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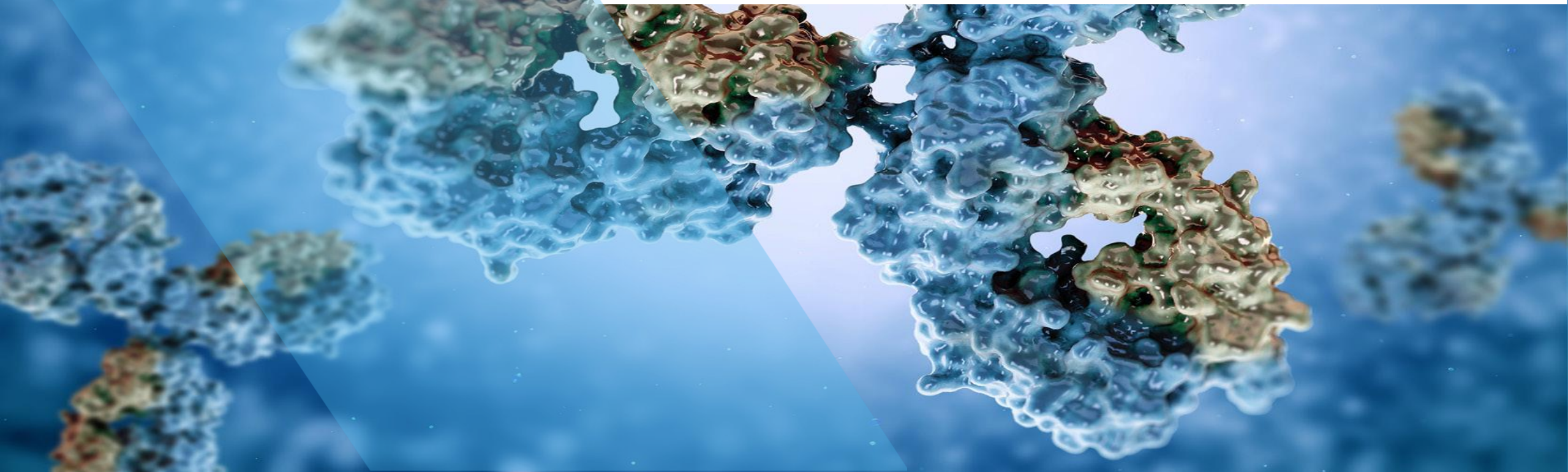
2. Harvest Technologies: Centrifugation & Filtration

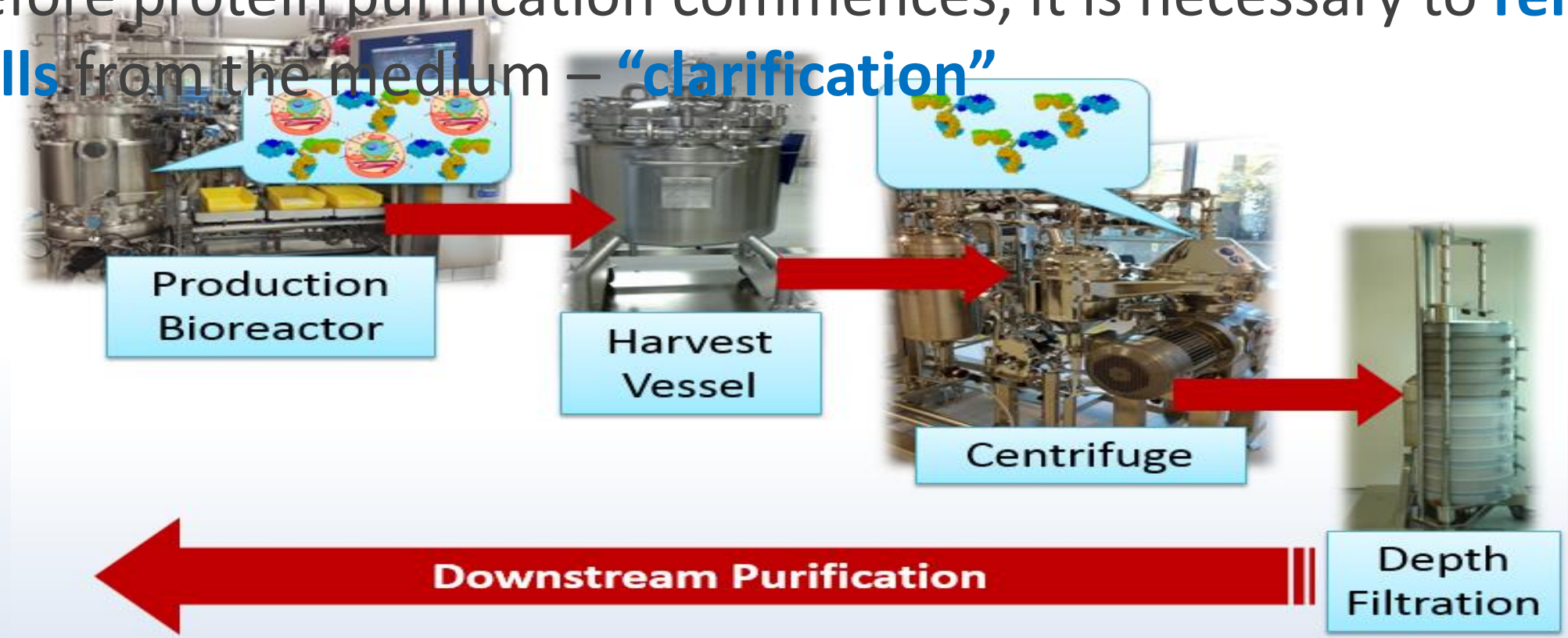


Topics

- Harvest Overview
- Microfiltration
- Centrifugation
- Depth Filtration

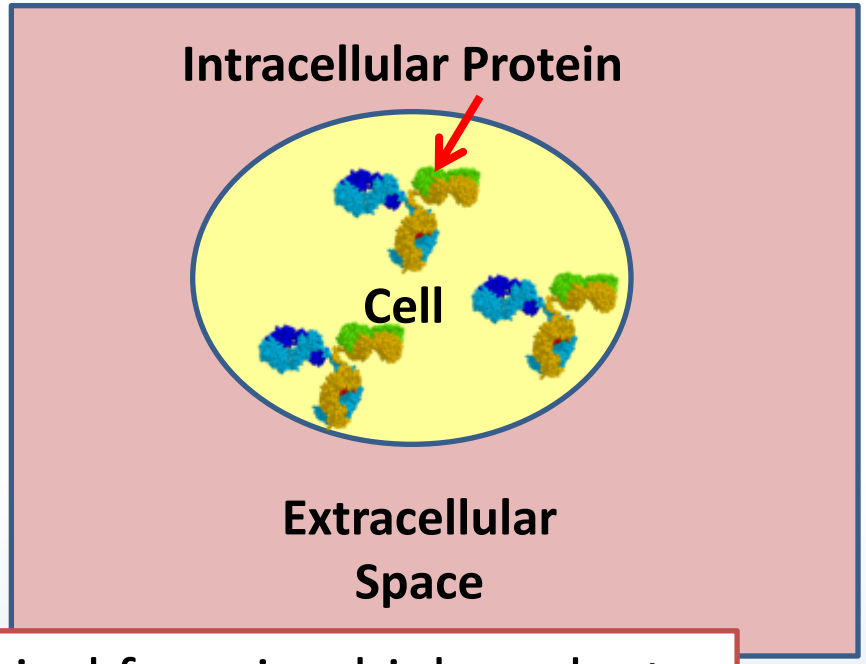
What is “Harvest”?

- Harvest begins when the production cycle in the bioreactor is complete
- Before protein purification commences, it is necessary to **remove cells** from the medium – **“clarification”**

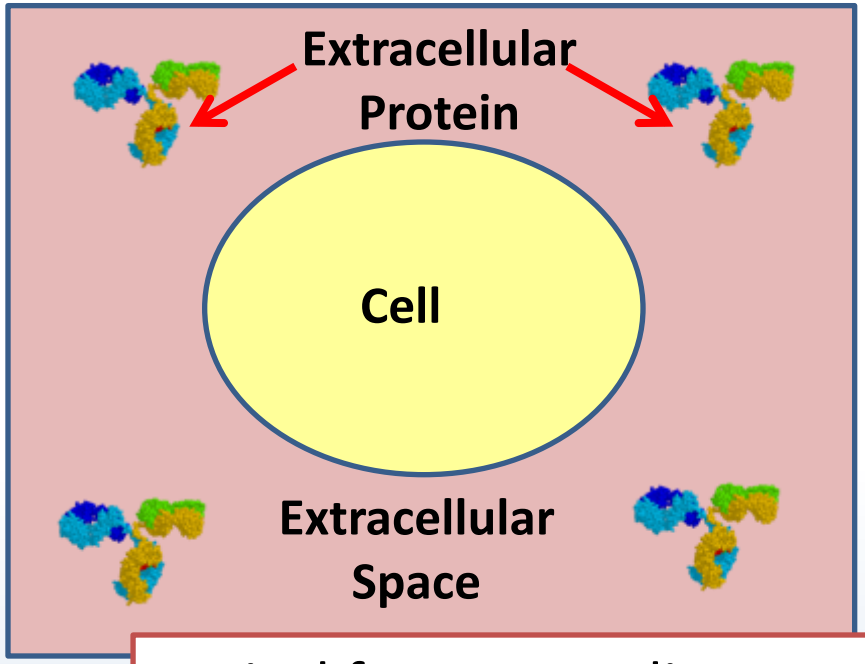


Know your Protein!

It is important to know your protein. Is it intracellular or extracellular?



Typical for microbial products



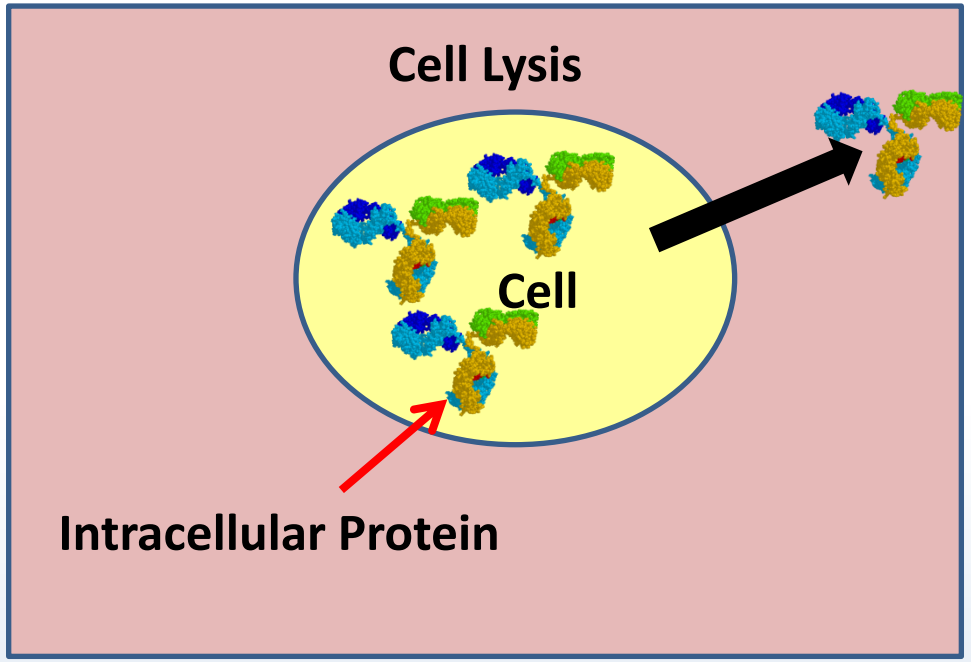
Typical for mammalian products

Harvesting Intracellular Proteins

The cell must be lysed to allow the intracellular protein to be released.

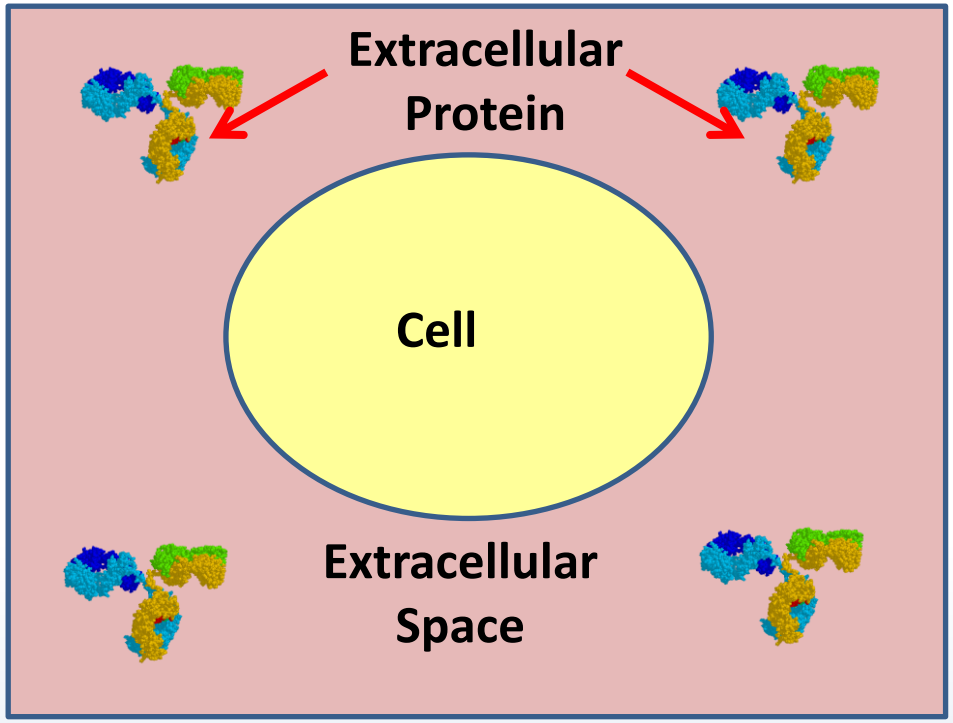
Can use the following methods:

1. Sonication
2. Lysis Buffer
3. Bead Milling
4. Homogenisation
5. Freeze/Thaw



Harvesting Extracellular Proteins

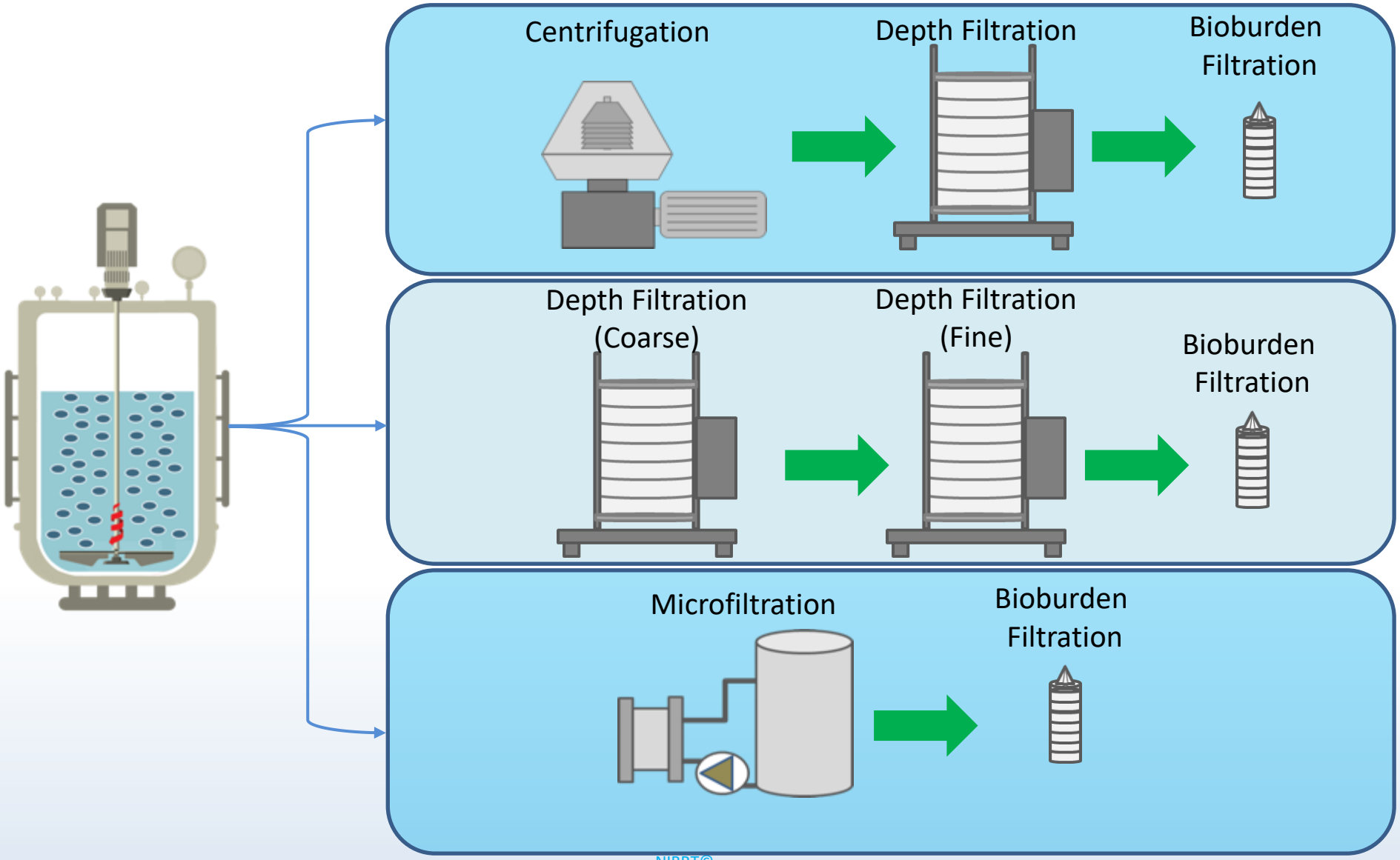
- Cell membrane/wall does not require lysis
- The protein is already in extracellular space
- **Care is required!** Cell lysis can release endogenous proteases and phosphatases which can degrade or modify the protein of interest



Not a Sterile Process!

- Unlike in bioreactor production, harvest is not designed to be a sterile operation
  - Designed for bioburden control rather than sterility
  - Use sanitary processing design and operating procedures
- Bioburden control dependent on:
  - Clean utilities
  - Cleaning of equipment and sanitisation of plant
  - Filtration of process streams

Harvest/Primary Recovery Options



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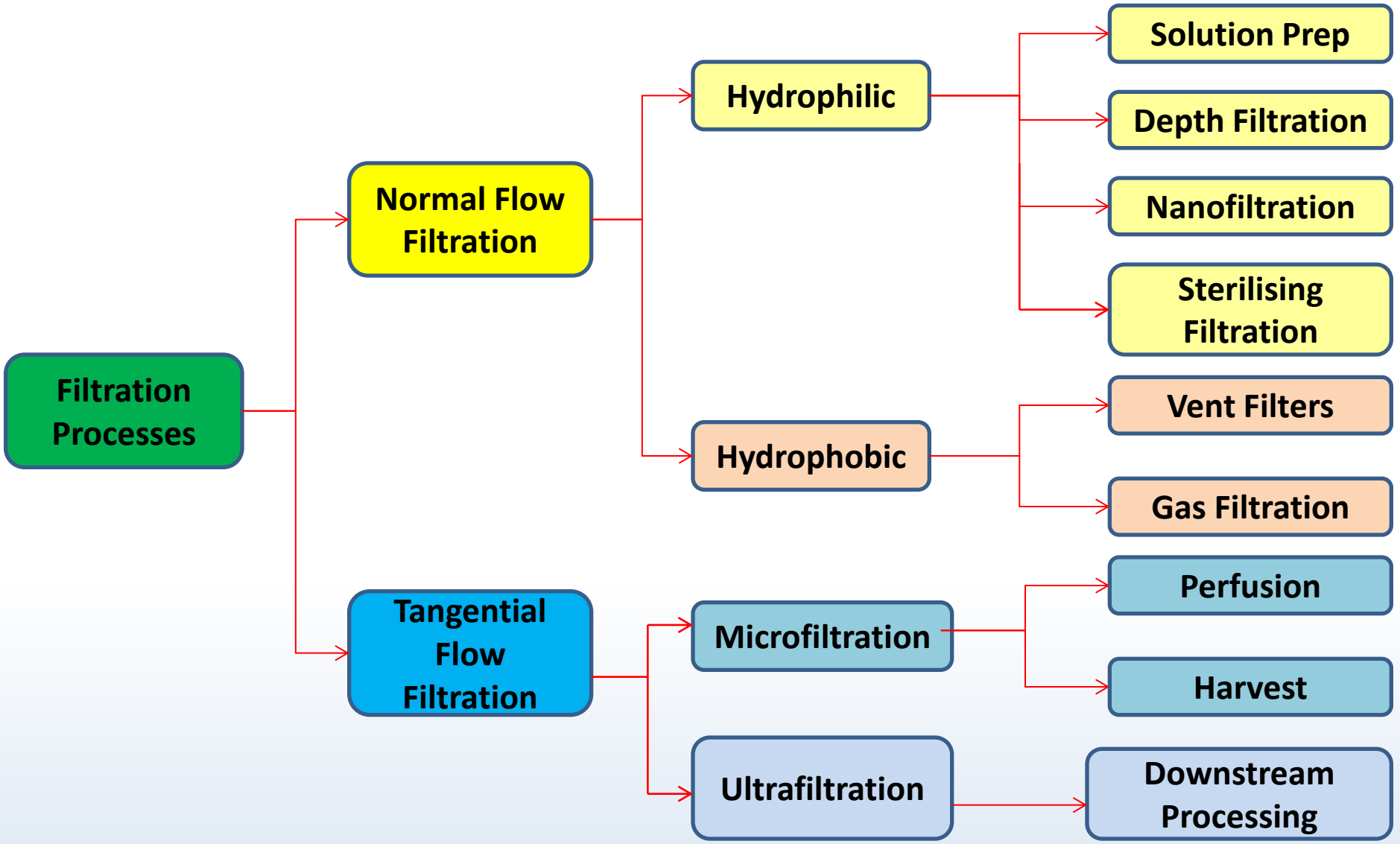
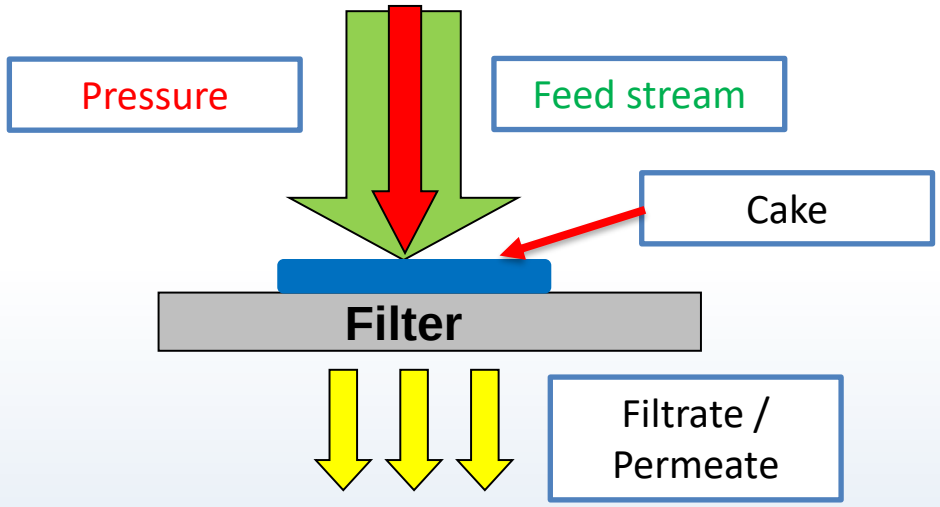
Filtration Processes

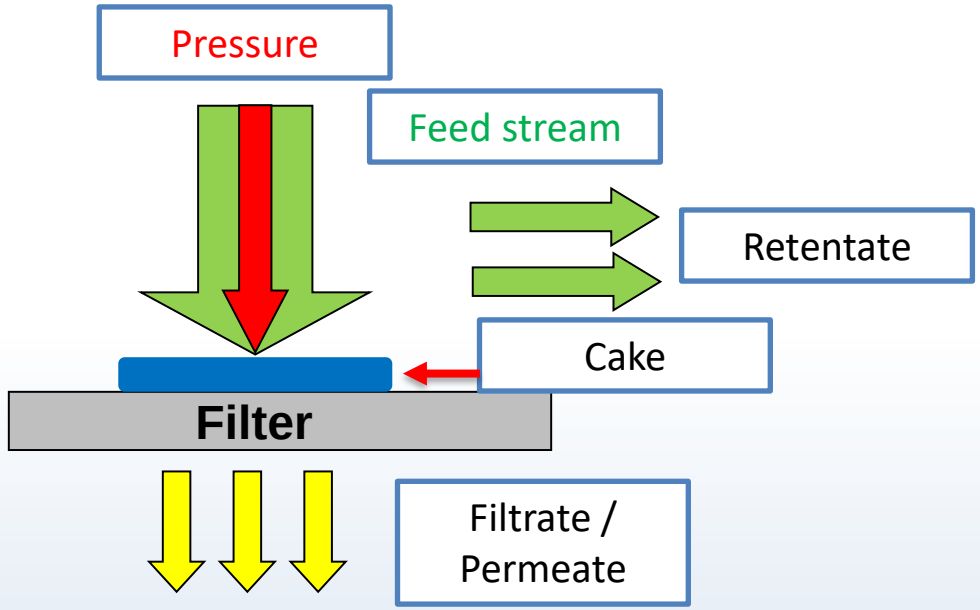


NFF vs TFF

- NFF – Pressure and feed are applied from the same direction, perpendicular to the membrane
- It might cause cake formation

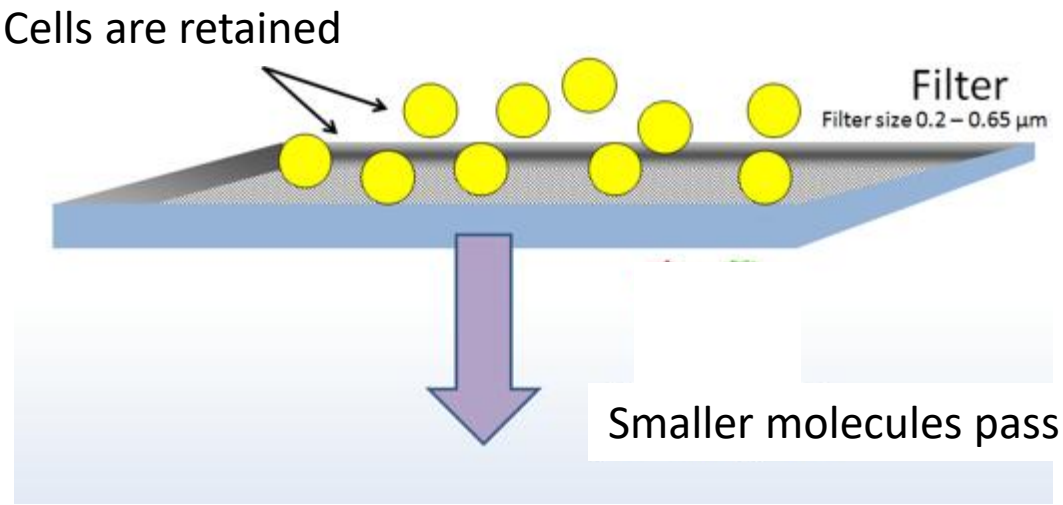


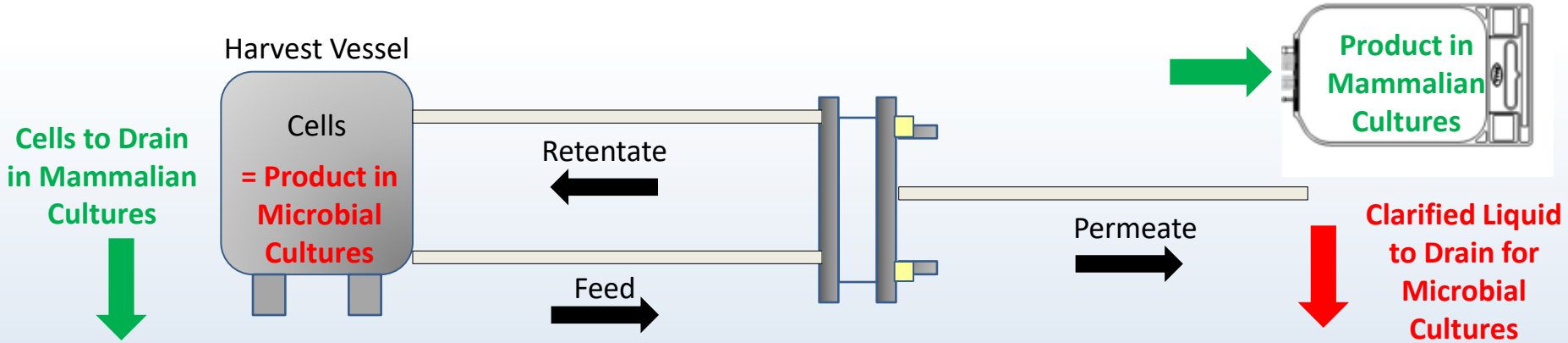
- TFF – Feed is applied parallel to filter membrane sweeping away material that builds up on the top of the membrane
- Part of the feed passes through the membrane as **permeate**
- Most of the solution is circulated back to the feed tank as **retentate**



Microfiltration

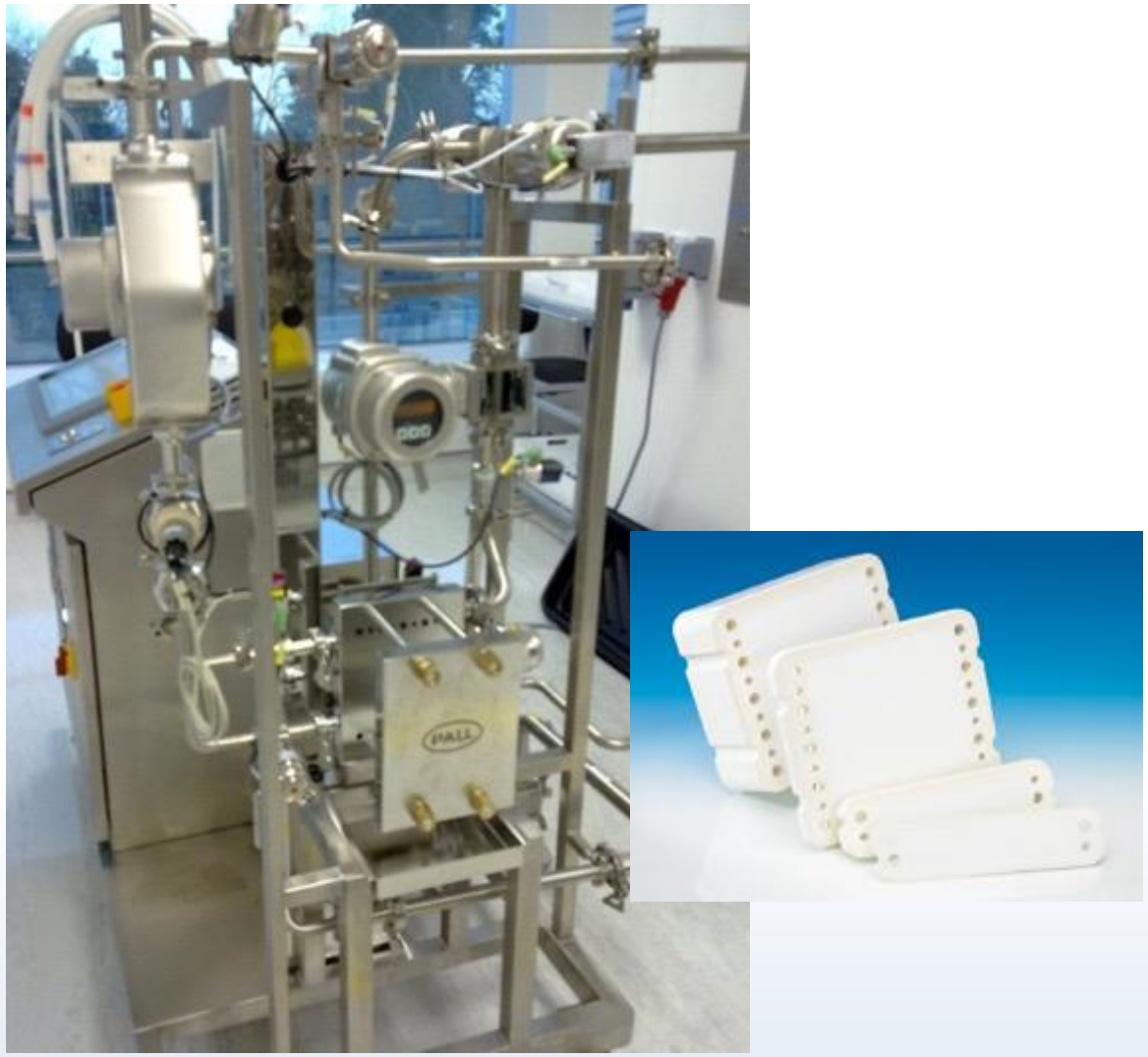
Separate cells from cell culture medium





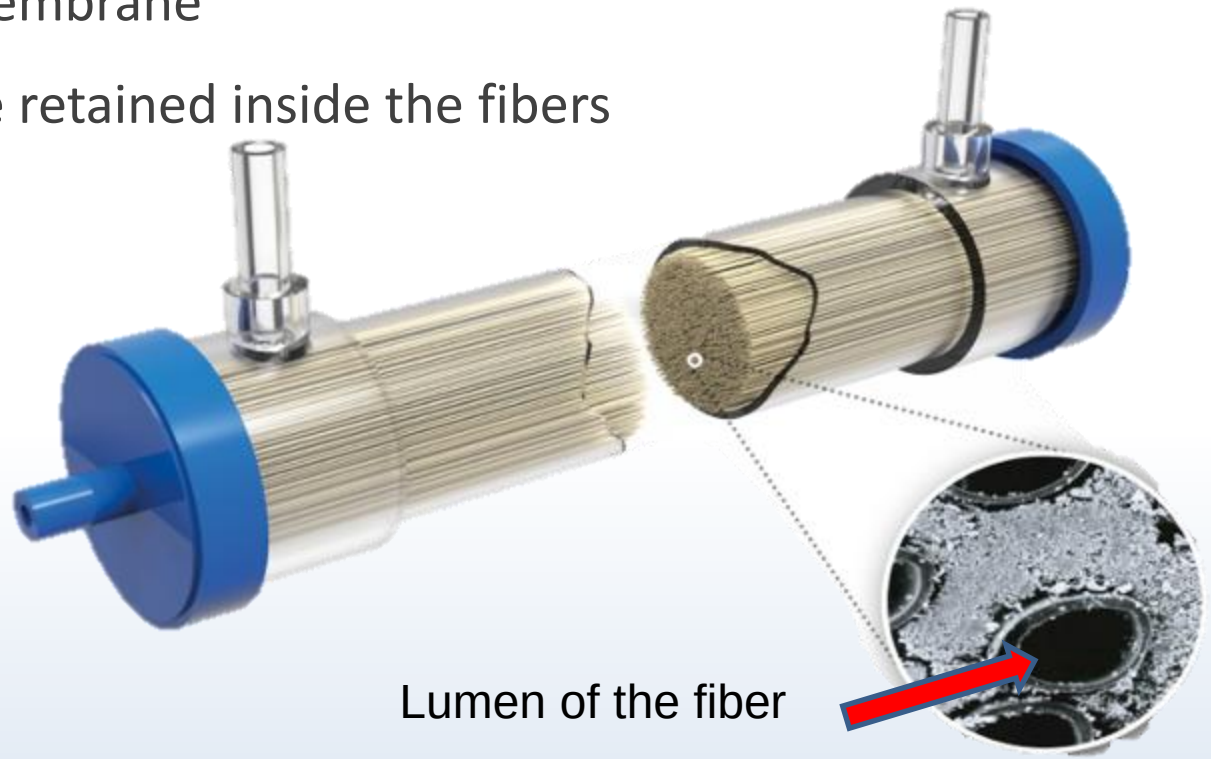
Microfiltration

- Highly efficient method of separating cells from liquid
- Pore size of filters average at 0.45µm
- Uses tangential flow filtration / cross flow filtration
- × Expensive
- × Long processing time



TFF filters – hollow fibre

- Hollow fiber filters
  - Consist of many “straws” tightly packed into the filter housing
  - Cell culture is pumped through the straws that are made of a porous membrane
- Small molecules will pass through the membrane
- Liquid with large molecules (cells) will be retained inside the fibers



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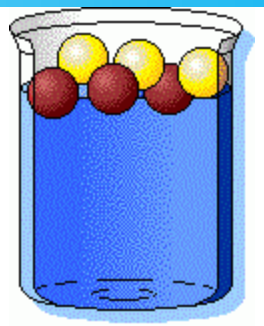
What is Centrifugation?

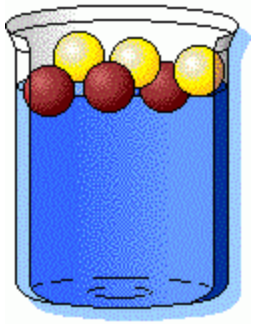
The separation of components in a liquid mixture using centrifugal force, generated by rotation around a central axis.

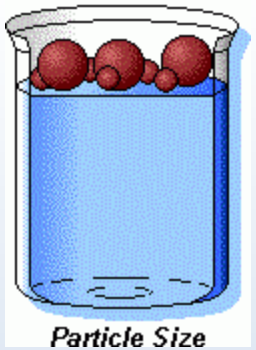
- Separation of a mixture will depend on several factors, including the mass or density of each component and the speed at which they are rotated.

Sedimentation

- Stokes' Law is a formula for determining the rate of sedimentation.
- Sedimentation is when a particle contacts a barrier and falls out of suspension – **particle capture.**
- It is important to control temperature, in order to control viscosity of the liquid.







Gravity Settling

Fine sediment

Sand

Gravel

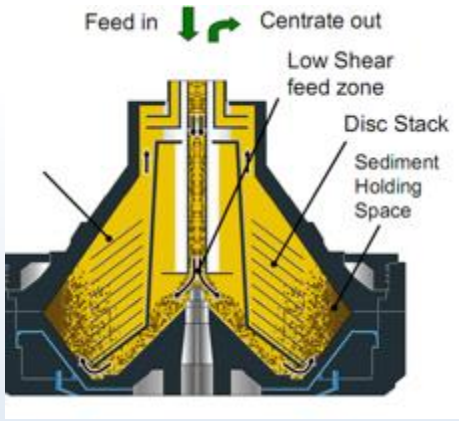
As in normal gravity settling, larger, heavier particles will separate out first.

Oil floats on water – different density.

Centrifugation uses this same principle but **accelerates** it!

Common Types of Centrifuge

- Rotary Centrifuge
- Tubular Bowl
- Disposable Centrifuges
- Disk Stack Centrifuge

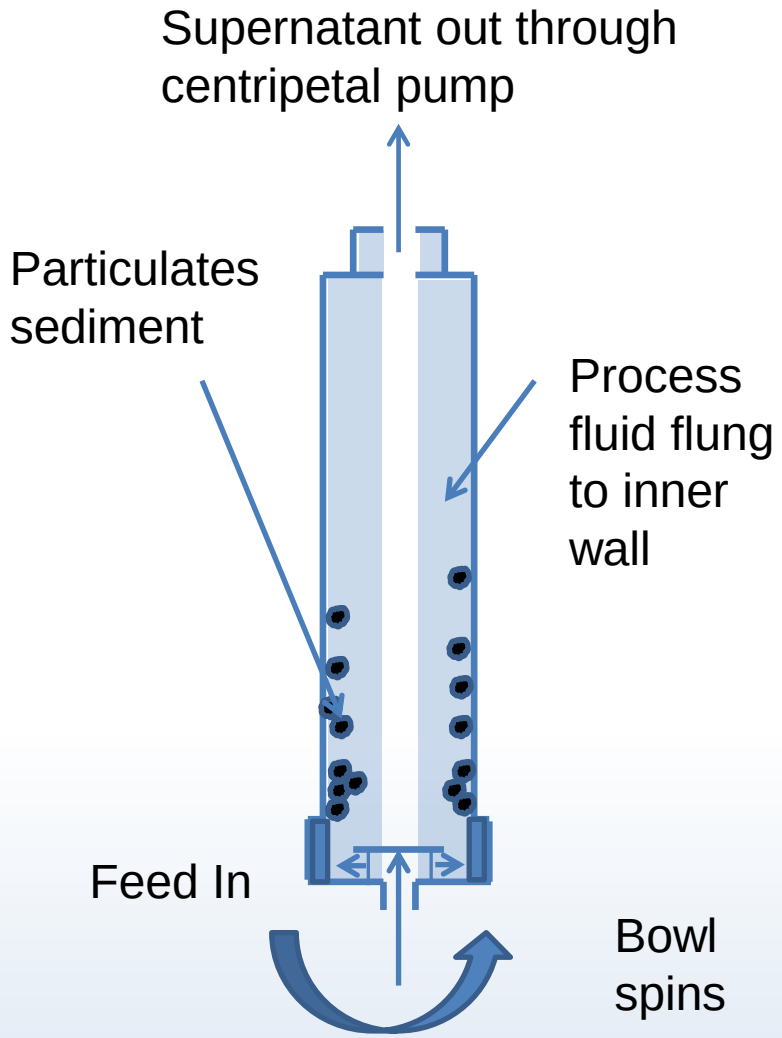


Rotary Centrifuge

- Bench-top / ultracentrifuges.
- Very effective for processing samples for assays or other bench-top applications.
- In biopharma, typically used at vial thaw stage to separate cells from media/cryoprotectant
- Can reach very high G forces.
- Advanced models have temperature control.
- Operate in **batch** mode.

Tubular Bowl Centrifuge

- Stainless steel tube spun at high speed
- Suspension fed in and moved through by **displacement**
- Material experiences centrifugal force while in the chamber
- Solids are thrown to outer wall
- Operates **continuously** – not limited by volume of the bowl
- **Residence time** is an important consideration



Disposable Centrifuge

- Unifuge (Carr / Pneumatic Scale Angelus)
- Tubular bowl centrifuge
- Disposable plastic lining in stainless steel housing – 1.6L volume bowl
- Maintains cell viability, thus reducing HCP & DNA release from cells.
- Solids can be removed by re-suspension with buffer and flushing out

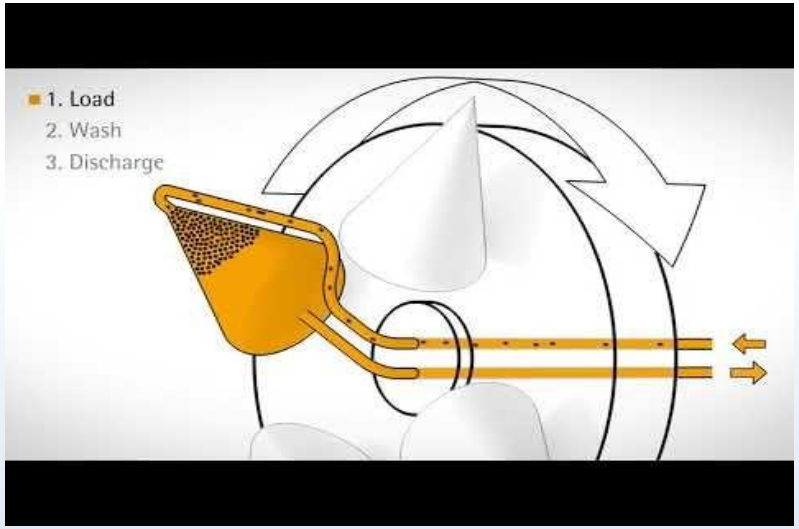
Disposable Centrifuges

- kSep6000S (KBI Biopharma Inc.)
- Processes up to 6000L
- Closed system
- Maintains viability of cells – can be used for perfusion

Disposable Centrifuges

- Product-contact area consists of disposable plastic inserts
- 4 conical chambers
- Vertical rotation
- Fluid flows in from the tip
- Force of fluid flow balances centrifugal force
- Creates a fluidised bed
- Cells remain suspended and intact.

https://www.youtube.com/watch?v=qodgYXxFb0g



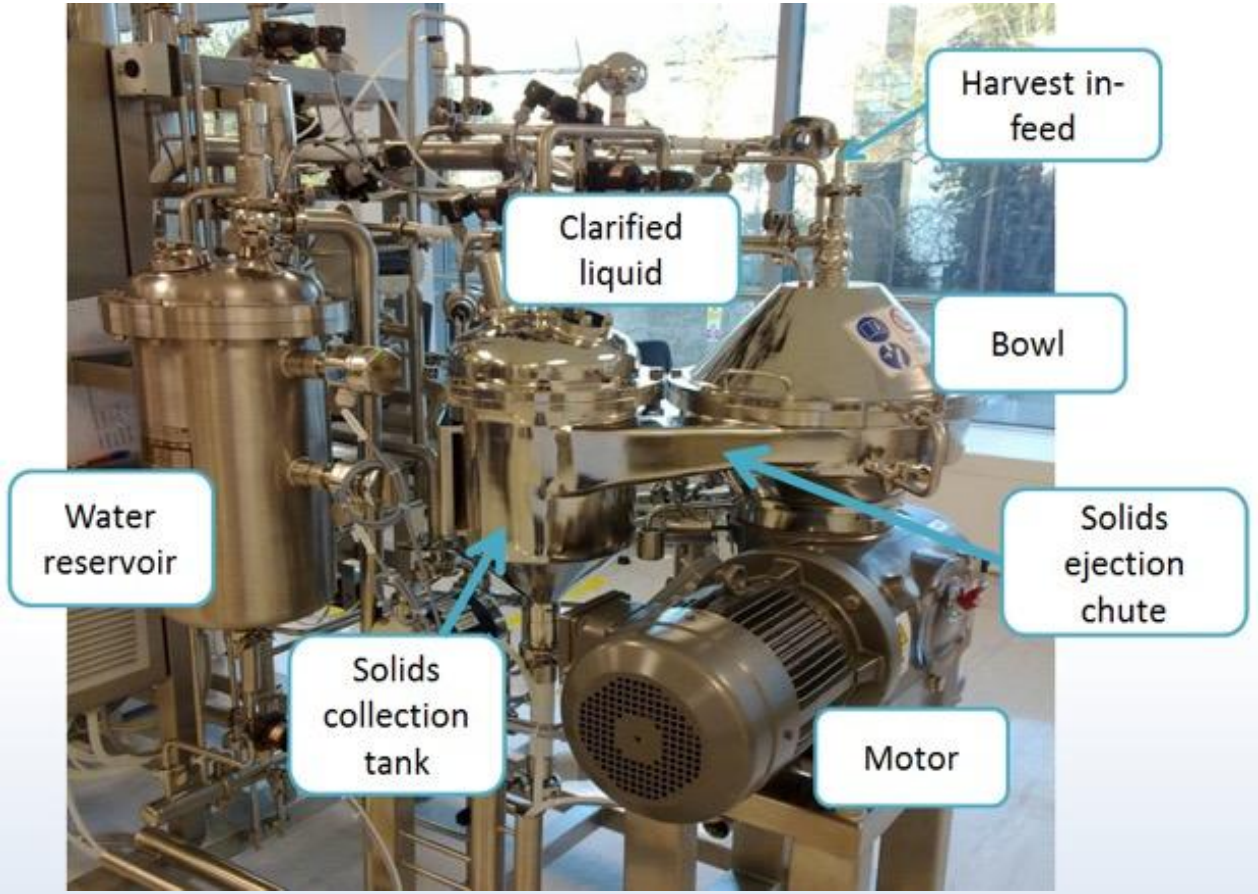
Disk Stack Centrifuge

- Commercial scale bioprocessing uses **disk stack centrifuges.**
- Principal suppliers are Westfalia and Alfa Laval

✓Low running costs.

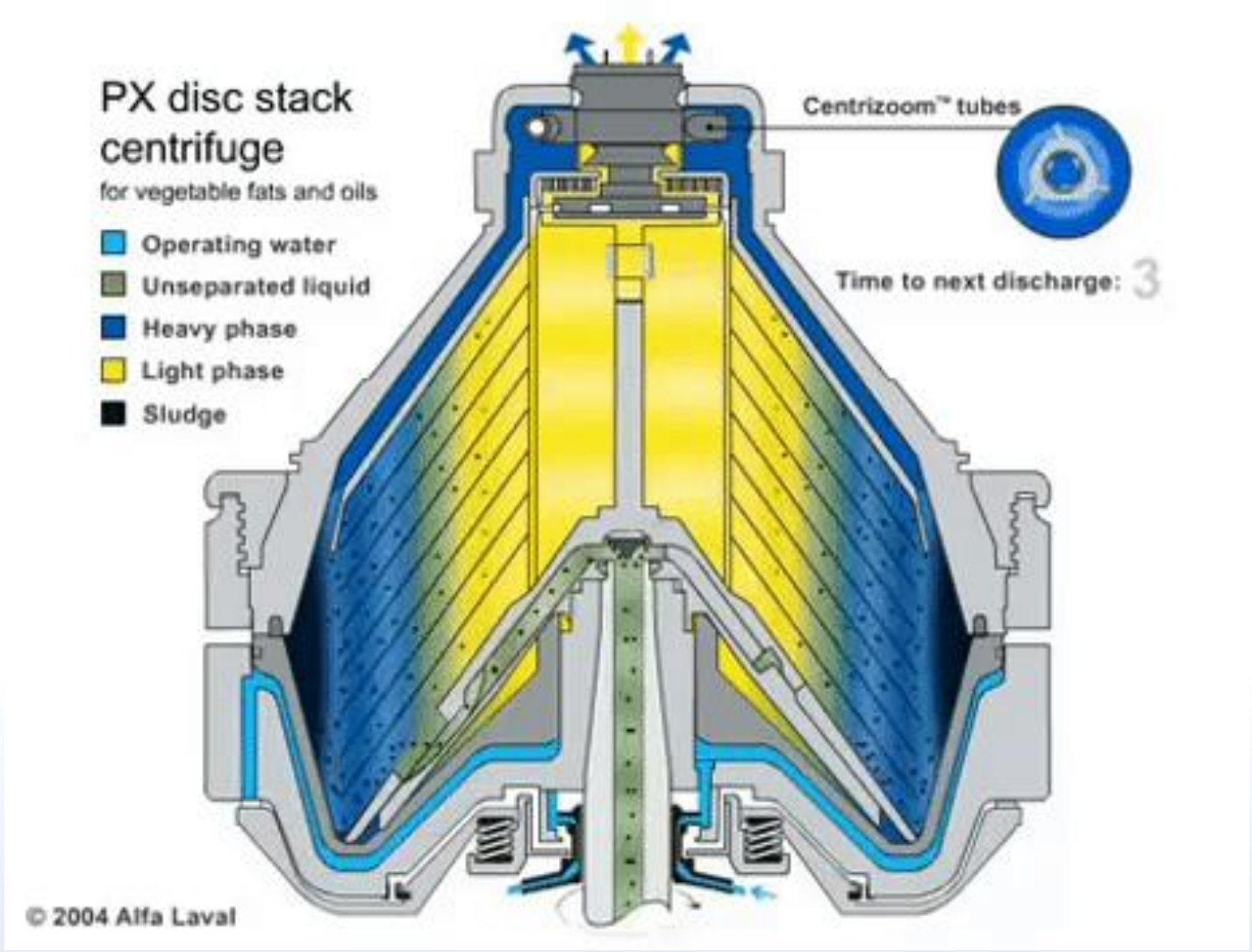
✓Continuous processing possible.

✓Quick processing time – runs at 12,000rpm.



Industrial centrifuge

- Liquid from bioreactor is directly fed into the centrifuge
- Separation process starts once the liquid enters the bowl
- Dense cells experience more centrifugal force, therefore they are forced towards the edge of the bowl
- Less dense protein travels with the **centrate** towards outfeed port



Centripetal Pumps

In centrifuges, the product infeed is pumped through the centripetal pump - a **stationary** component submerged in **rapidly rotating** process fluid within the bowl.

The rapidly rotating clarified process fluid is forced into veins in the outfeed of the centripetal pump and routes out to supernatant line.

Distance

Two tanks with equal volumes – in which will particles settle by gravity more quickly?

In tank B particles have shorter distance to travel, therefore sediment quicker

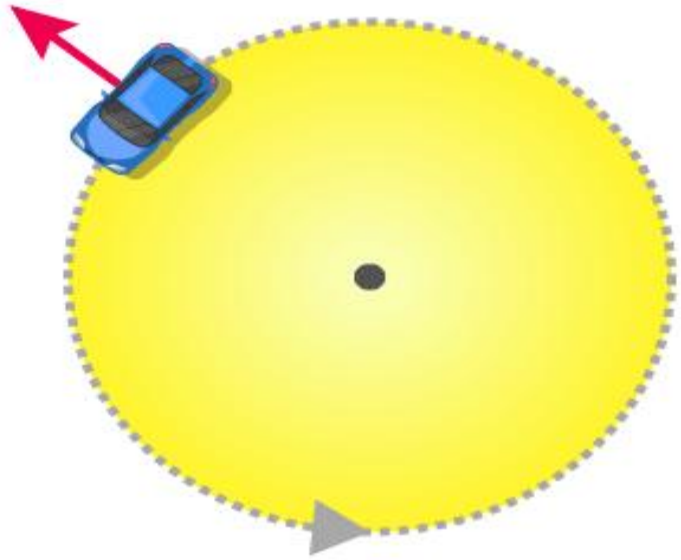
In Centrifuge, distance which particles need to travel is decreased by the addition of **separating disks**.

Centrifugal Force Formula

An object traveling in a circle behaves as if it is experiencing an outward force. This force is known as the centrifugal force.

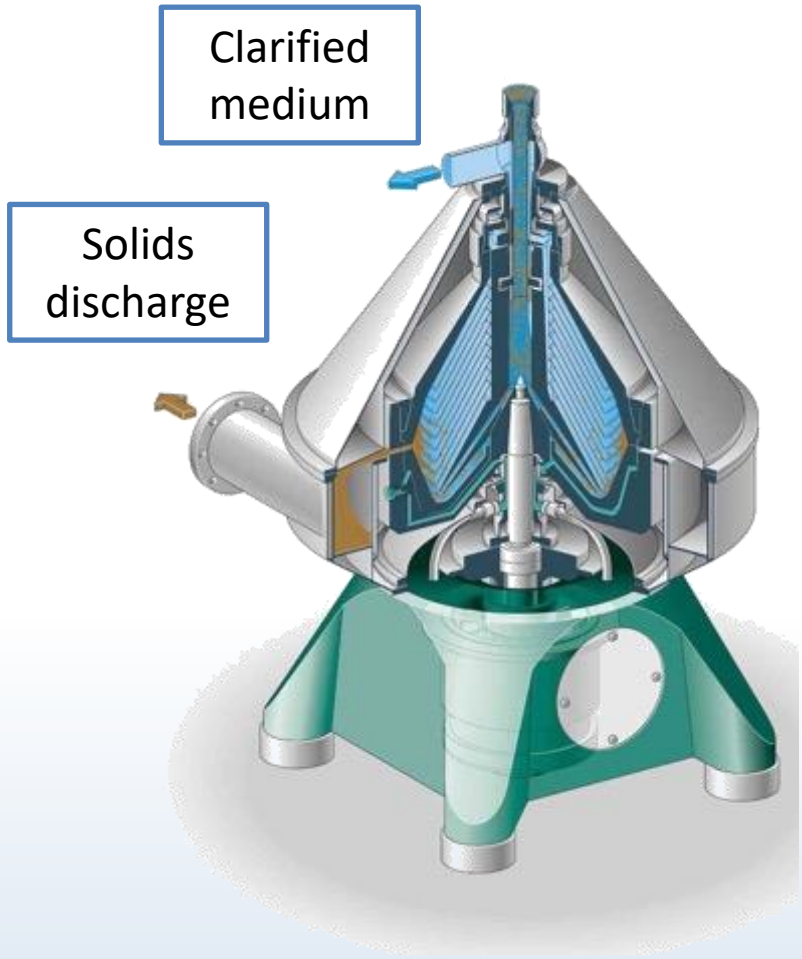
$$Fc = mv^2/r$$

Fc = centrifugal force,
m = mass,
v = speed,
r = radius



Solids Ejection

- Disk stack centrifuges can perform partial and / or total ejections to remove cell waste
- The two halves of the bowl can temporarily separate to allow cells to be discharged towards a waste collection tank
- This allows us to run the centrifuge in continuous mode, without the need to clean the bowl when it fills up



When is the correct time to perform solid ejection?

Separation Efficiency

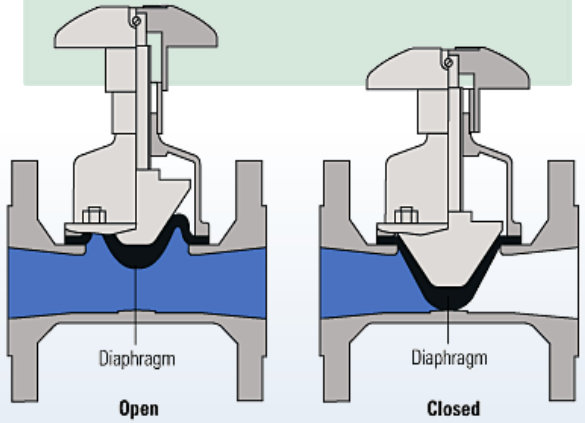
How efficiently separation occurs depends on a number of variables:

The Harvest

- Viscosity
- Temperature
- Cell Viability

Residence Time

- In-feed pump speed
- Back pressure



Equipment

- Speed of rotation
- Number of separating disks

Solids Ejection

- Frequency of solids removal

Inadequacies of Centrifugation

- The centrifugation step is not 100% effective at removing particles

- It is normal to place a lenticular depth filter downstream of the centrifuge to capture any stray cells or cellular material that may have escaped the centrifuge

Depth

Polishing

Topics

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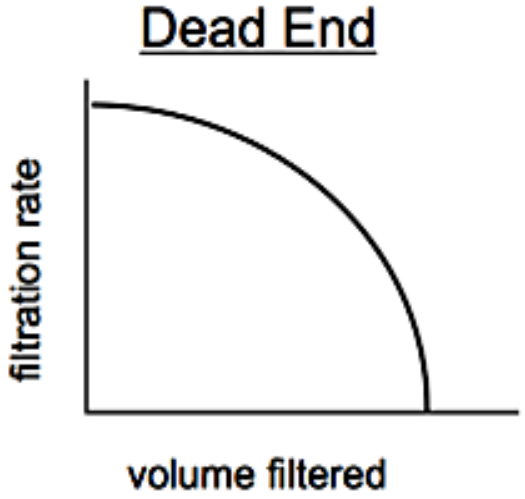
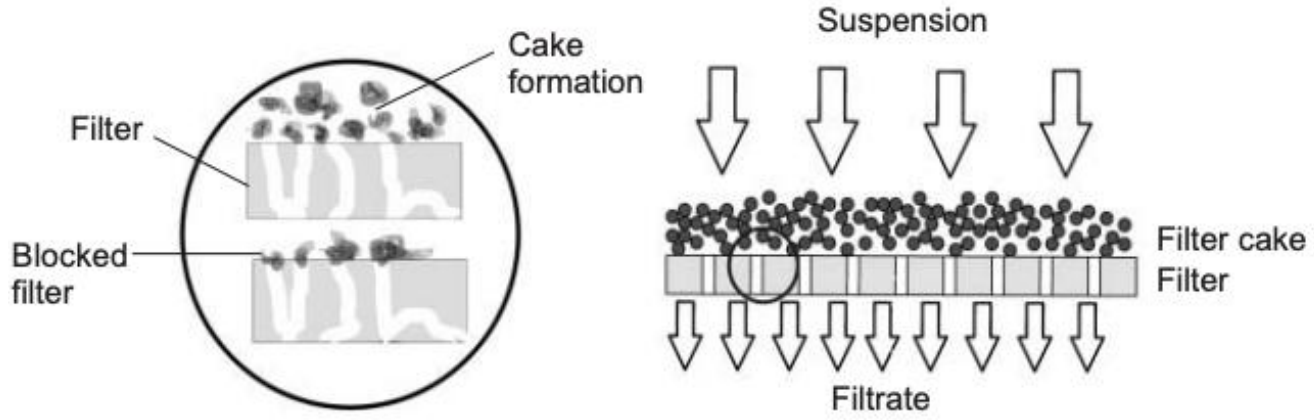
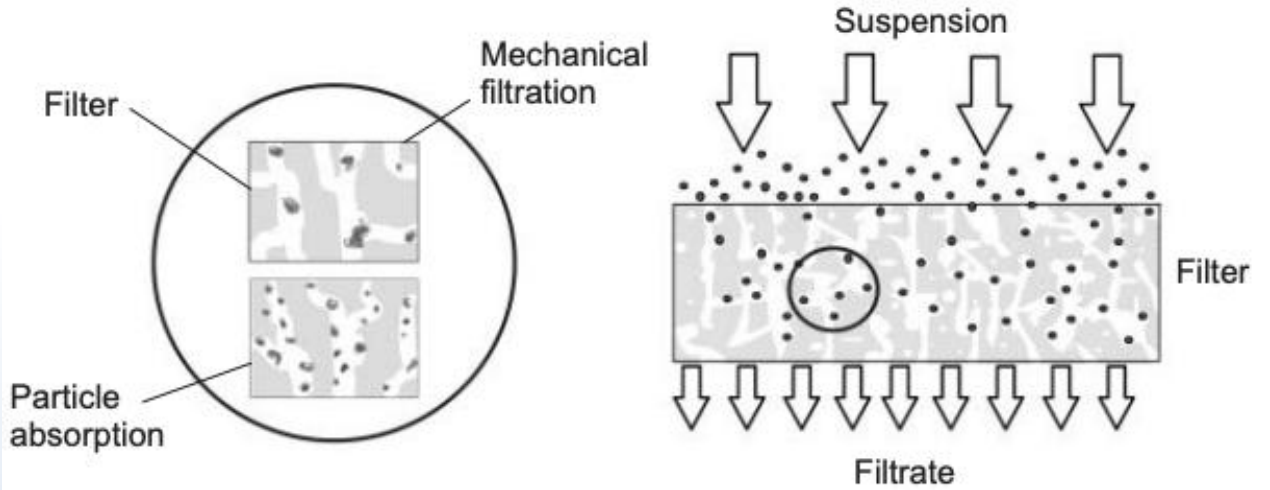
Normal Flow Filtration

- Potential for **fouling layer** to build
- Flow is restricted and eventually stops
- TFF is one method to avoid this
- Another is **depth filtration**



It’s all about the depth

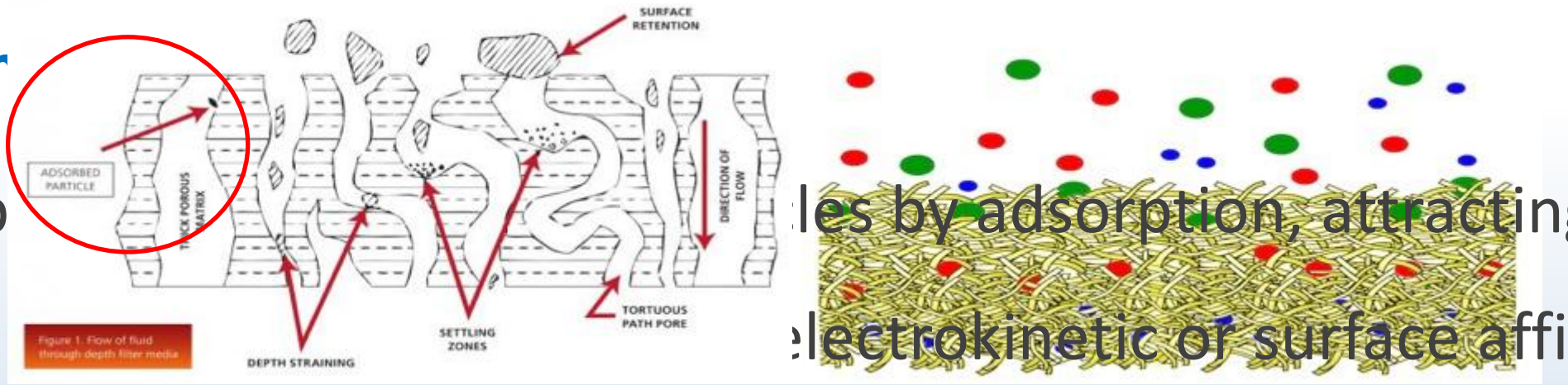




- Membrane filter – collects material on the surface of the membrane
- Depth filter – traps particles within the membrane

Depth Filtration

- Thick mat of **randomly arranged fibres** – unlike membranes with specific pores
- Creates a **torturous path** for material to travel
- Traps particles throughout the medium – good for liquids with **high**
- Dep les by adsorption, attracting the ectrokinetic or surface affinity



Ref: Nixon, B. Troubleshooting depth filtration. 2012. Pharmaceutical Manufacturing.

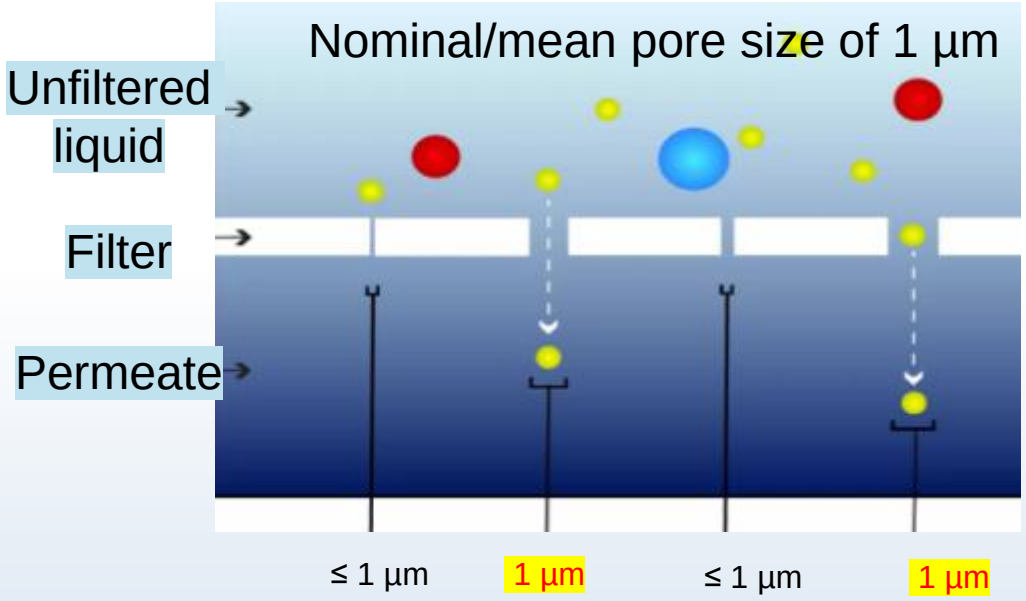
Depth Filters – Pros and Cons

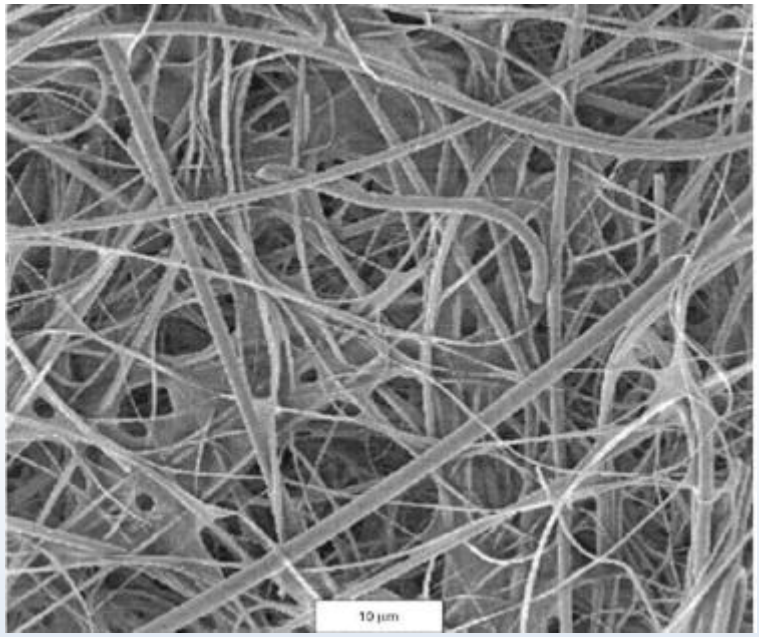
Advantages

+ Lower cost
+ Higher throughputs
+ Higher capacity

Disadvantages

× Nominal pore size
× Leaching
× No integrity testing





Depth Filtration Principles

Lenticular Filters

Stainless Steel housing

Housing cleaned post-use

Potential for leaks upon disassembly

Single-use filter cartridges

Number of cartridges required depends on product volume

Fully disposable post-use.

No cleaning required

Horizontal assembly, vertical operation.

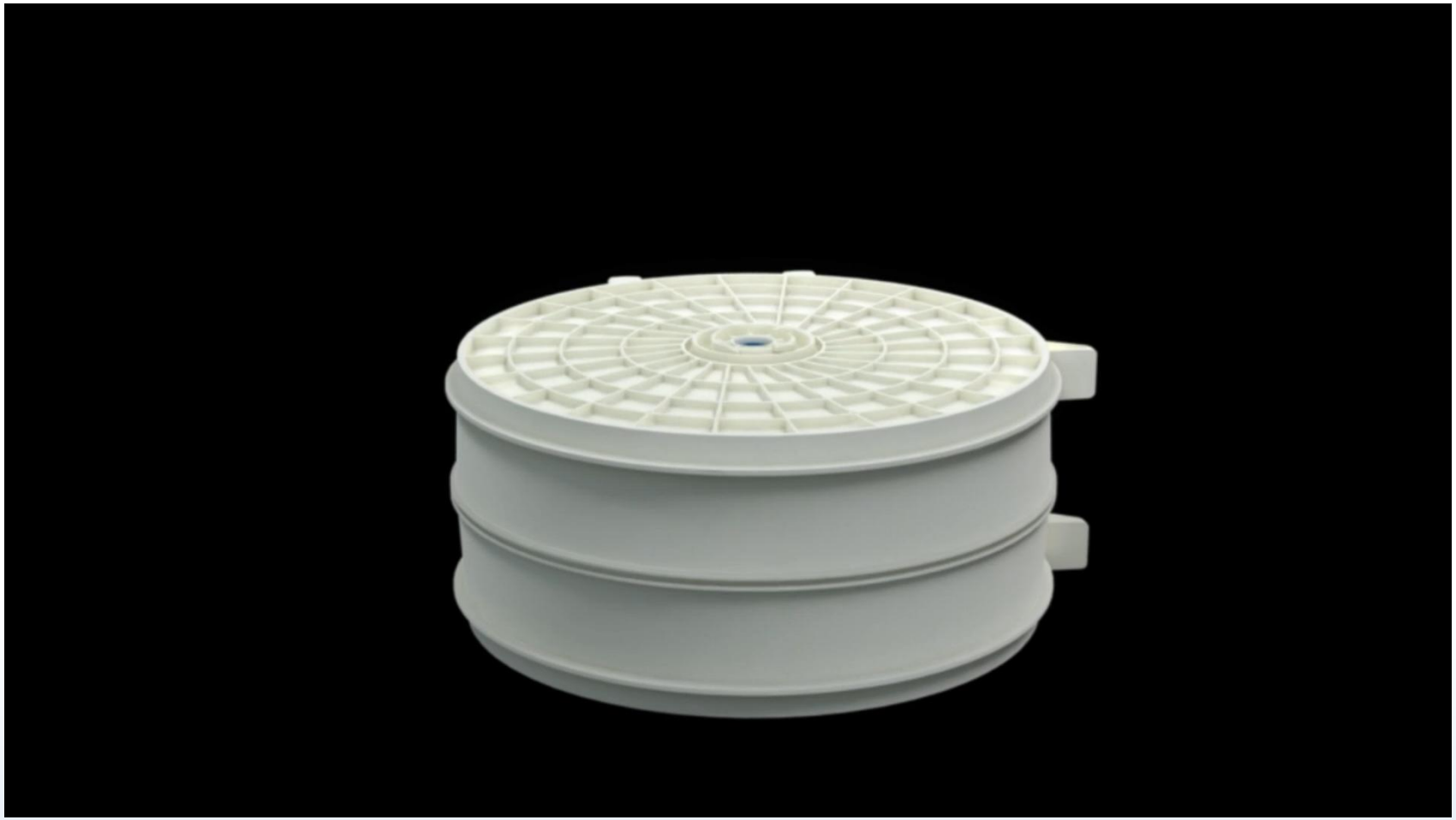
Depth Filters

Single use pods with lenticular shaped matrix

Combination of cellulose and cellulose esters

Pods can be stacked together to increase the surface

Depth Filtration – Flow Pattern



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Thank You