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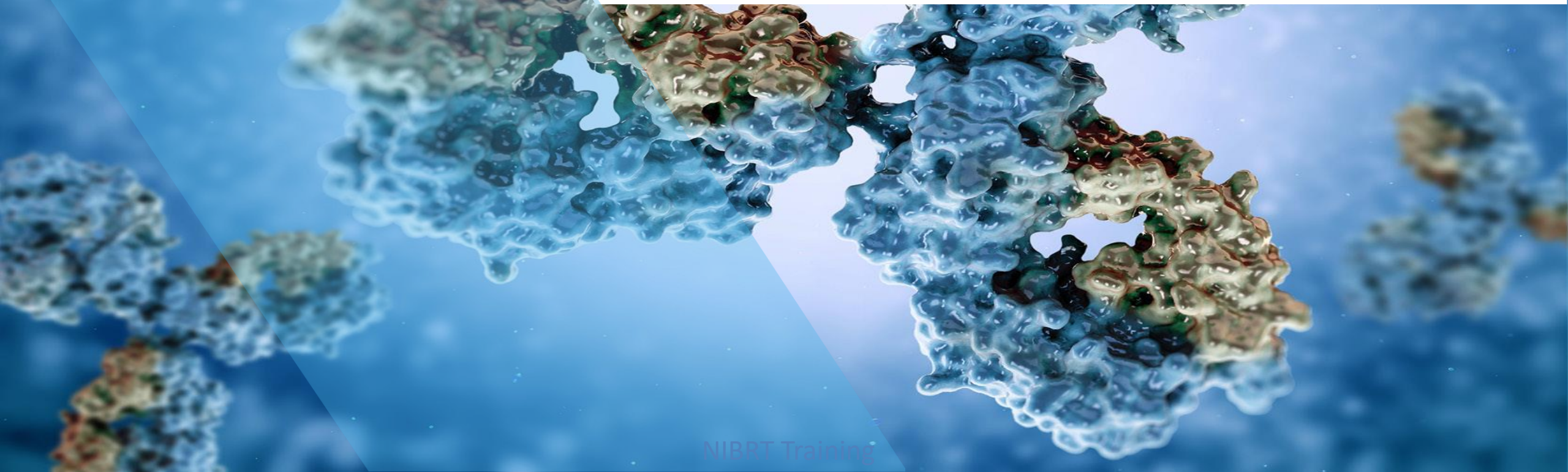
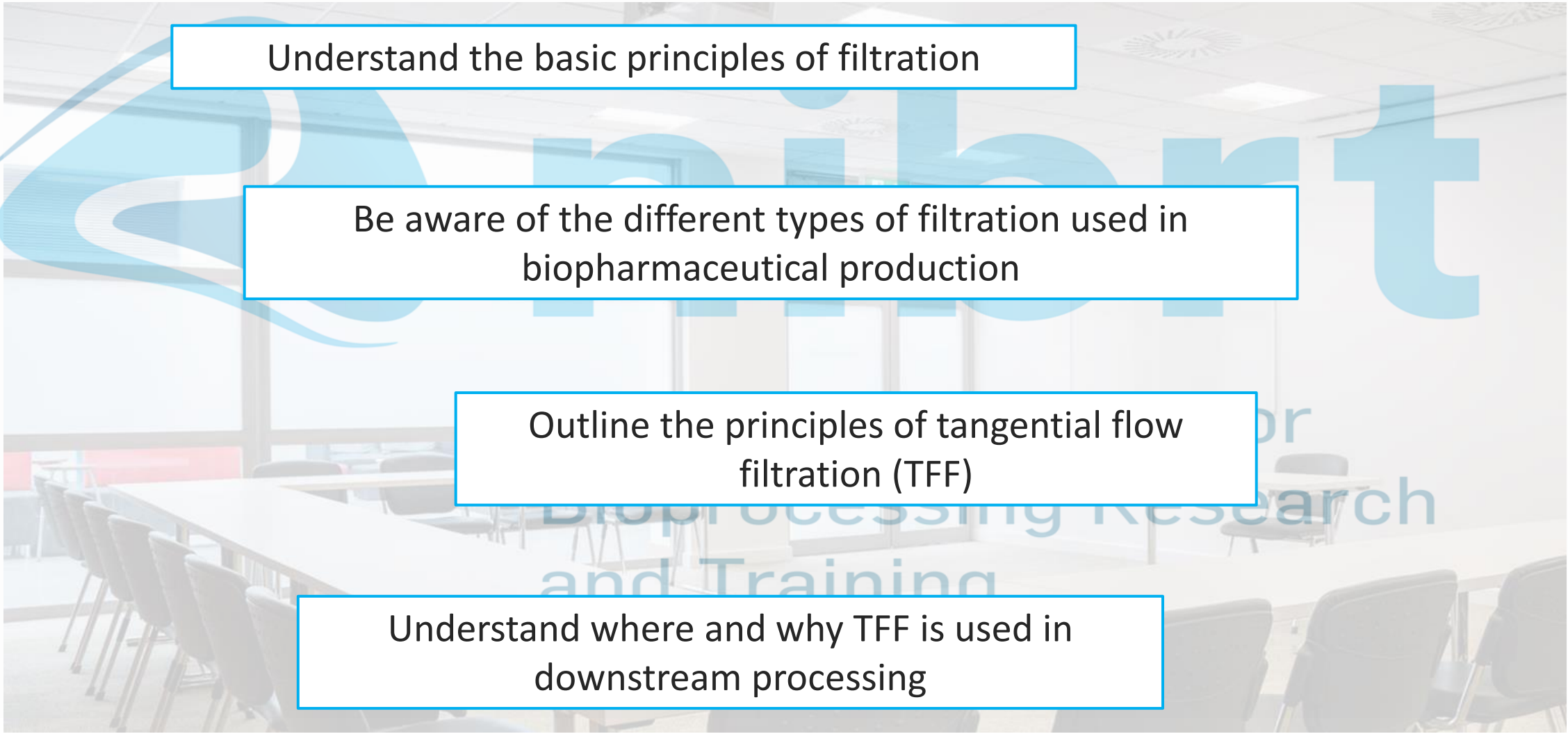
Filtration



Learning Objectives



- Understand the basic principles of filtration
- Be aware of the different types of filtration used in biopharmaceutical production
- Outline the principles of tangential flow filtration (TFF)
- Understand where and why TFF is used in downstream processing

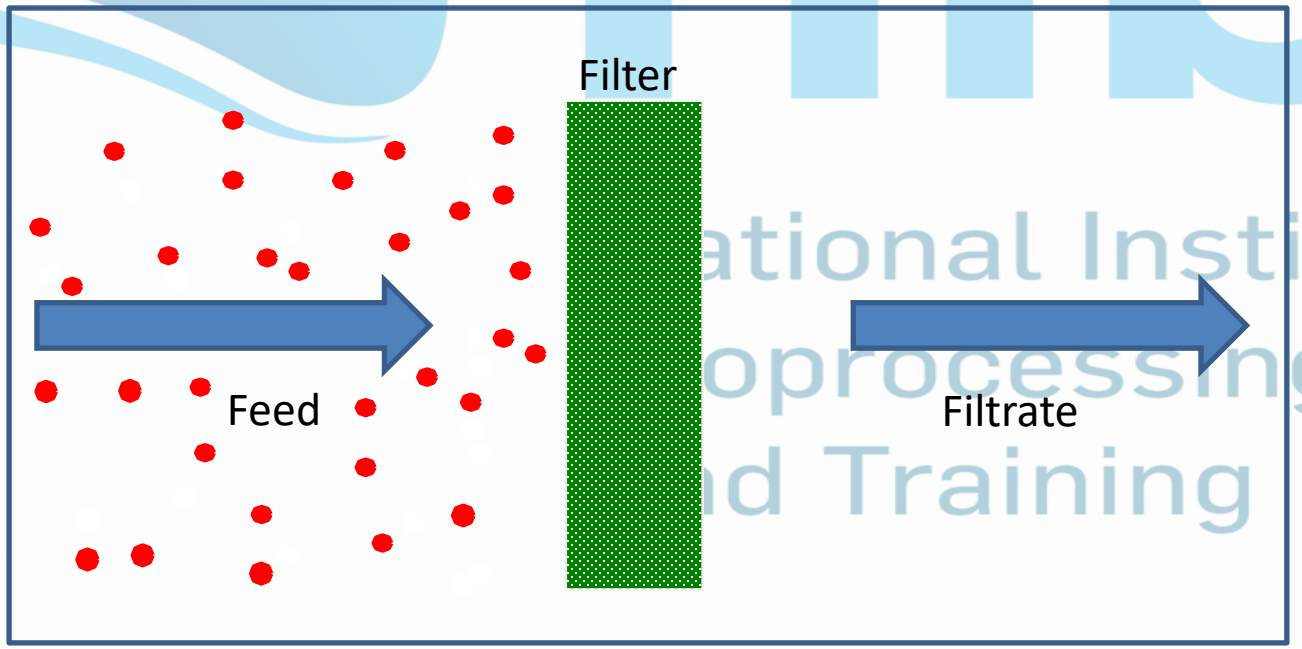
Topics

- Principles of Filtration
- Normal Flow Filtration
- Tangential Flow Filtration UFDF
- UF/DF Operations

General Filtration Principles

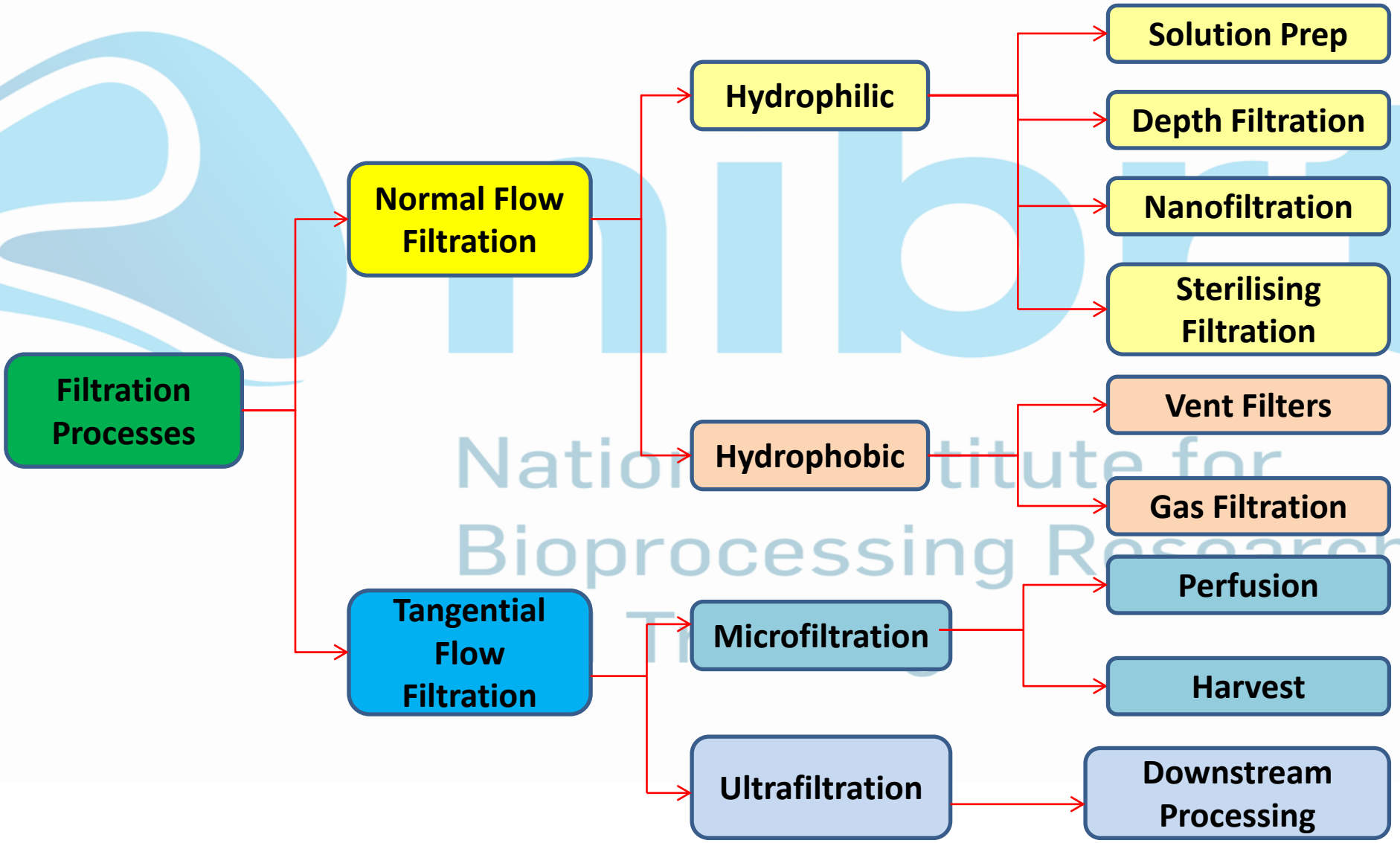
Filtration

The removal of particles from a fluid (gas or liquid) stream through the use of a porous medium.



Needs a driving force:
Pressure
or
Concentration

Filtration Processes



Filtration in Biopharma

Filtration is widely used in Biopharma to clarify and/or to sterilize solutions

Sterilizing grade filters are widely used due to:

- speed compared to other sterilisation processes
- the heat-sensitive nature of proteins

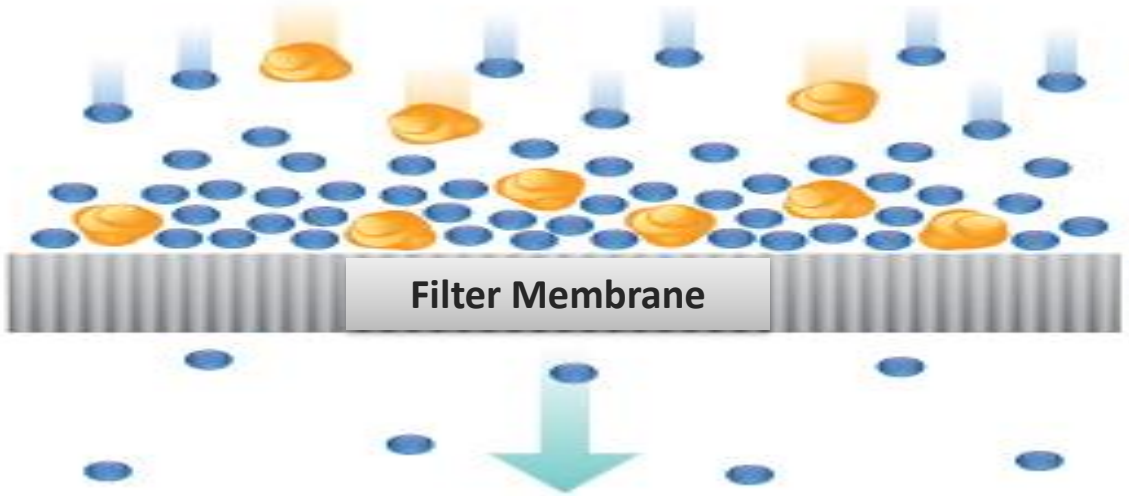
Mainly filters can be categorized into **depth** and **membrane filters**

Depth are not used as a means of sterilisation due to particle generation

Factors that can affect filter performance	
Viscosity	Flow rates
Surface tension of feed	Maximum use time
pH	Temperature
Compatibility of feed	Osmolality
Pressures	Hydraulic shock.

Membrane Filtration

- A membrane is a selective layer that allows certain components to pass while rejecting others

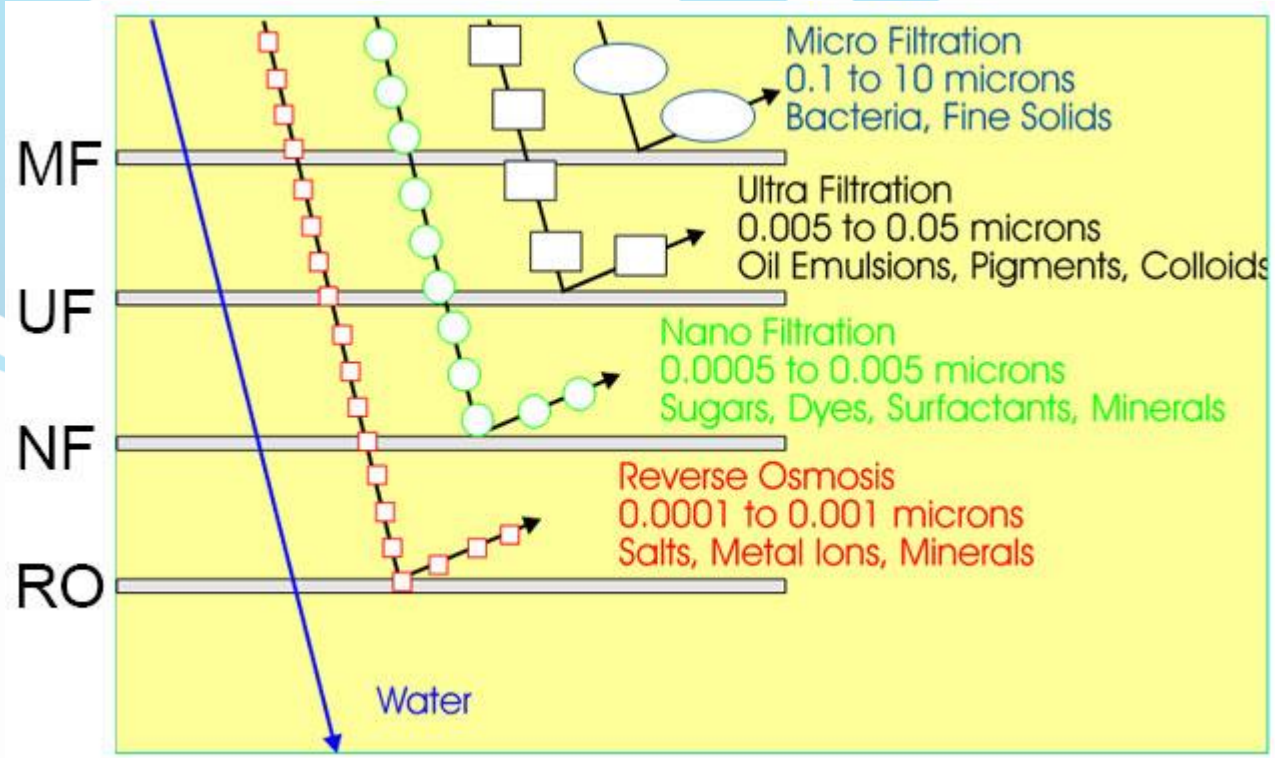


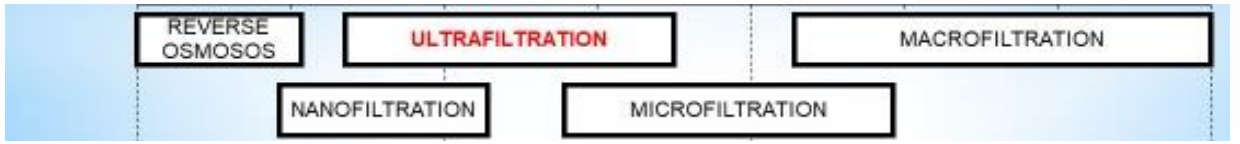
- Membranes can operate with liquid or gaseous feed streams and can be either dense or porous

Filter Polymer type:
e.g. cellulose acetate, polyamide polyether sulfone, polyvinylidene fluoride, polytetrafluoroethylene, polypropylene (PP), polyethylene (PE), glass fiber

Filter Pore Sizes

Micro | Ultra | Nano | Reverse Osmosis





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http://www.masterwater.com/wp-content/uploads/2018/09/Contaminant-Size-Scale.jpg

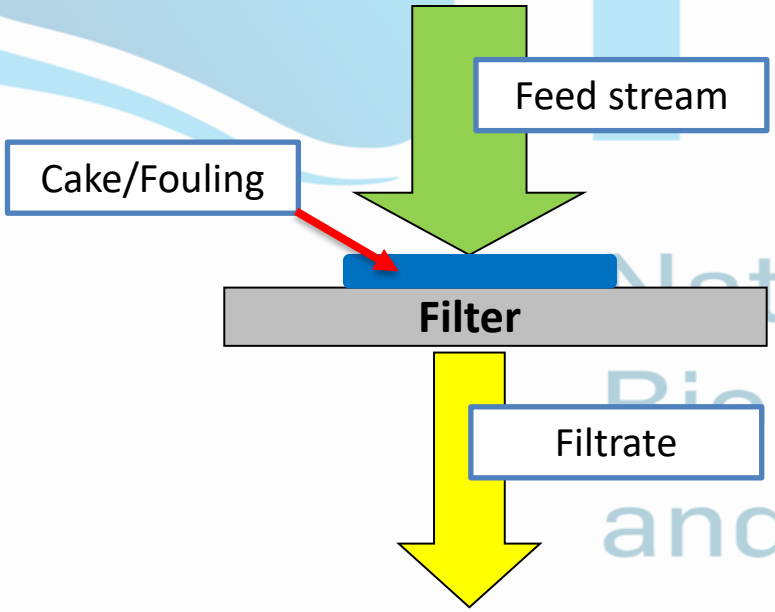
Topics

- Principles of Filtration
- Normal Flow Filtration
- Tangential Flow Filtration UFDF
- UF/DF Operations

Normal Flow Filtration

Particles collect on upstream side of filter during normal flow filtration (or frontal flow)

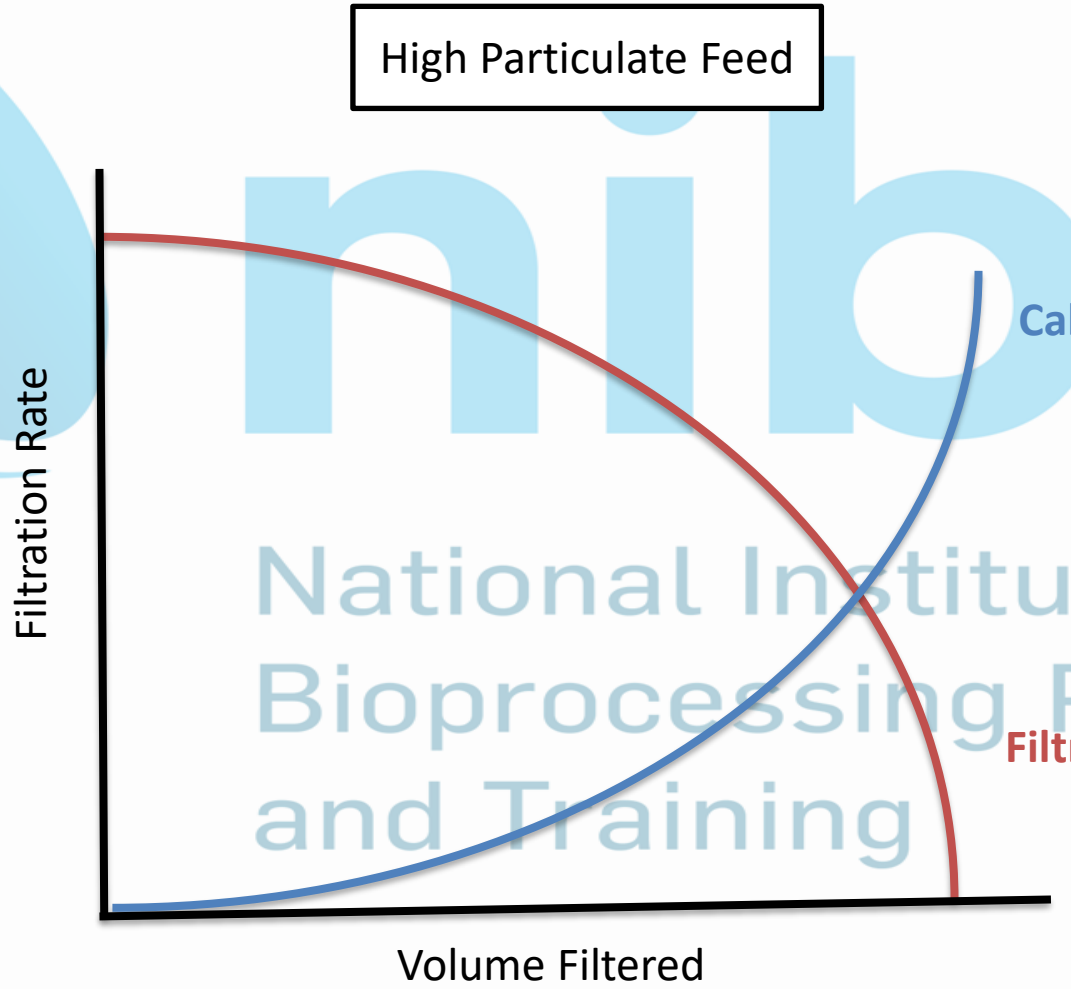
- Can result in fouling/clogging/caking of the filter surface



Normal Flow Filters

Normal flow filters are generally not reused after use

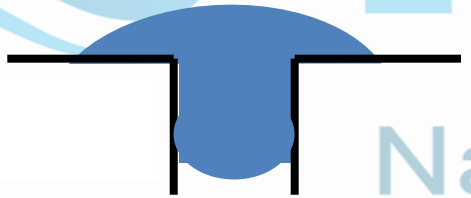
Normal Flow Filtration



Hydrophilic vs. Hydrophobic Filters

Hydrophilic Filters

"Water-loving"

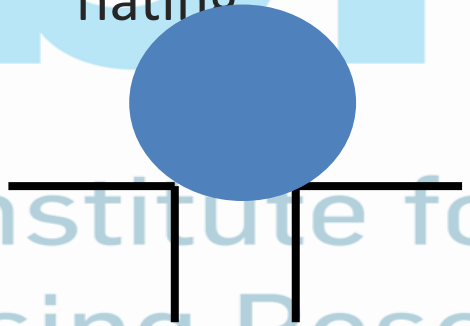


Applications

- Filtration of aqueous or aqueous/organic solutions

Hydrophobic Filters

"Water-hating"



Applications

- Vessel vent filters
- Gas filters

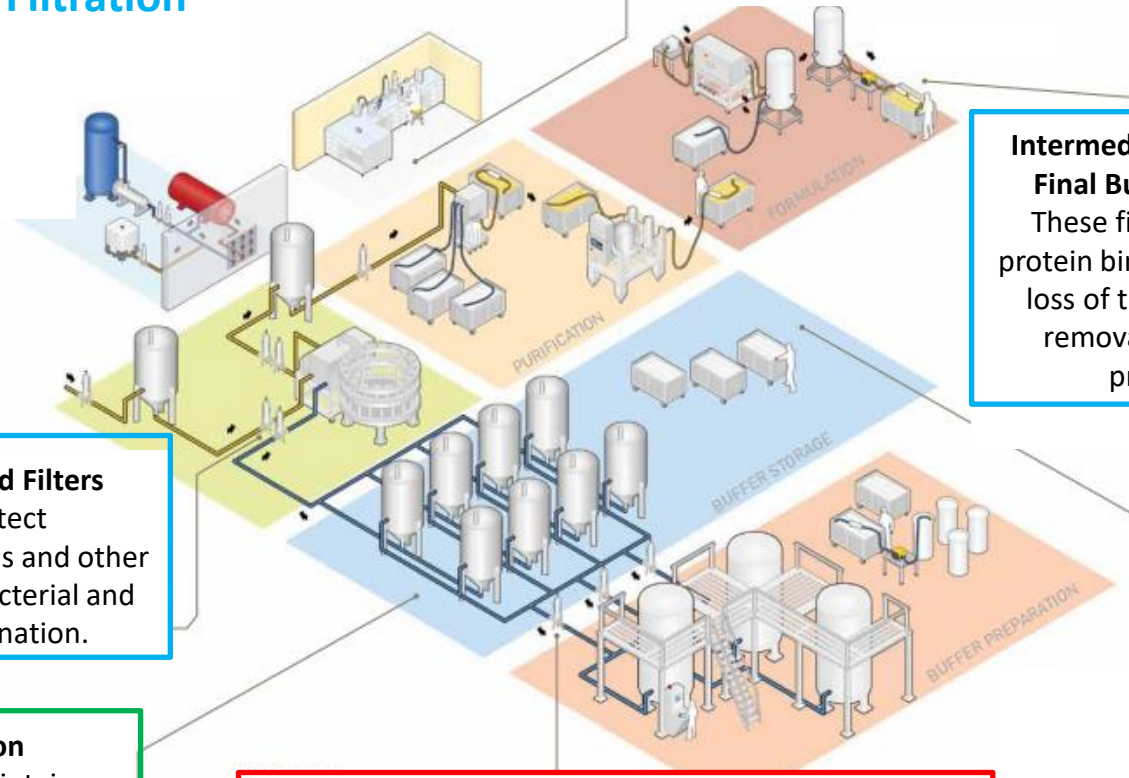
Normal Flow Filtration at Downstream

Buffer Filtration

Product Stream Filtration

Vent Filtration

Filter Integrity Testing
FIT is a fundamental requirement to ensure the safety of the final product



Intermediate Product Stream & Final Bulk Product Filtration
These filters should have low protein binding to ensure minimal loss of the product during the removal of bioburden from product streams.

Chromatography Skid Filters
These filters protect chromatography columns and other d/s equipment from bacterial and particulate contamination.

Vent Filtration
Vent filtration maintains sterility in buffer, cell culture and intermediate holding tanks

Buffer Filtration
Filtration of buffers is essential to protect downstream equipment and ensure quality of the final product

Ref: www.parker.com

Topics

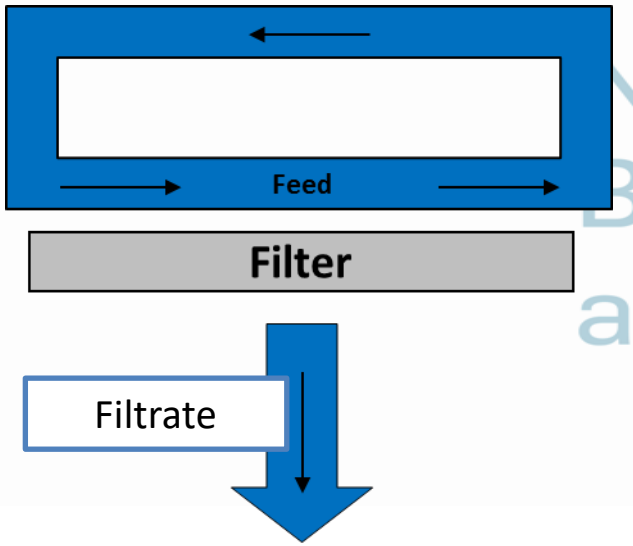
- Principles of Filtration
- Normal Flow Filtration
- Tangential Flow Filtration UFDF
- UF/DF Operations

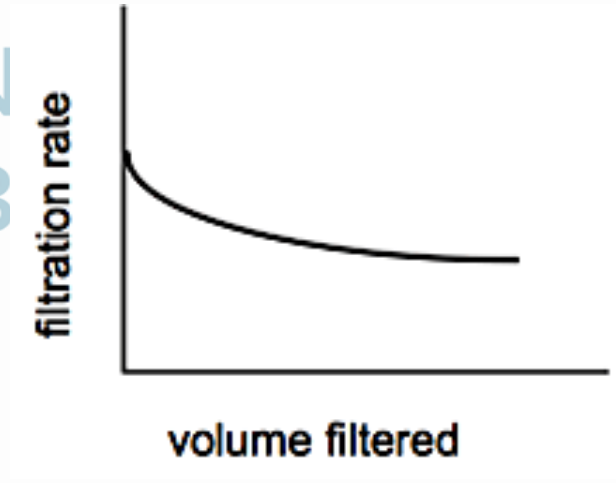
Tangential Flow Filtration (TFF)

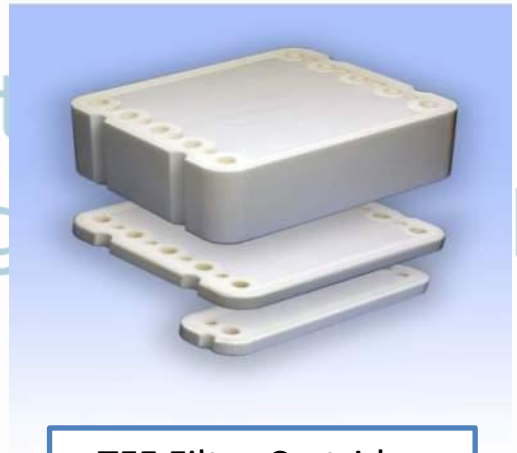
Tangential flow filtration (TFF) reduces cake formation/clogging

TFF **increases throughput** and **prevents material from building** on the membrane

Filter is “self-cleaning” and can be re-used for several batches

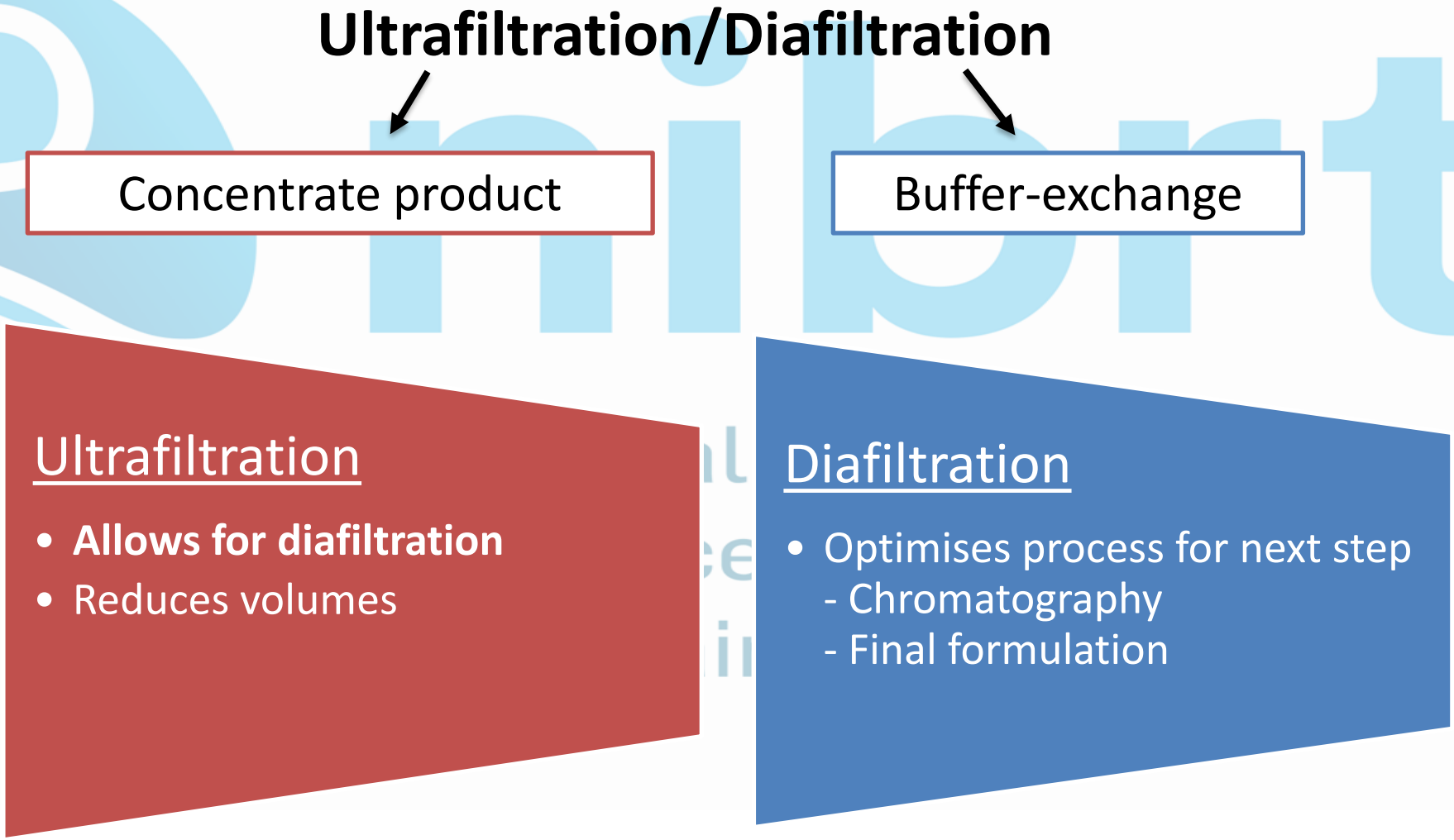




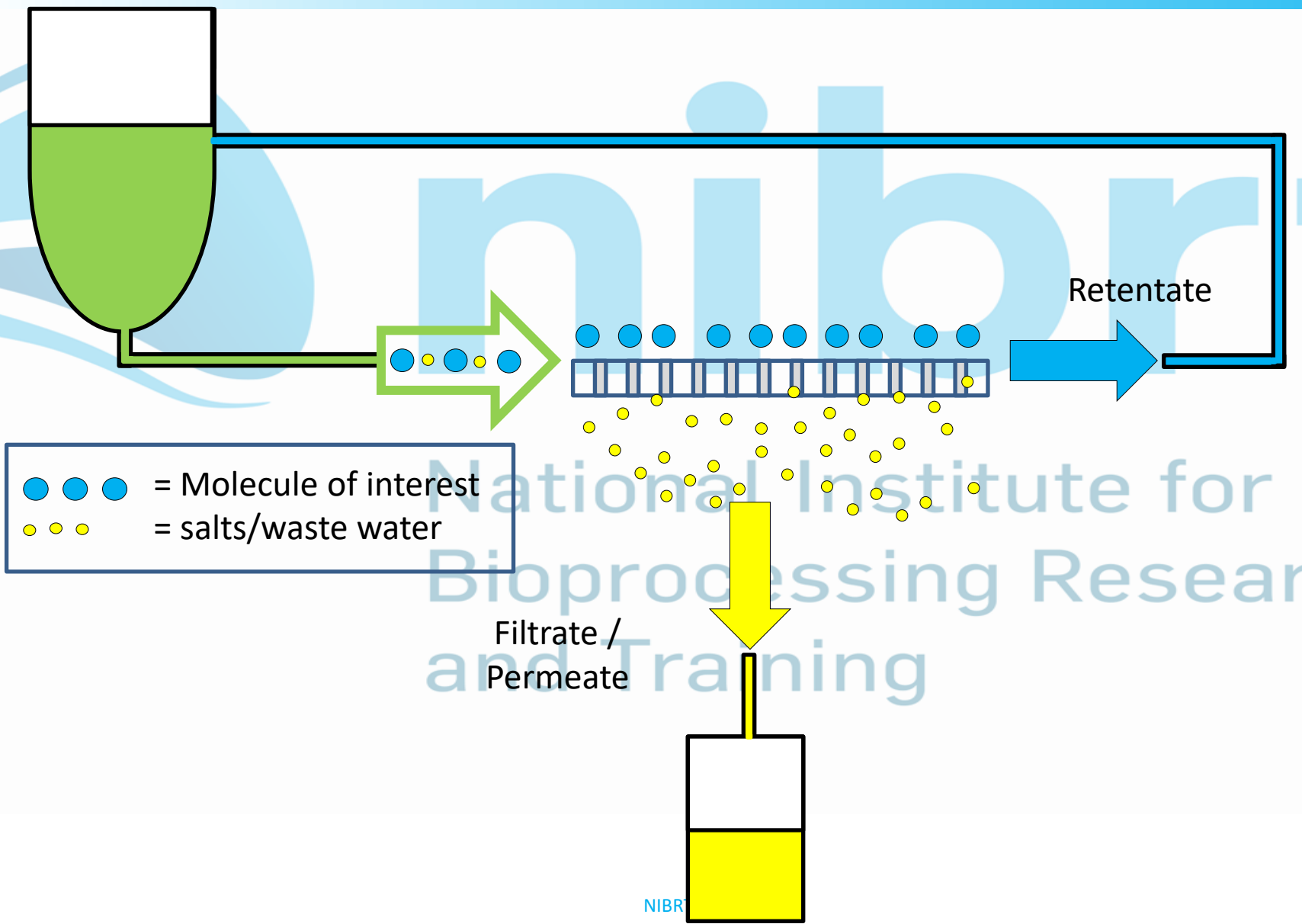


TFF Filter Cartridge

Ultrafiltration/Diafiltration

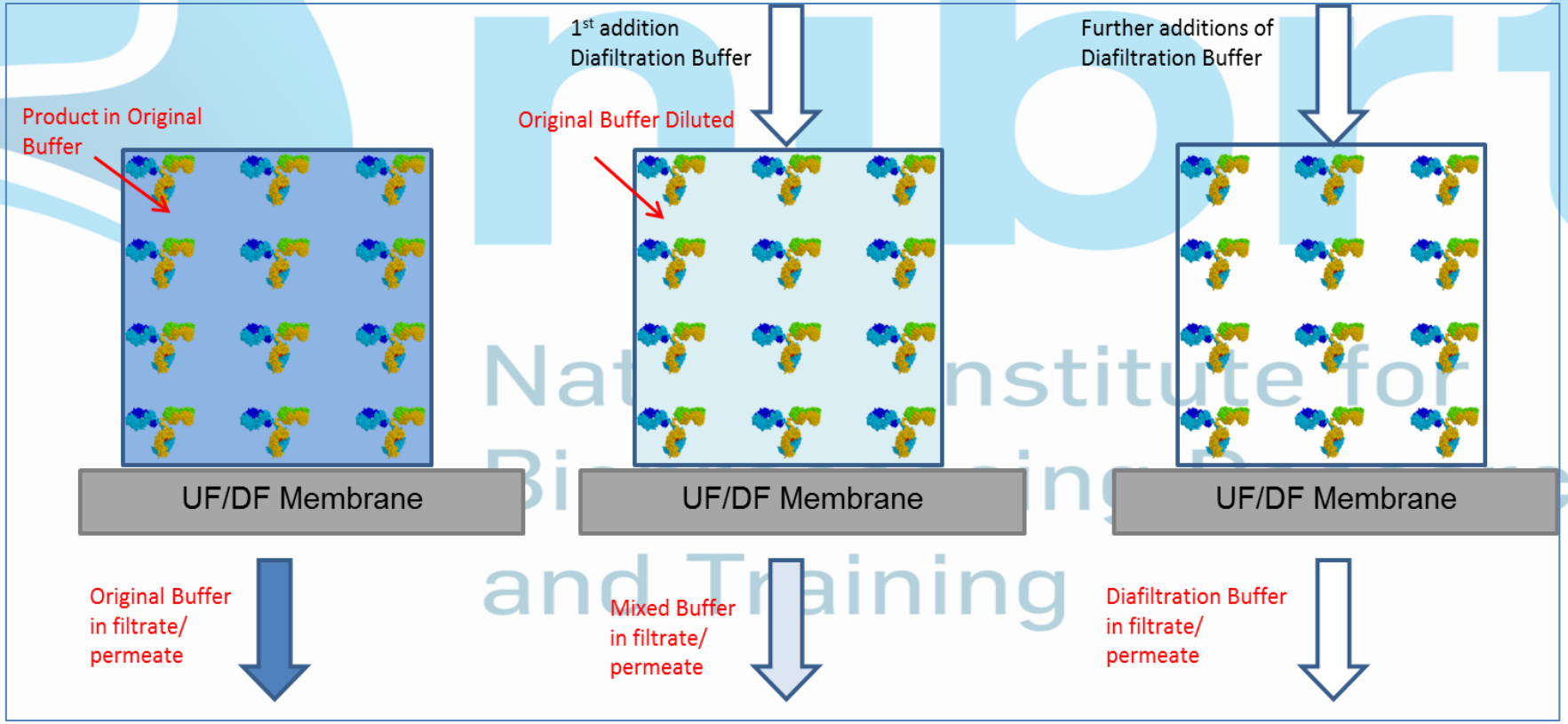


Concentration by Ultrafiltration



What is Diafiltration?

- Diafiltration = Buffer Exchange
- Replacement of old buffer with new buffer



Diafiltration continues until DF buffer = filtrate (pH/conductivity)

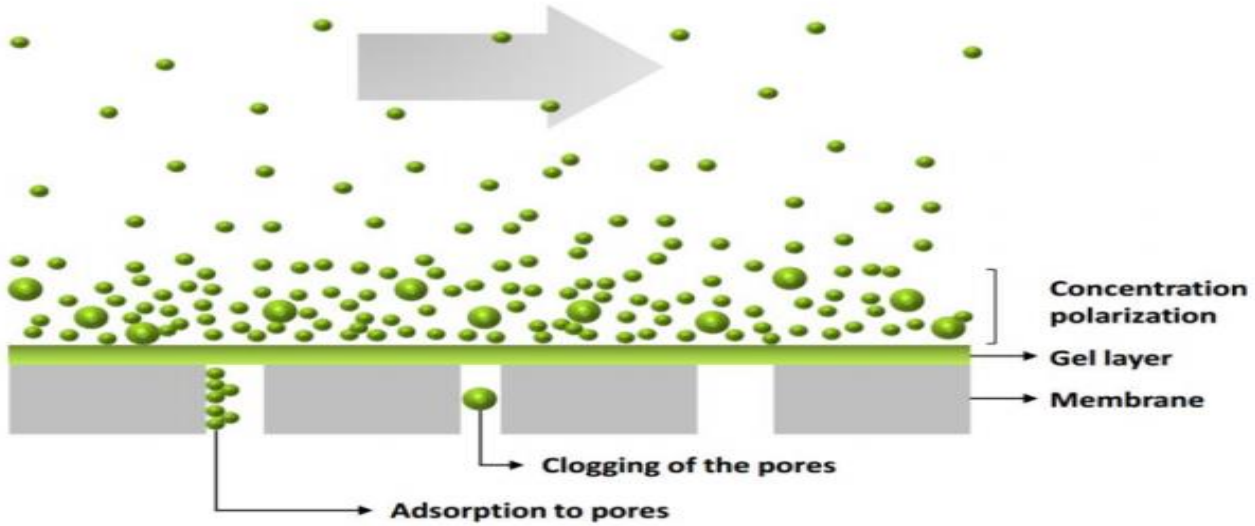
Membrane Fouling

Fouling is a phenomenon which can reduce flow rates through the filter

AFFECTING FACTORS:
+ Concentration gradients
+ Slow feed flow
+ High pressures
+ Feed solution make up

EFFECT CAUSED:
+ Adsorption on pores
+ Gel/cake layer formation
+ Plugging/blocking of pores

RESULTS IN:
+ Reduction in permeate flow
+ Difficult cleaning
+ Lower lifespan of membrane



Topics

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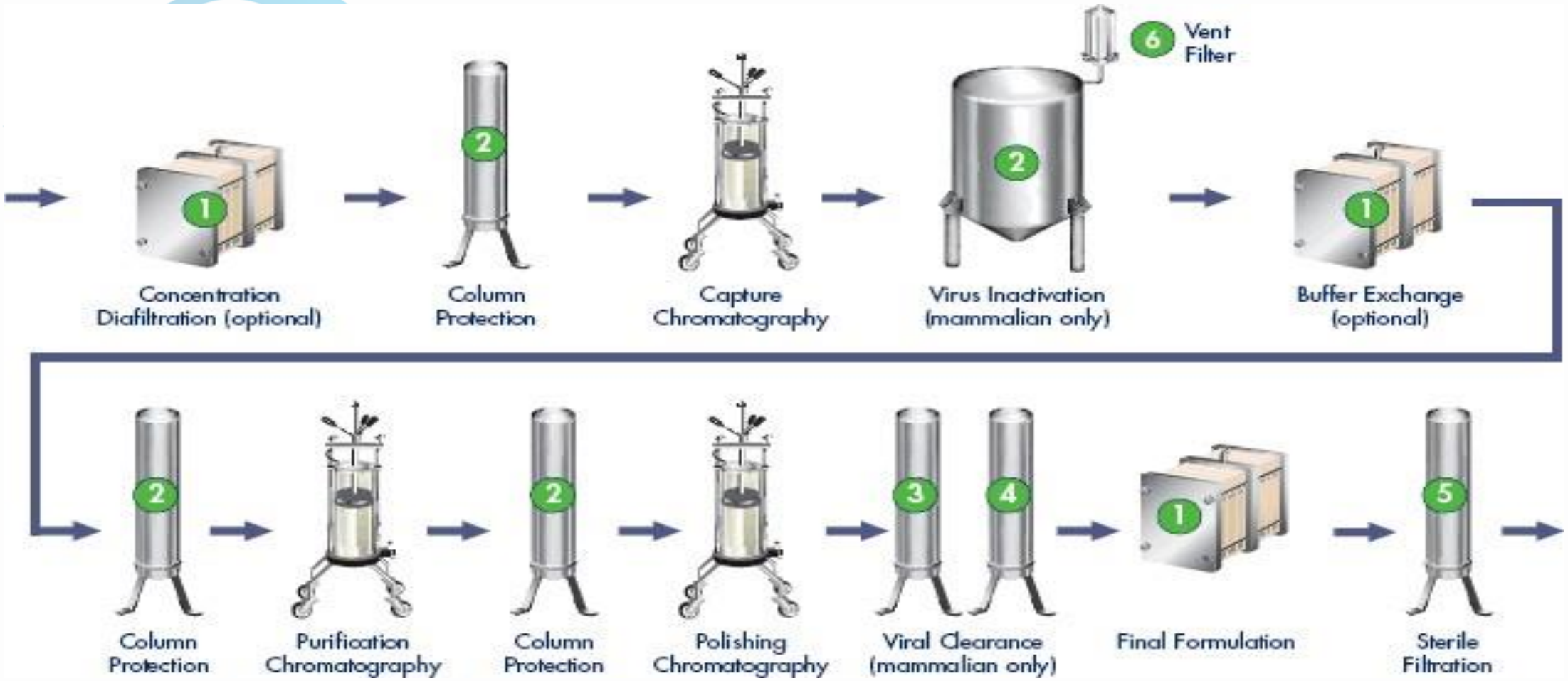
Protein & Mab DSP - Typical Process Flow



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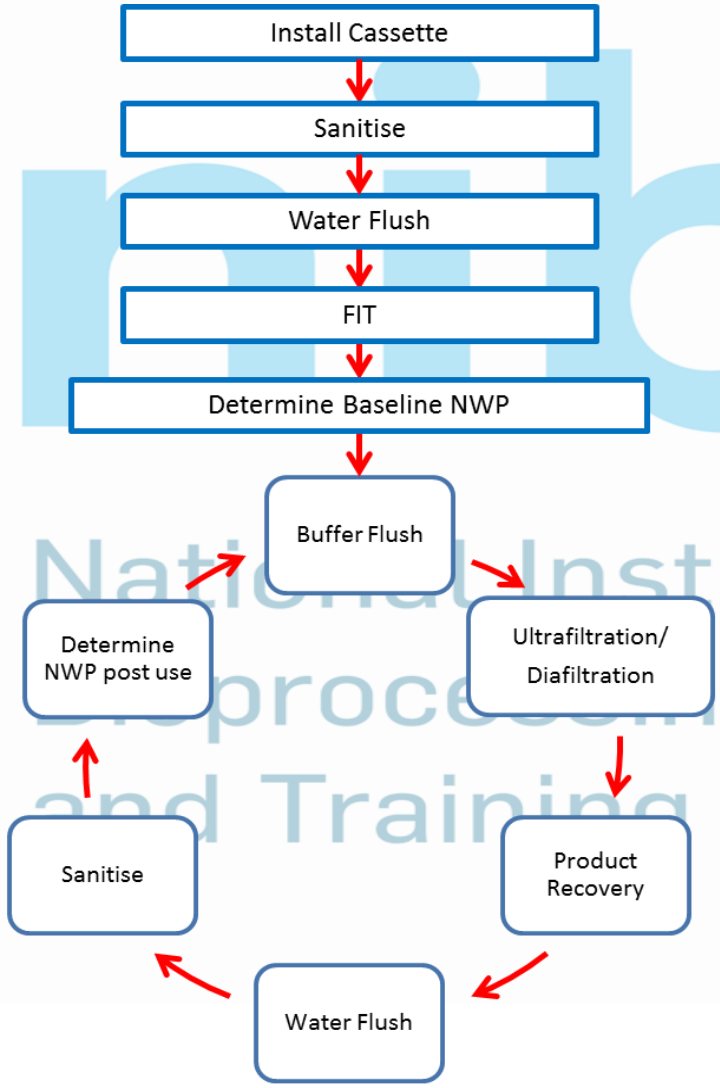
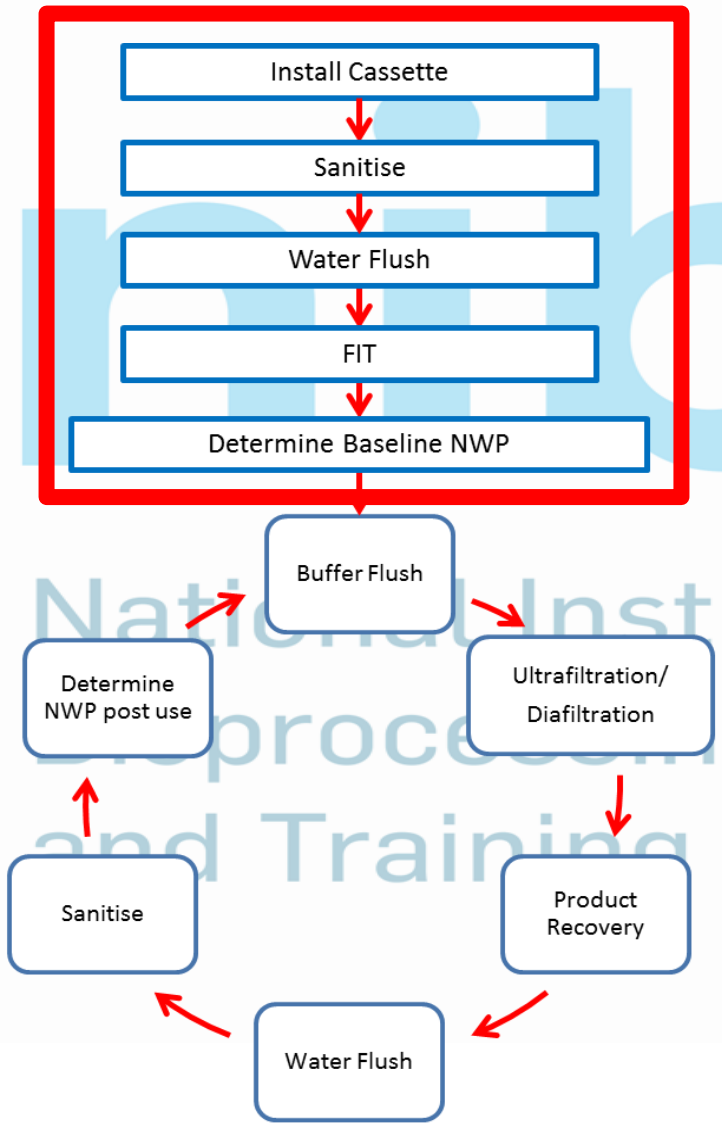
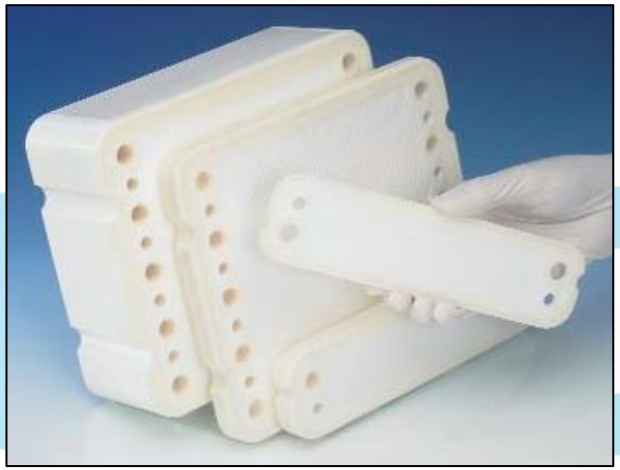
Typical UF/DF Process

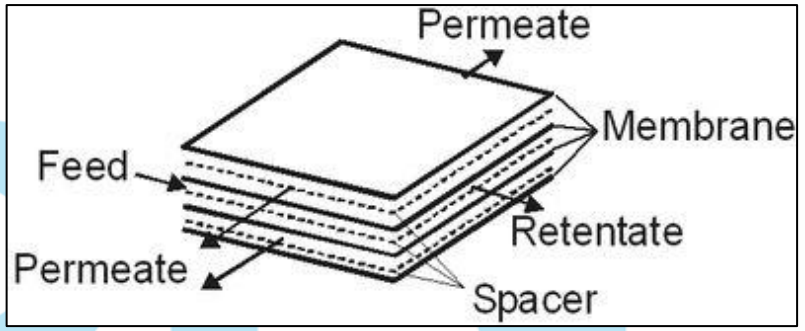


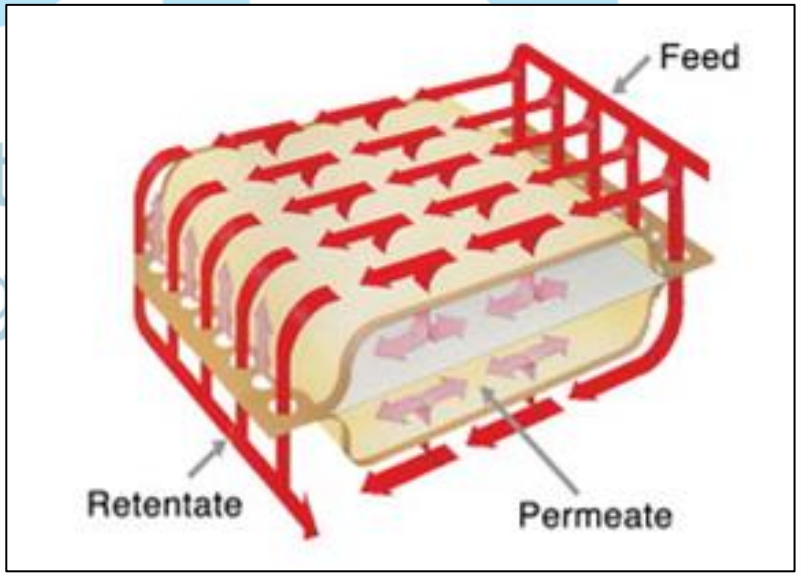
Set-Up



Installation







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Sanitisation

Membranes can be cleaned and re-used for another batch of the same product

Cleaning procedures generally include the following steps:

1. Flushing with water to remove protein residue
2. Sodium hydroxide (~0.2M) recirculated through the system and held for specified amount of time
3. These flush, circulate and hold cycles are often repeated several times
4. Some systems may need the addition of sodium hypocholorite depending on the protein being cleared from the membranes
5. Rinse with water afterwards

UF/DF Integrity Test

Why Integrity Test?

Check filter is conforming to manufacturer specifications

Check set up correct (Cassette systems typically use compression to seal the cassettes together)

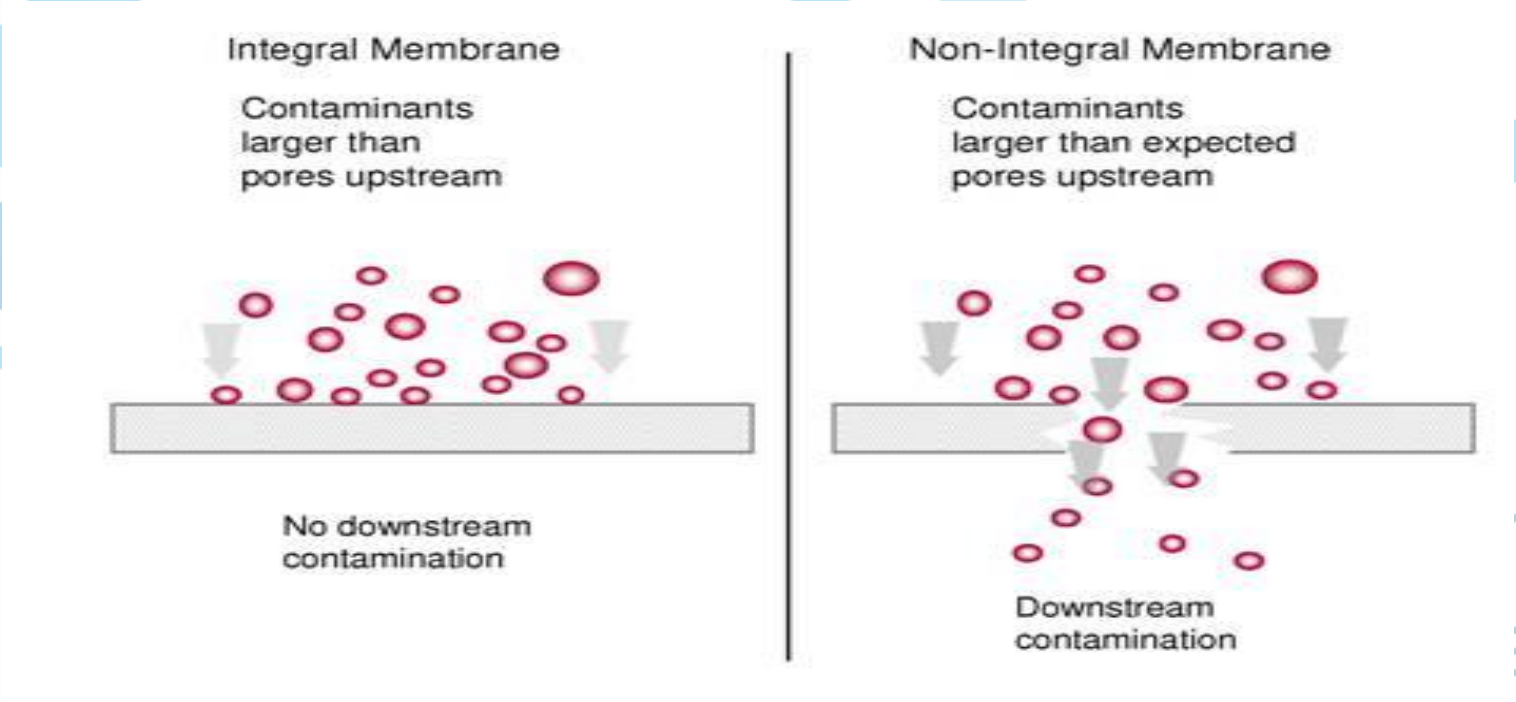
What tests can we run on UF/DF cassettes?

Diffusion testing

Cannot perform a bubble point

UF/DF Filter Cassette

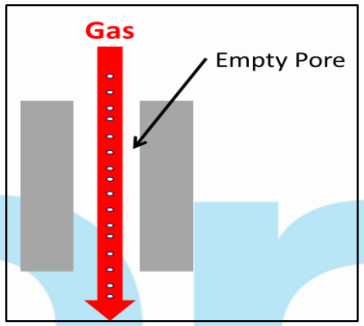
What is Membrane Integrity?



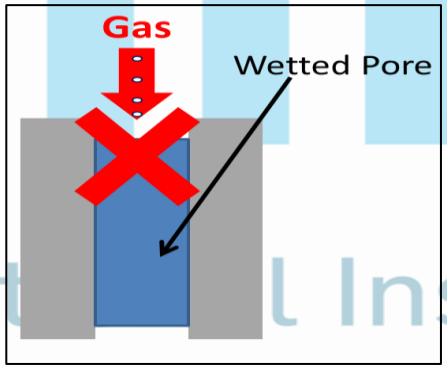
Membrane integrity refers to the ability of the filter membrane to do its job properly

Testing Filter Integrity Using Gas Flow (Diffusion)

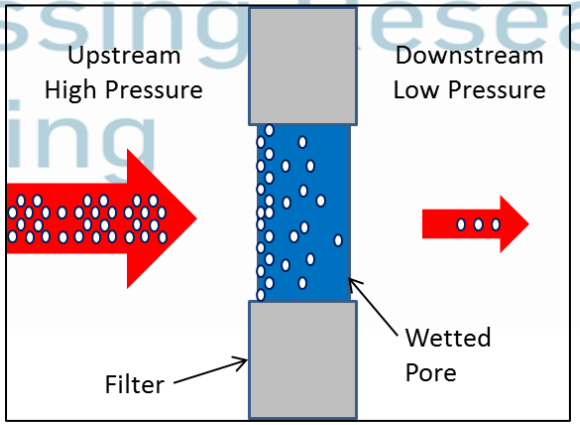
1. Using gas to determine integrity in a dry filter doesn't work as the gas flow is too fast



2. By wetting a pore there is now resistance to gas flow



3. Gas dissolves into the wetting solution in accordance with a concentration gradient and out the downstream side



Baseline Normalised Water Permeability

Provides a “health check” on the filter cassette

Can my used cassette by reused?

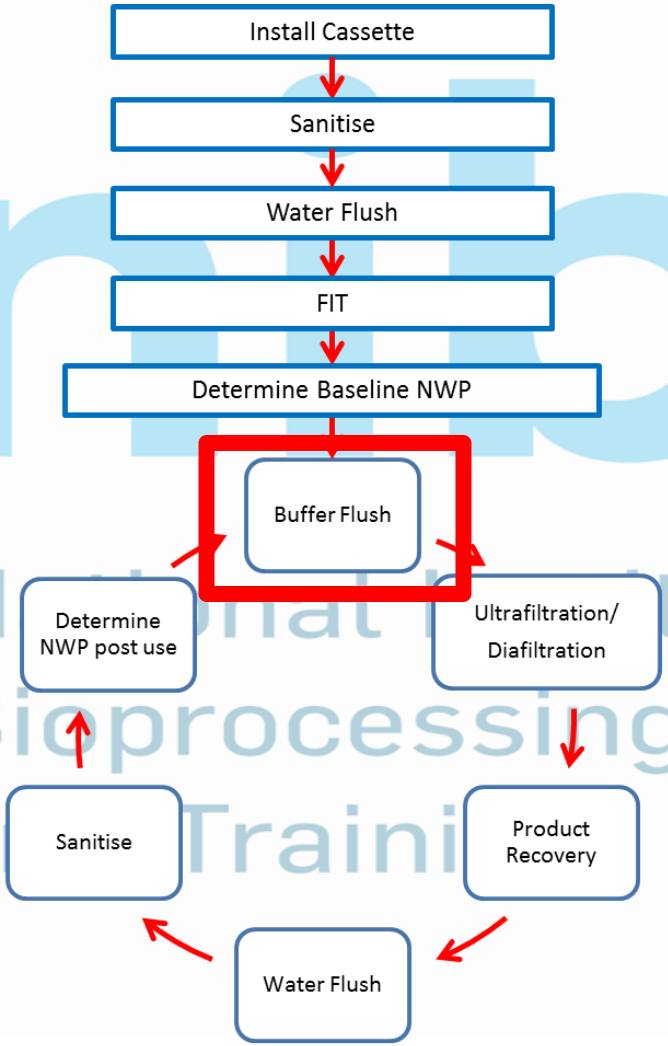
Was my cleaning successful?

NWP test is carried out on the newly installed cassette (baseline).
After each process run another NWP test is carried out (post).
The Post and Baseline NWP values are the compared.

Normalised:
Need to standardise the testing

Water Permeability:
Pressure across the membrane
Size of membrane
Temperature of solution

Preconditioning



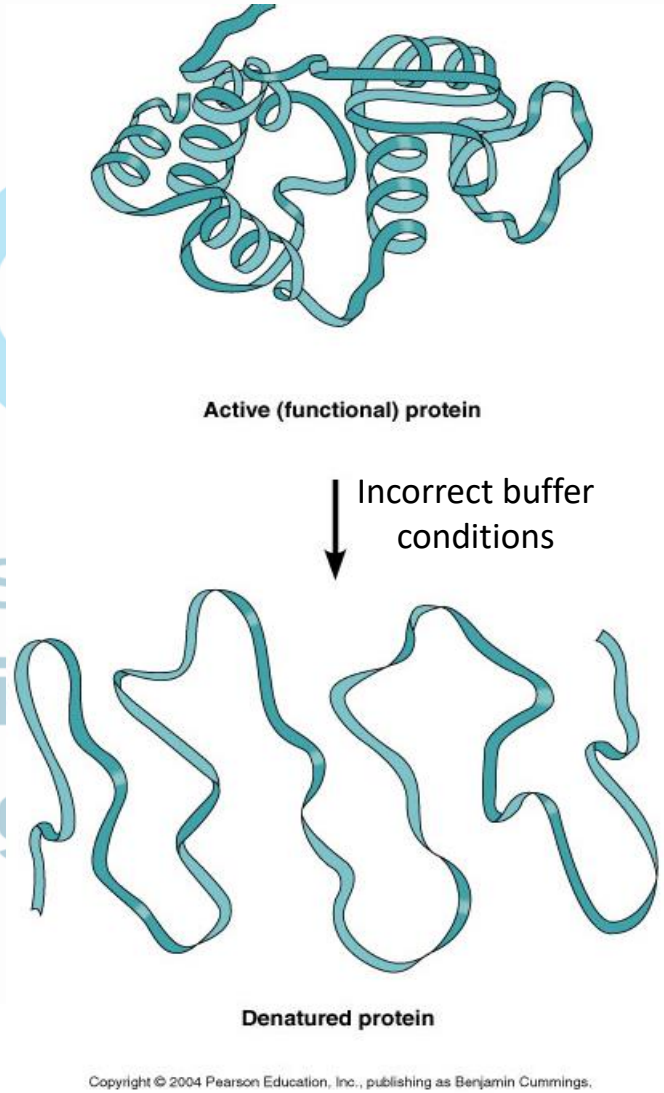
Buffer Flush

Before processing, the flow path may be wet and/or dry.

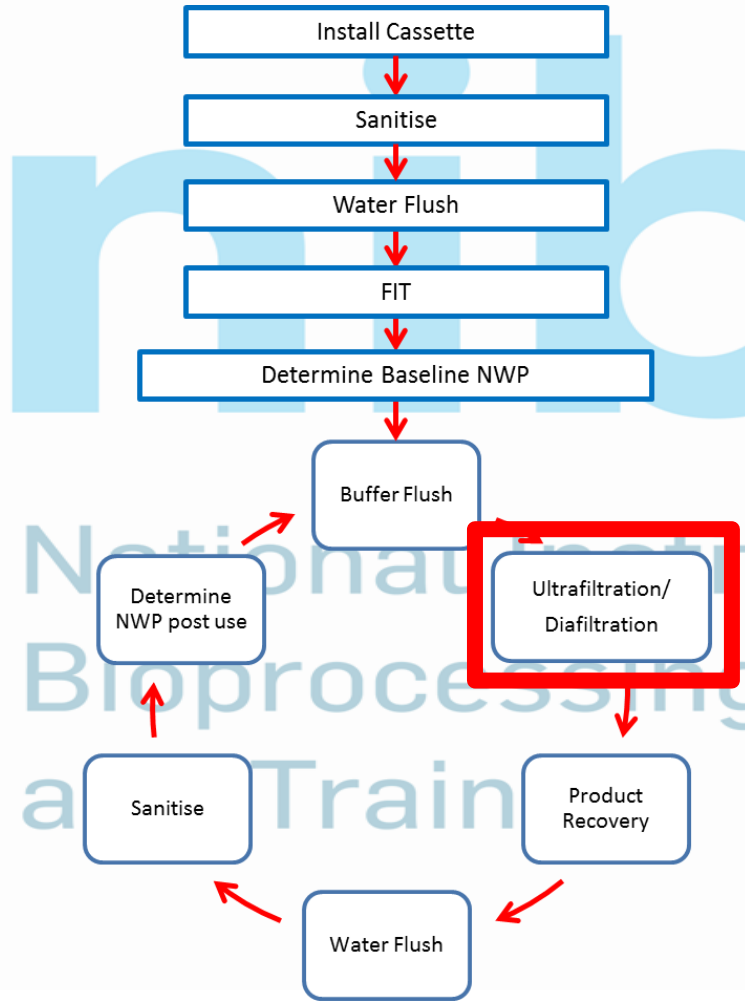
If conditions are not optimal, protein can become destabilised which leads to:

- Clipping
- Denaturation
- Aggregation
- Precipitation

Buffer **primes the line** with an optimal environment to ensure protein is protected when introduced



UFDF Processing

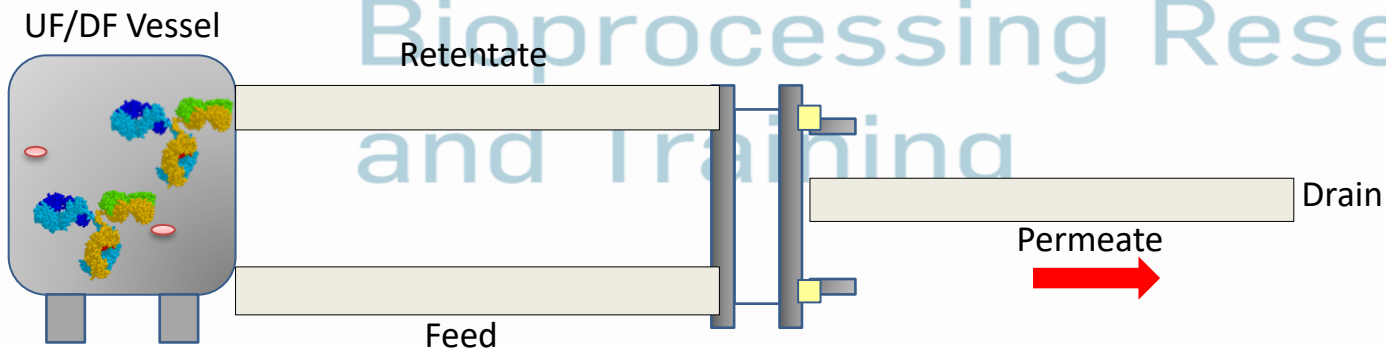


Example of Production Stage

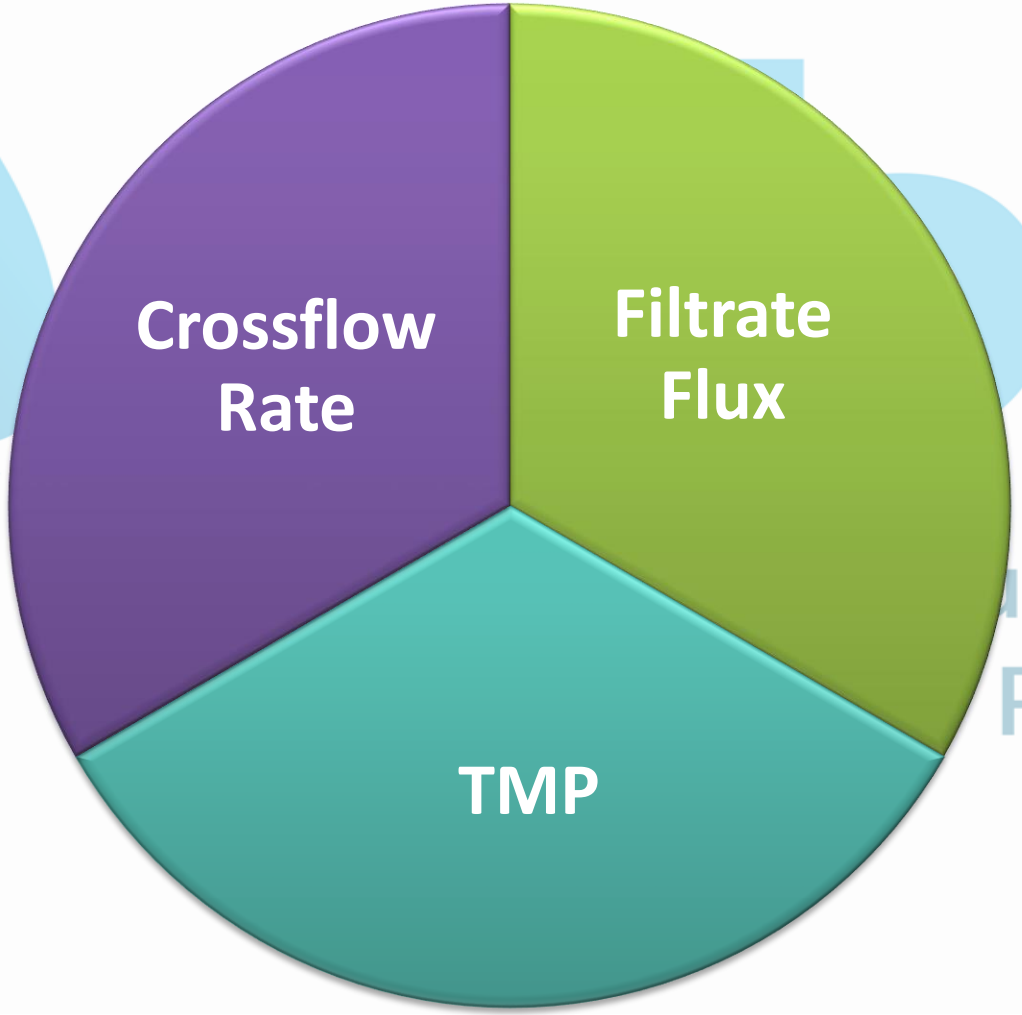
Step	Vessel Volume
Starting solution	100L
Concentration 5X (UF)	20L
Buffer addition 3XDV (DF)	80L
Concentration 4X (UF)	20L
Buffer addition 3XDV (DF)	80L
Concentration 4X (UF)	20L
Buffer addition 3XDV (DF)	80L

DV = Diavolume

1 DV = volume in the vessel



UF/DF Process Parameters

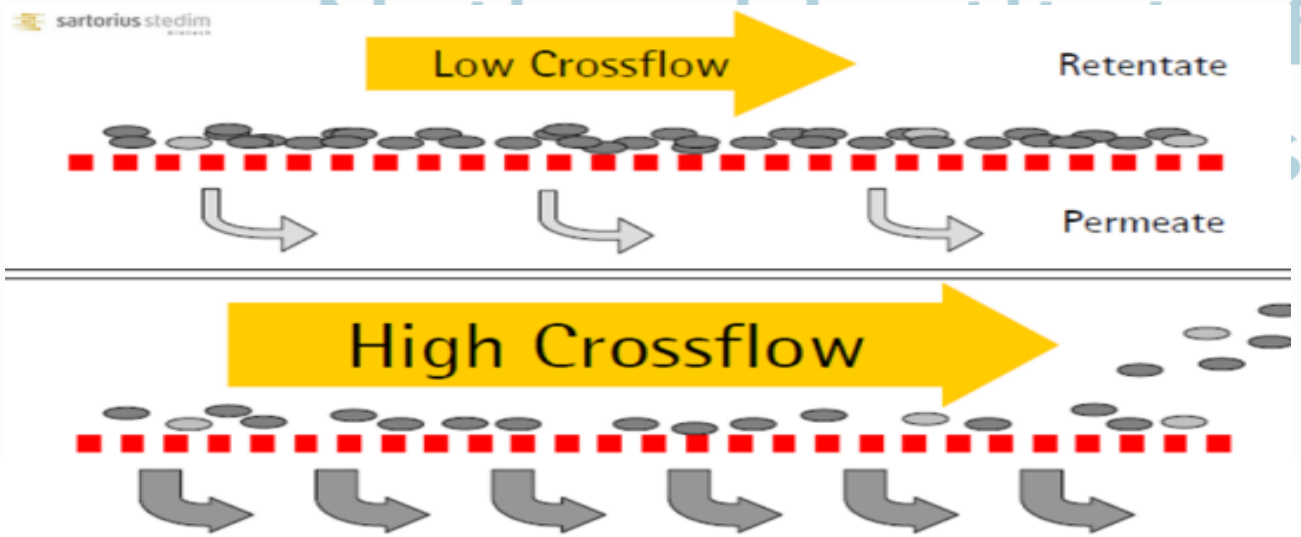


Crossflow Rate

The Crossflow or Feedflow rate is the rate of solution flow through the feed channel and across the membrane

It provides the force that sweeps away the molecules that can foul the membrane

Higher turbulence and **potential shear** may be caused by a crossflow rate that is too high

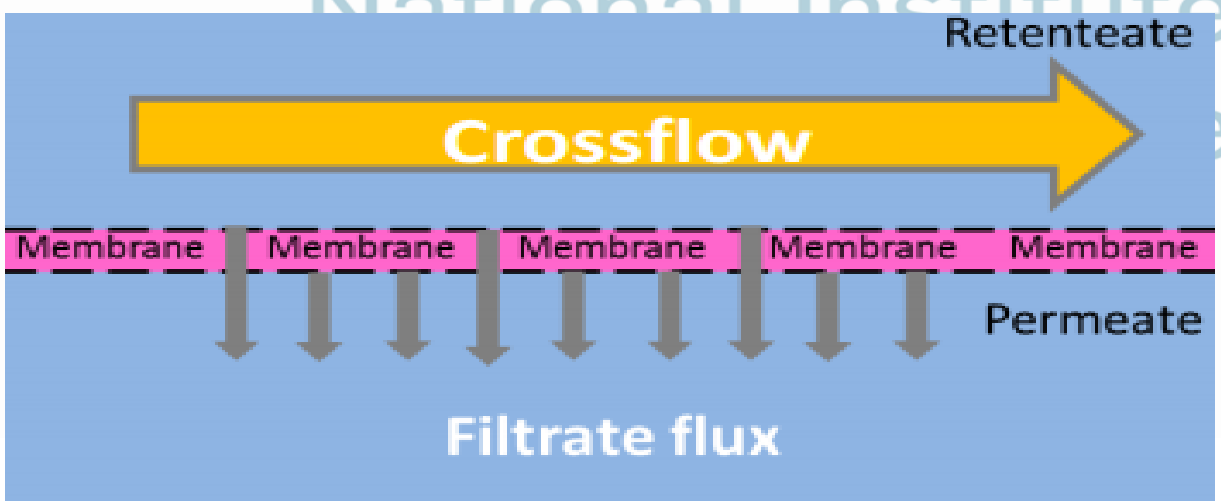


Filtrate Flux

The **rate at which permeate moves through** the membrane is called the filtrate flow rate or permeate flow rate

Filtrate flux is the filtrate flow rate **normalised for the area of the membrane** [m^2] through which it is passing

Filtrate flux is measured in **LMH (litres/m^2/hour)**

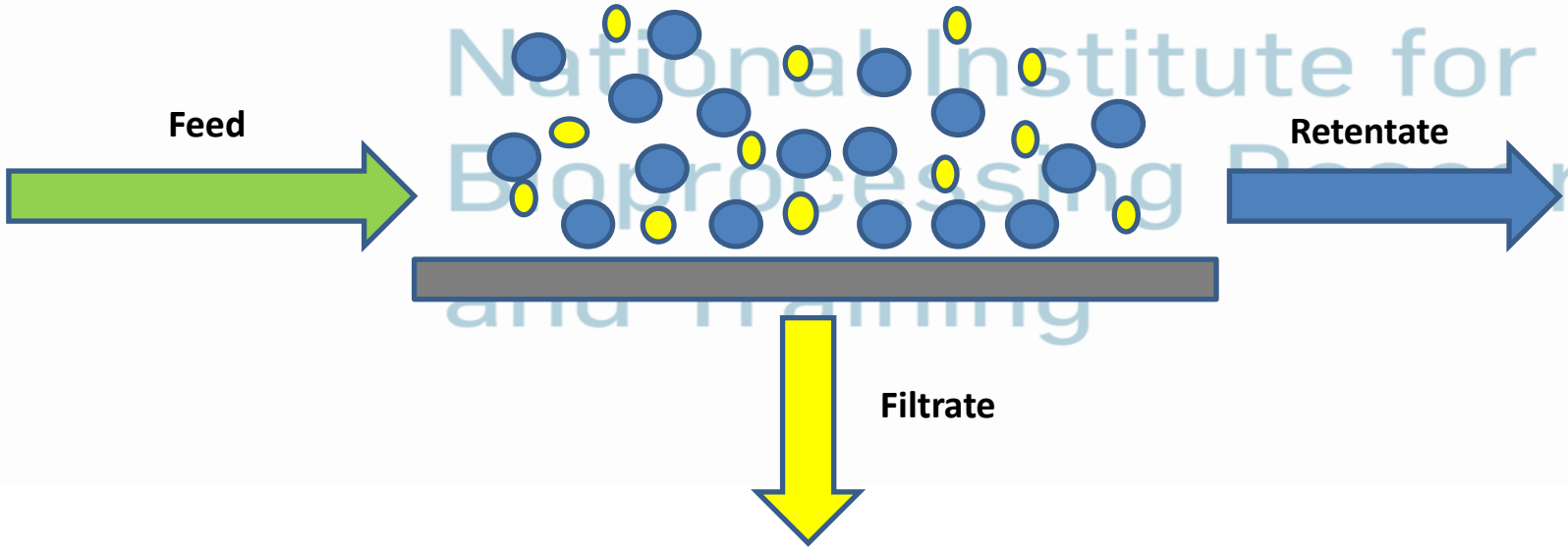


Transmembrane Pressure (TMP)

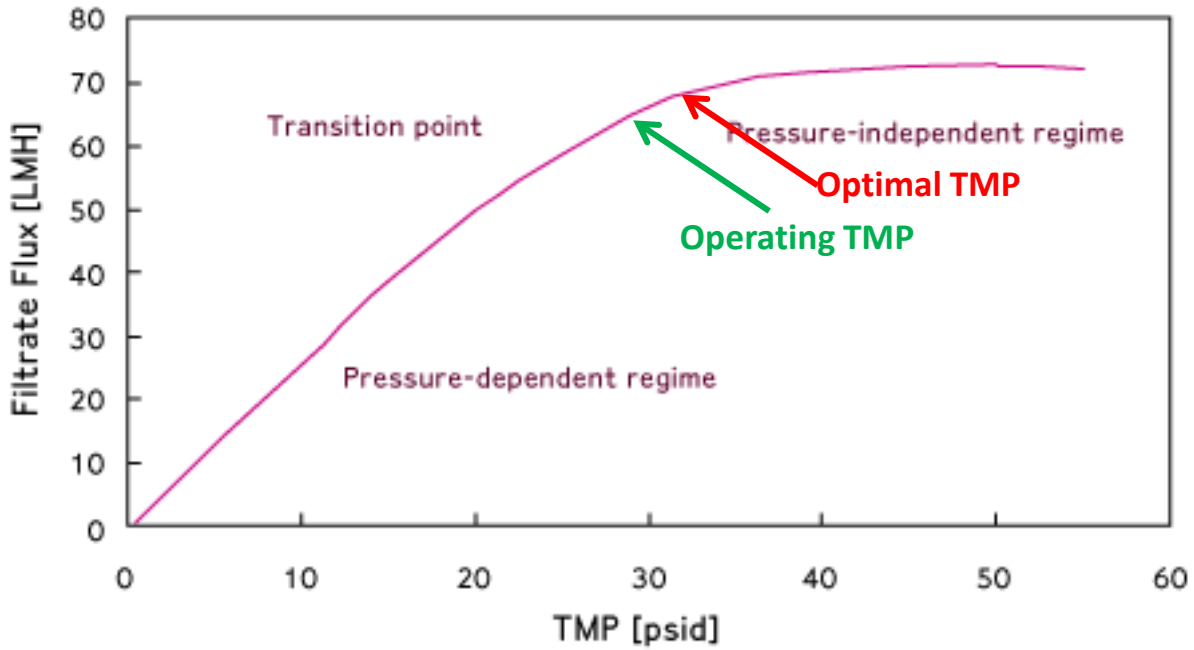
TMP = The force that drives fluid through the membrane

ie. the difference in pressure above and below the membrane

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



Determining Optimal TMP



When **flux starts to plateau** at the knee of the curve, the optimal process TMP operating point has been identified

The operating TMP is chosen just **below this point**

TMP Must Be Regulated

TMP varies depending on **membrane pore size** and **process.**

High TMP enables **faster flow** across the membrane.

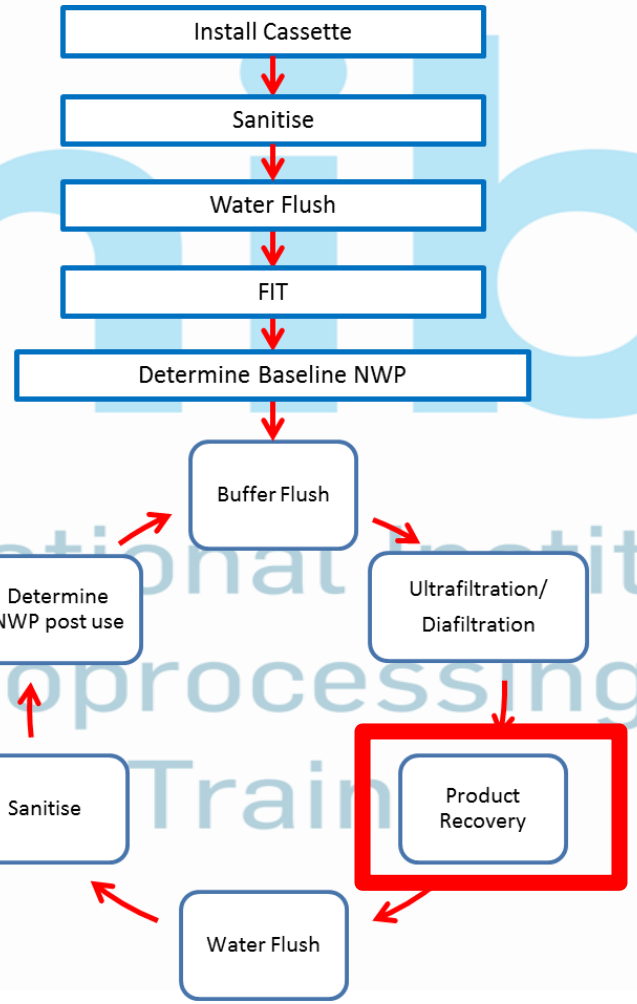
Having a TMP that's **too high** can encourage fouling.

TMP value is **balanced** between flow rate and fouling of membrane.

Usually maintain a small to moderate TMP across the membrane.

The priority is to **reduce fouling**

Product Recovery



Buffer Rinse

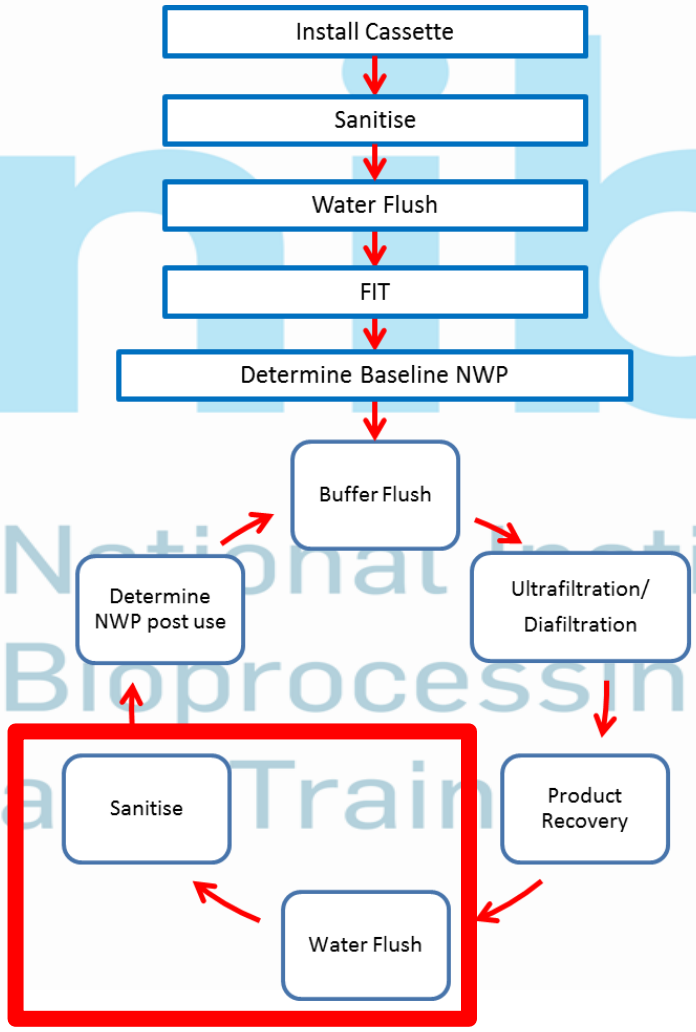
Need to run buffer through the system as there will be considerable hold up of process solution

Buffer rinse can remove some adsorbed protein material

Larger membranes will have more losses

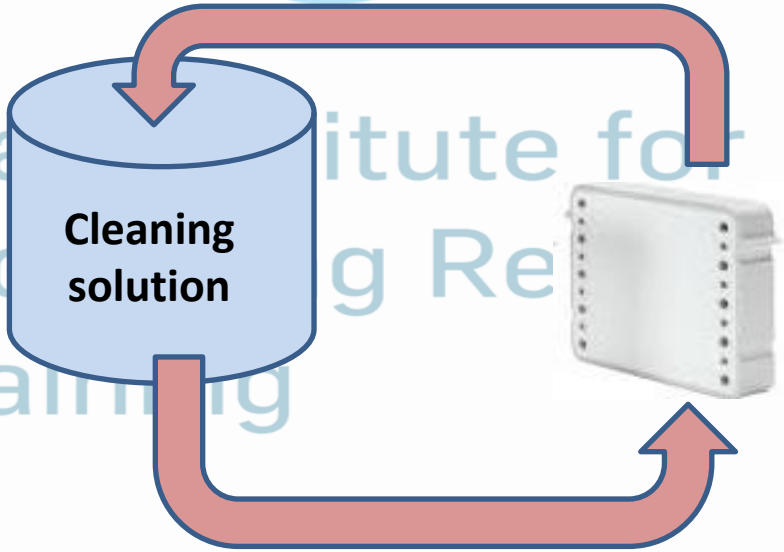
After the buffer rinse ~99% should be the target recovery

Regeneration of Cassette

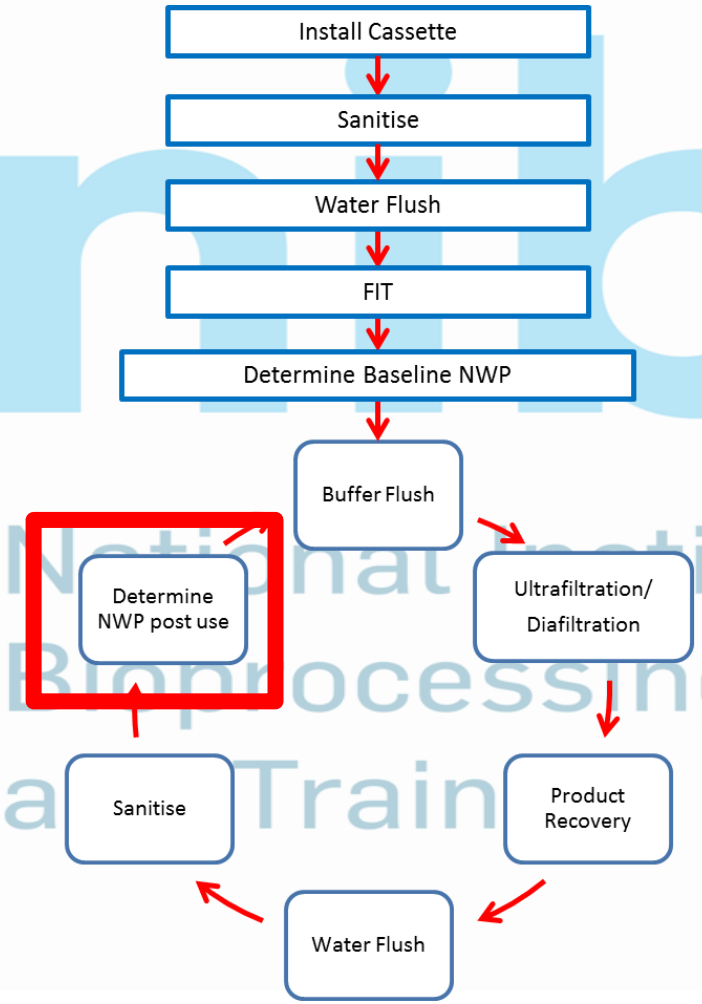


Sanitisation

After each membrane use and product recovery, the UF/DF assembly must be cleaned using the same cleaning protocol that was performed before use

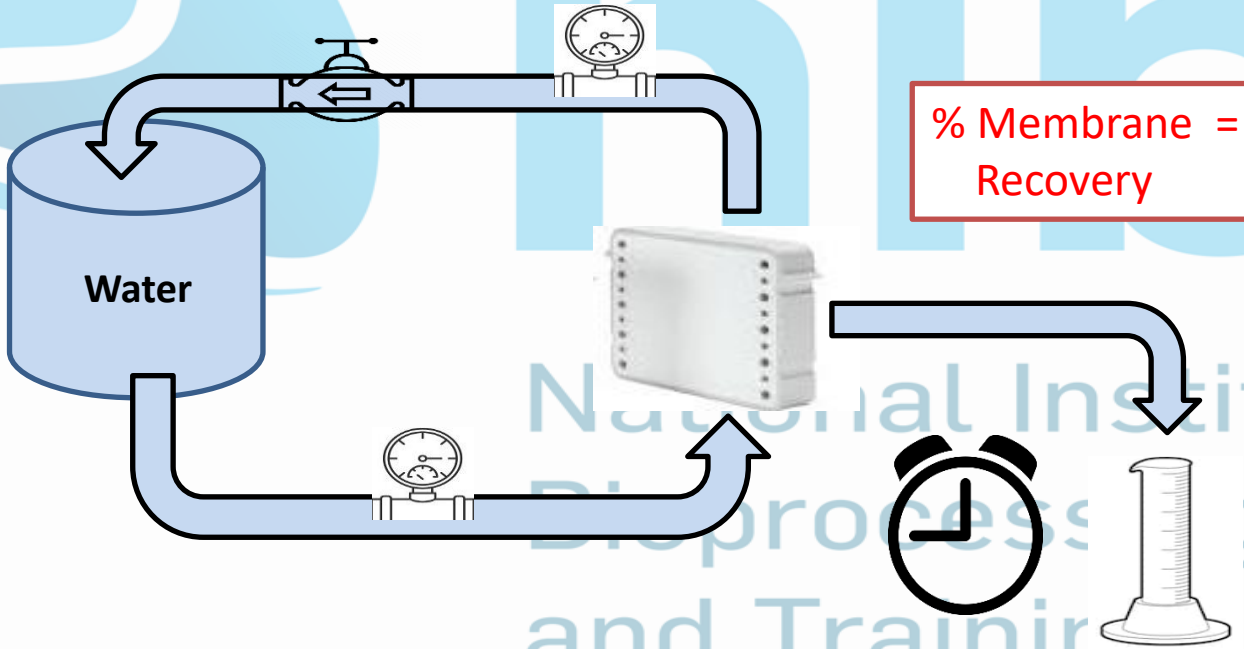


NWP Post-Use



NWP Post-Use

Comparing the post-use cleaned membrane permeability with the original value indicates the effectiveness of the cleaning



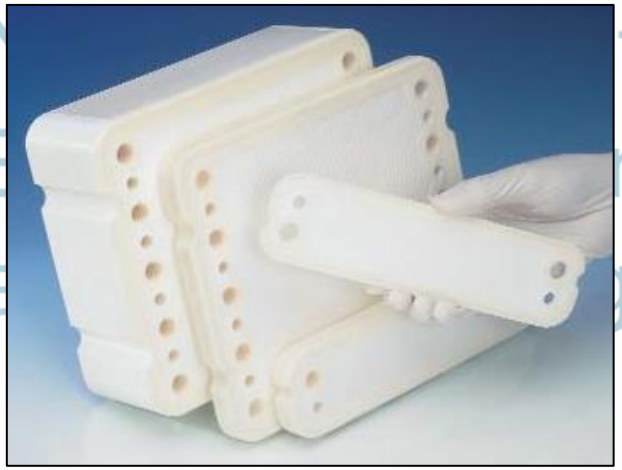
$$\% \text{ Membrane Recovery} = \frac{\text{NWP Post-Use}}{\text{NWP Pre-Use}} * 100$$

Trends can be used to gauge expected membrane lifetime and to set a limit on maximum number of cycles per membrane. Membrane recoveries are company set and range from 80 – 120%.

Storage

Cassettes can be stored long-term if they are not to be reused immediately

- An appropriate solution must be recirculated
- Cassettes removed and stored wetted
- The solution should be anti-microbial



Summary

- **Filters - semi-permeable barrier layers permitting certain components of solutions or suspensions to permeate more rapidly than others** – sieving or adsorption effect
- Separation of particles from liquid by applying a pressure to the solution to force the solution through a filter
- Depth filtration (NFF) – clarification and/or bioburden control
- Cross-flow or tangential flow filtration (TFF)
  - Concentration – controlled reduction of process sample volume whilst retaining target molecules / particles
  - Fractionation – separation of complex mixtures in discrete fractions (MWCO points)
  - Diafiltration – buffer exchange

Summary

- Two kinds of filtration – normal flow and tangential flow
  - **Normal flow** is predominantly for clarification of the feed-stream
  - TFF is used for concentration of sample (volume reduction) or for diafiltration (buffer exchange)
- Filters must be tested for performance and integrity
  - Forward flow / diffusion tests post use
  - Bubble point tests pre and post use
  - Bacterial retention tests – post use (or more commonly batch samples are tested by the manufacturer)

Sample Questions

- **SAQ**: Compare depth and tangential flow filtrations with respect to capacity, applications and stage of use in DSP.
- **SAQ**: What is the difference between micro- and ultra filtration? In each case, provide an example of to illustrate the objective of each method.
- **SAQ**: Comment on the development of single use technology filtration systems – what advantages do they offer?
- **LAQ**: Explain diafiltration works and comment on factors to be considered in setting up such a protocol.

Issues to address , might include some/all of the following:

- *Define diafiltration*
- *Batch versus continuous*
- *Purpose and examples of where used*
- *Membrane selection - MWCO*
- *Filter characteristics*

Topics

- Principles of Filtration
- Normal Flow Filtration
- Tangential Flow Filtration UFDF
- UF/DF Operations

Thank You