of sample flow through a given membrane area per unit time. Commonly expressed as LMH (liters/m^2/hr).

- **TCF** (default set is at 20 ^{o}C) values for different water temperatures:
  1.14 at 15 ^{o}C
  1.11 at 16 ^{o}C
  1.08 at 17 ^{o}C
  1.05 at 18 ^{o}C
  1.03 at 19 ^{o}C
  1.00 at 20 ^{o}C
  0.98 at 21 ^{o}C …

NWP

- ***Transmembrane Pressure (TMP):*** the force that drives fluid through the membrane, carrying along the permeable molecules. Calculated as the average pressure applied to the membrane minus any filtrate pressure.

$$TMP = \left(\frac{P_{feed} + P_{retentate}}{2}\right) - P_{permeate}$$

In most cases, $P_{permeate} = 0$

✓ **Membrane MWCO or Pore Size and TMP**

UF: 0.65 kDa – 100 kDa (MWCO) - 0.7 bar (10 psi)
UF: >100 kDa (MWCO) - 0.3 bar (5 psi)
MF: 0.1 µm – 1.2 µm - 0.1 – 0.2 bar (2 psi)

NWP

- ***Example problem:***
  Water permeate flux rate (110 LMH) was measured for a 10kDa UF membrane cassette at ***transmembrane pressure*** *of 10 psi.*
  The temperature of water was 16 ^{o}C*.*
  Determine the initial NWP at 20 ^{o}C.

  Water permeability = 110 LMH x TCF at 20 ^{o}C (1.109) / 10 psi
  = 121.9 LMH / 10 psi
  = 12.19 LMH / psi

Filter Integrity Test

- Why Integrity Test?
  - ✓ If filter is not intact, your separation will not be performed properly and product may be lost.
  - ✓ Required to satisfy regulatory concerns

- Principle of Integrity Testing
  - ✓ Wet the membrane completely with water
  - ✓ *Measure pressure decay through the wetted membrane at low pressure (30 lbs for 5 minutes).*

Buffer Equilibration

- What is Buffer Equilibration?
  - ✓ Wetting the membrane with a buffer that is compatible with the feed solution
  - ✓ Prevents any solutes from being precipitated or denatured when they contact the membrane

- How is it done?
  - ✓ Circulate an appropriate buffer through the membranes under pressure.
  - ✓ Take a sample for a pH check.

Product Processing

- Types of Processing:
  - ✓ Concentration
  - ✓ Diafiltration

- Procedures:
  - ✓ Fill tank with process fluid
  - ✓ Start pump and adjust system to recommended flows/ pressures
  - ✓ Concentrate and diafilter, add diafiltration fluid where required

Final NWP Test

- Final Normalized Water Permeability Test
  - ✓ Repeat same measurements (NWP) as initial NWP
  - ✓ Calculate percentage of recovery after cleaning

    NWP Recovery = $\frac{\text{NWP after cleaning (final)}}{\text{Initial NWP}}$

    Percentage Recovery = Recovery x 100

    The rate varies (client select) but should be 60% or above.

- Why is it Useful?

  Determines the effectiveness of the cleaning procedure.

Storage

- Why
  - ✓ Keep the membranes wet
  - ✓ Prevent growth of bacteria, mold, fungi, etc. when the system is not in use
- Procedure
  - ✓ Prepare preservative solution in tank
  - ✓ Circulate solution through membranes in total recycle mode
  - ✓ For long term storage, membranes can be removed from the system

Important Parameters during Operation

- ✓ Pressure
- ✓ Flow rate
- ✓ TMP
- ✓ Product level, permeate reservoir
- ✓ Weight
- ✓ Temperature (depends on the system)
- ✓ Sample taking
- ✓ Connections
- ✓ Pump speed
- ✓ Flow in-between processing steps
- ✓ Massaging the bags to encourage mixing

Questions or Comments

Review for today

TFF stands for?

a. Total Flow Filtration
b. Total Filtration Flow
c. Tangential Flow Filtration
d. Tangential Filtration Flow
e. None of the above

Review for today

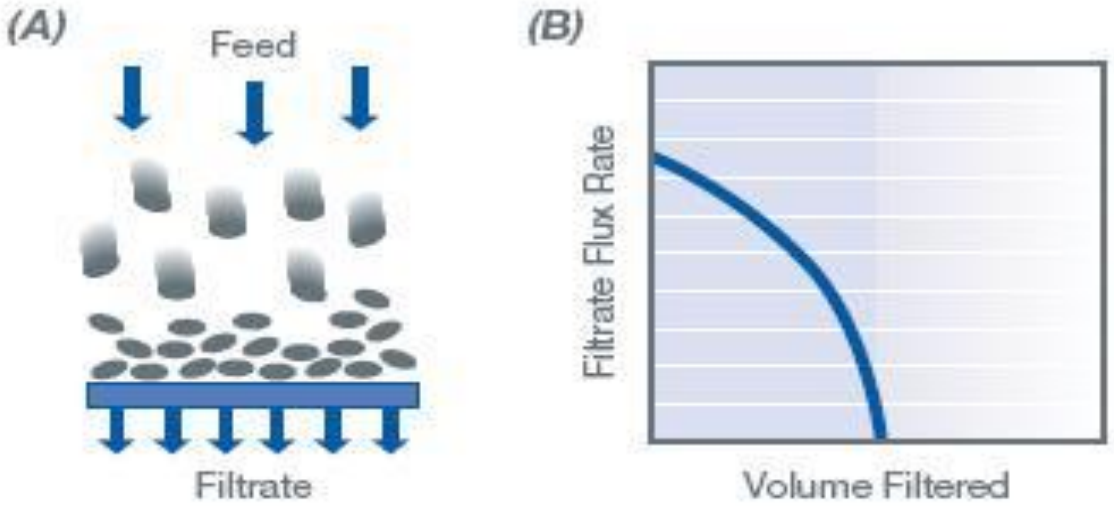
Fill in the blanks with the most appropriate terms below.

- (_____) is the fluid or particle that is smaller than the size of the pores in the filter passes through a filter while (_____) is the bigger particles that will be trapped by the filter.
- (_____) is a build-up of retained or adsorbed species on the membrane surface resulting in decreased flux and possibly an increase in retention of permeable solutes.
- (_____) is the rate of volume flow across a unit area. Liters/ second x area
- (_____) is act of using a chemical cleaning protocol to clean the membrane and membrane assembly free of any foulants.
- (_____) of a membrane is defined by its ability to retain a given percentage of a solute of a defined molecular weight.
- (_____) filter can remove particles as small as 0.3 μm from air and used in biological safety hood and clean room.

A. Fouling
B. HEPA
C. Flow Rate
D. CIP
E. Retentate
F. Molecular Weight Cut Off
G. Filtrate

Review for today

The diagram below shows filtration principle of which of the following?



A. TFF
B. DFF
C. Cross Flow filtration
D. None of the above

Review for today

Which of the following statement ***is not correct*** about the concentration operation using TFF?

A. A process involves removing fluid from a solution while retaining the solute molecules.

B. The concentration of the solute increases in direct proportion to the decrease in solution volume.

C. A macrofiltration membrane with a MWCO that is substantially lower than the molecular weight of the molecules to be retained is normally used.

D. A membrane with a MWCO that is 3 to 6 times lower than the molecular weight of the molecules to be retained is used.

Review for today

Match the filtration with its description.

Separation of particles with molecular weight 1 – 1000 kDa and have pore diameters from 1 to 100 Å (angstrom=100 picometers). Used for fractionation, concentration, and desalting.

Separation of particles about 0.1 – 10 µm. Particles that are same or larger than the pore size are 100% retained (bacteria and whole cells). Very often used to remove contaminating bacteria, fungi, and yeast from heat sensitive solutions.

Separation of particles **10 µm or larger**. Filters are made out of glass fibers, sand, cloth (depth filters or general filters), and lab filter papers. Often used as pre-filters.

A. Macrofiltration
B. Microfiltration
C. Ultrafiltration

Review for today

Which of the following ***is incorrect*** about the buffer equilibration step in the TFF operation?

A. Involves wetting the membrane with a buffer that is compatible with the feed solution

B. Prevents any solutes from being precipitated or denatured when they contact the membrane

C. Involves in circulating an appropriate buffer through the membranes under pressure.

D. None of the above.

Review for today

Which of the following ***is incorrect*** about the CIP step in the TFF operation?

A. It removes product residue from the system: prevents cross-contamination of batches

B. It allows maintaining low level of bioburden: kills bacteria, mold, viruses

C. It restores membrane permeability: ensure reproducible filtration from run to run

D. 1 N HCl is commonly used as a cleaning agent.

Review for today

"Filter integrity test is normally performed by monitoring (_____) for about 5 minutes."

A. Pressure decay

B. Pressure increase

C. Intake flow rate

D. Retentate flow rate

Review for today

Calculate Normalized Water Permeability for a *10 kDa UF membrane cassette at* ***transmembrane pressure*** *of 10 psi. Water permeate flux rate was 110 LMH at 20 ^{o}C.*

IT'S TIME FOR A BREAK!

THEN LAB SESSION

Hands-on Lab

TFF application using a ultrafiltration membrane

1. **Purification** of yellow dye molecules from blue dye molecules (green mixture: yellow + blue)

2. **Concentrating** diluted blue dye solution

Basic information about the dye molecules and the membrane

TFF membrane MWCO is10 KDa

The yellow dye (Acid Yellow with 300 Da MW)

The blue dye (Dextran Blue with 2000 Kda MW)

* 1 kDa= 1,000 Da

Order of Operation

1. Flush the TFF device with 70 ml of water.
2. Measure pressure decay while flushing
3. Conditioning is not necessary since dye molecules are dissolved in water
4. Process the sample (concentration or purification)
5. Clean in Place (CIP) with 50 ml of 0.1N NaOH
5. Flush with 70 ml of water
6. Measure pressure decay

Work in Two Groups

1. Group A: Concentration of Dextran Blue Solution
2. Group B: Purification of Acid Yellow from Dextran Blue

Group A. Concentrating Dextran Blue

1. Flush the system with 70 ml of water.
2. Measure pressure decay
3. Add 75 ml of diluted Dextran Blue solution (25 ml of DB + 50 ml of water). Save 2 ml of initial material in a tube for a spectrophotometer assay at 600 nm.
4. Perform TFF operation at 15 psi retentate pressure.
5. Recirculate the retentate until it becomes 10 ml volume in the tank.
6. Save 5 of permeate fractions with 5 ml volume each.
7. Collect the retentate
8. Perform CIP (50 ml of 0.1N NaOH)
9. Measure pressure decay with 100 ml of H_2O

Group B. Purifying Acid Yellow

1. Flush the system with 70 ml of water.
2. Measure pressure decay
3. Add 100 ml of green solution (50 ml of DB and 50 ml of AY dye solution mix). Save 2 ml of green solution in a tube for spectrophotometer assay
4. Continue TFF filtration at 15 psi retentate pressure
5. Collect permeate fractions with 5 ml volume each
6. Recirculate the retentate until it becomes 10 ml volume in the tank.
7. Add extra 50 ml of water to the fill tank for continuous separation.
8. Perform CIP with 50 ml of 0.1N NaOH
9. Measure pressure decay with 100 ml of H_2O

What To Do

- Monitor the amount of dye molecules in your initial, retentate, and permeate fractions using spectrophotometer measurements at A460 (yellow) and A600 (blue).

- Present the fractionation principle and the results from your procedures at the end of the lab!