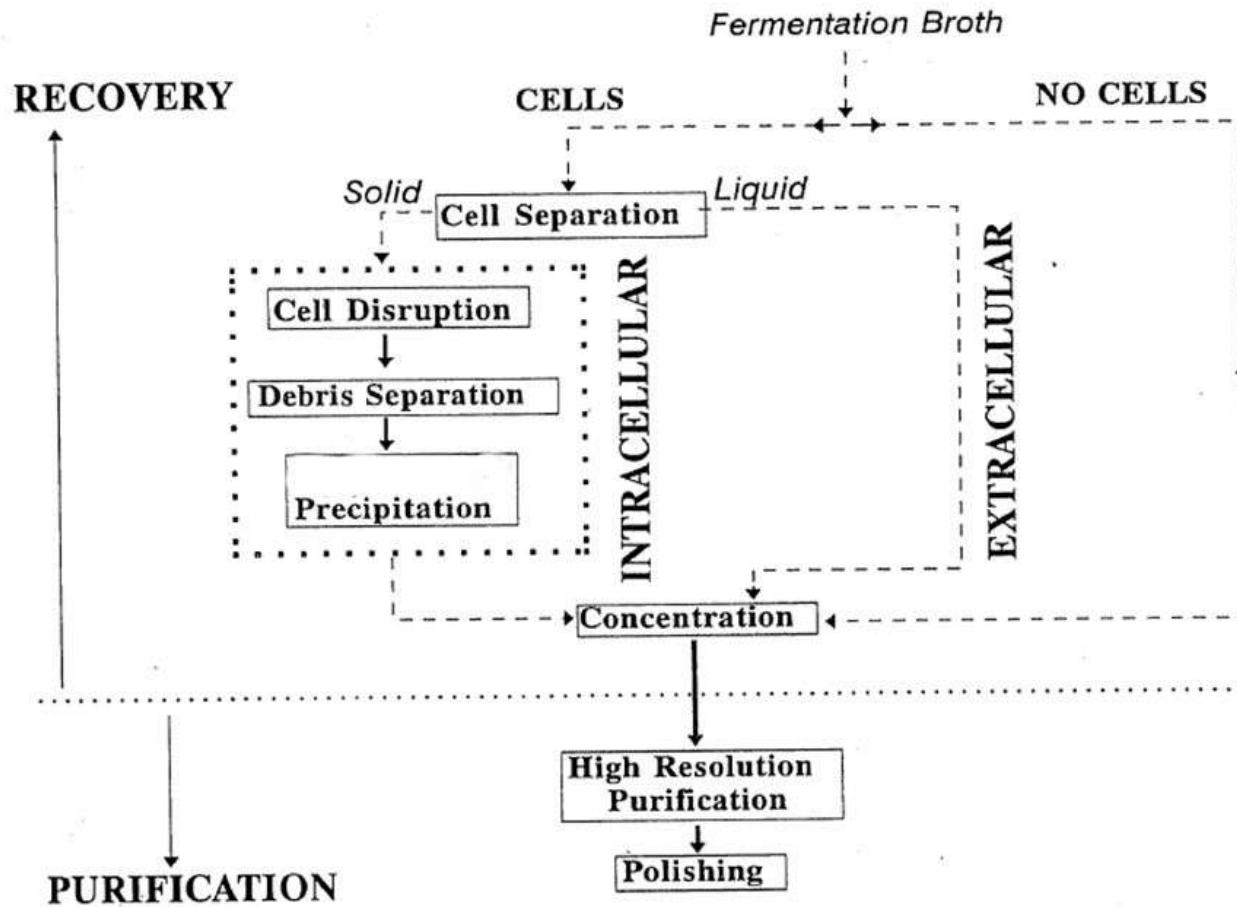




# Downstream Processing

Sailee Gurav  
MSc Biochemistry Part - 1

# General Steps in Downstream Purification



# Downstream processing

- The various stages of processing that occur after the completion of the fermentation or bioconversion stage, including separation, purification, and packaging of the product.

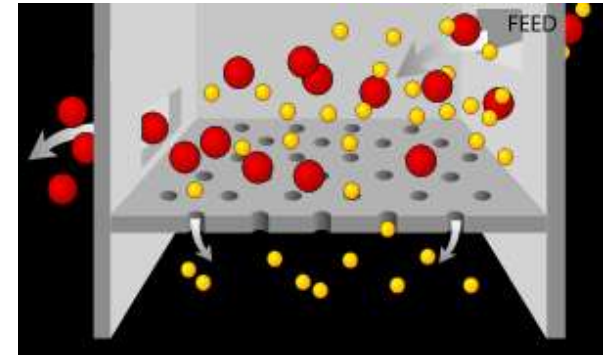
# Stages in Downstream Processing

- Removal of Insolubles
- Product Isolation
- Product Purification
- Product Polishing
- A few product recovery methods may be considered to combine two or more stages.

For example, expanded bed adsorption accomplishes removal of insolubles and product isolation in a single step. Affinity chromatography often isolates and purifies in a single step.

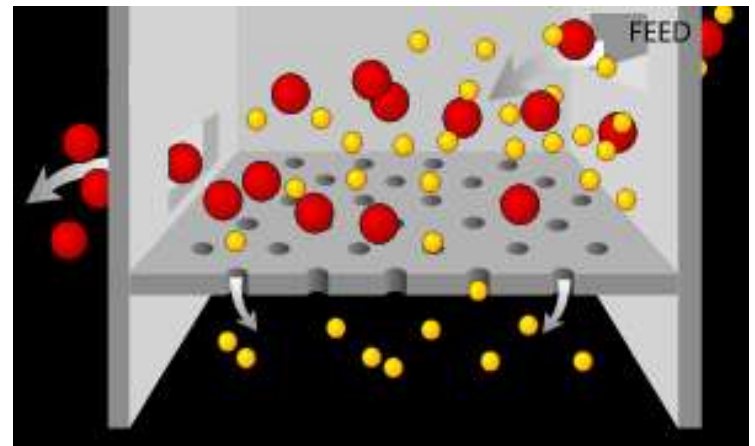
# Removal of Insolubles

- Separation of cells, cell debris or other particulate matter
- Typical operations to achieve this:
  - 1) Filtration
  - 2) Centrifugation
  - 3) Sedimentation
  - 4) Flocculation a process where a solute comes out of solution in the form of floc or flakes.
  - 5) Gravity settling



# Filtration

- A mechanical operation used for the separation of solids from fluids (liquids or gases) by interposing a medium to porous membrane through which the fluid can pass, but the solids in the fluid are retained.

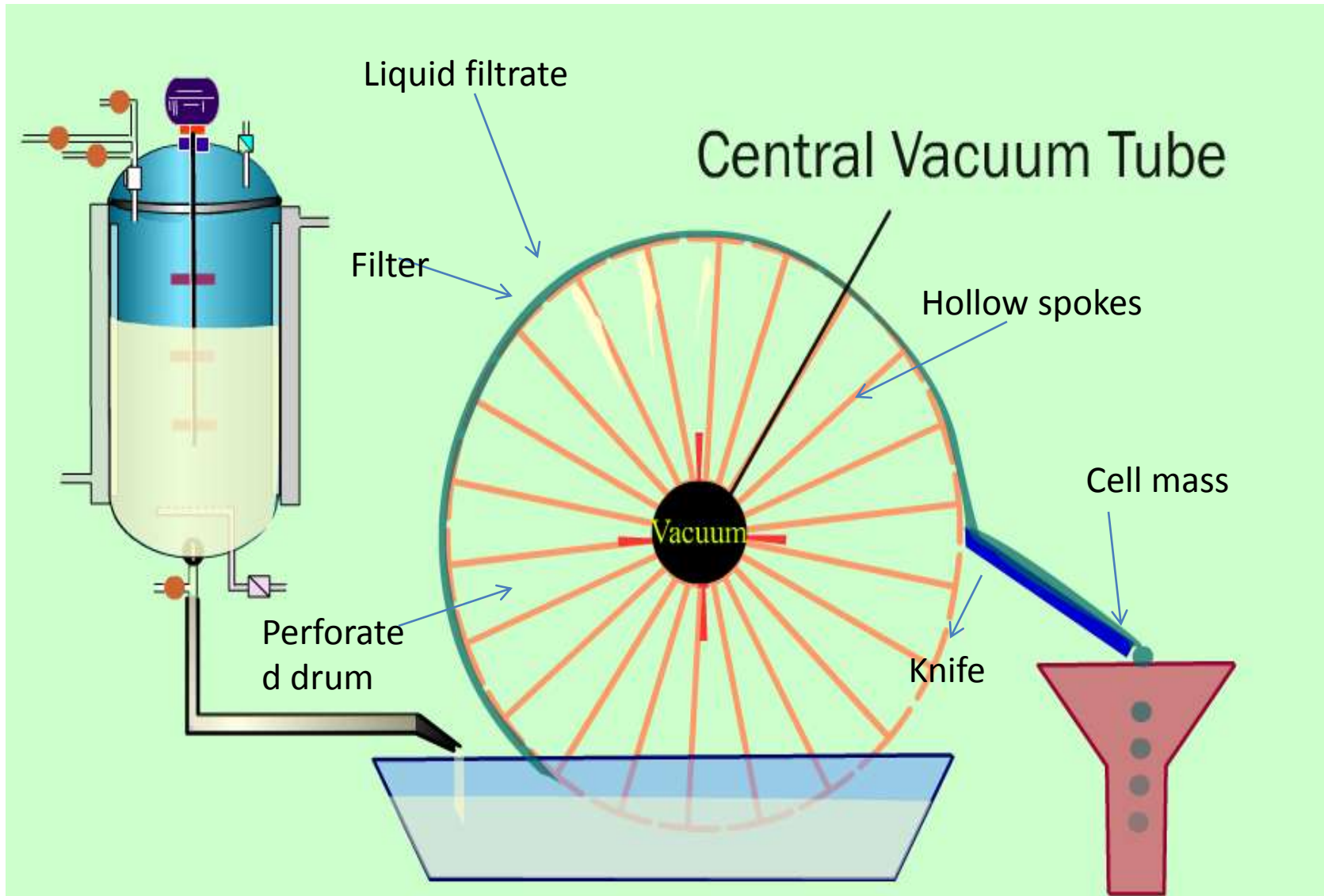


# Filtration

- The solid particles deposited on the filter form a layer, which is known as filter cake.
- All the solid particles from the feed are stopped by the cake, and the cake grows at the rate at which particles are brought to its surface.
- All of the fluid goes through the cake and filter medium.



# Continuous Rotary filter





# Continuous Rotary Vacuum filter

- It is one of the most commonly used type of filter in fermentation.
- The drum is pre coated prior to filtration.
- A small agent of coagulating is added to the broth before it is pumped into the filter.
- The drum rotates under vacuum and a thin layer of cells sticks to the drum.
- The thickness of the layer increases in the section designed for forming the cake.

# Points to be considered while selecting the filter medium:

- Ability to build the solid.
- Minimum resistance to flow the filtrate.
- Resistance to chemical attack.
- Minimum cost.
- Long life

# Centrifugation

- Centrifugation is used to separate particles of 100 – 0.1 micrometer from liquid by gravitational forces.
- It depends on particles size, density difference between the cells and the broth and broth viscosity.
- Use of the centrifugal force for the separation of mixtures
- More-dense components migrate away from the axis of the centrifuge
- Less-dense components migrate towards the axis.
- Types of centrifuges used are Tubular bowl centrifuge, multichamber centrifuge, disc bowl centrifuge etc.

# Sedimentation

- It is applicable only for large particles greater than 100 micrometer flocs.
- It is a slow process and takes ~3 hours.
- It is used in process like activated sludge effluent treatment.
- It's a free settling process depends only on gravity.
- Particles settling is a high particle density suspension(hindered settling).

# Flocculation

- Process where a solute comes out of solution in the form of flocs or flakes.
- Particles finer than  $0.1 \mu\text{m}$  in water remain continuously in motion due to electrostatic charge which causes them to repel each other
- Once their electrostatic charge is neutralized (use of coagulant) the finer particles start to collide and combine together .
- These larger and heavier particles are called flocs.

# Product Isolation

- Removal of those components whose properties vary markedly from that of the desired product.
- Water is the chief impurity
  - a) Isolation steps are designed to remove it (i.e.dialysis)
  - b) Reducing the volume
  - c) Concentrating the product.
  - d) Liquid –liquid extraction, adsorption, ultrafiltration, and precipitation are some of the unit operations involved.



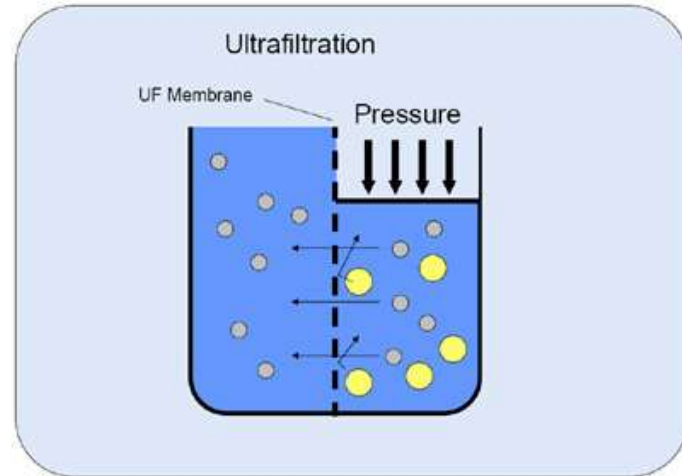
# Liquid -Liquid extraction

- It is a separation process that takes the advantage of the relative solubilities of solute in immiscible solvents.
- Solute is dissolved more readily and becomes more concentrated in the solvent in which it has a higher solubility.
- A partial separation occurs when a number of solutes have different relative solubilities in the two solvents used.
- Solvent should be non toxic, selective, inexpensive and immiscible with broth and should have a high distribution coefficient for the product.

# Adsorption

- is a surface phenomenon
- It is the binding of molecules to the surface and different from absorption.
- The binding to the surface is weak and reversible.
- Compounds containing chromogenic group are usually strongly adsorbed on activated carbon.
- Common adsorbent used are activated carbon, silica gel, alumina becoz they present enormous surface areas per unit weight.

# Ultrafiltration



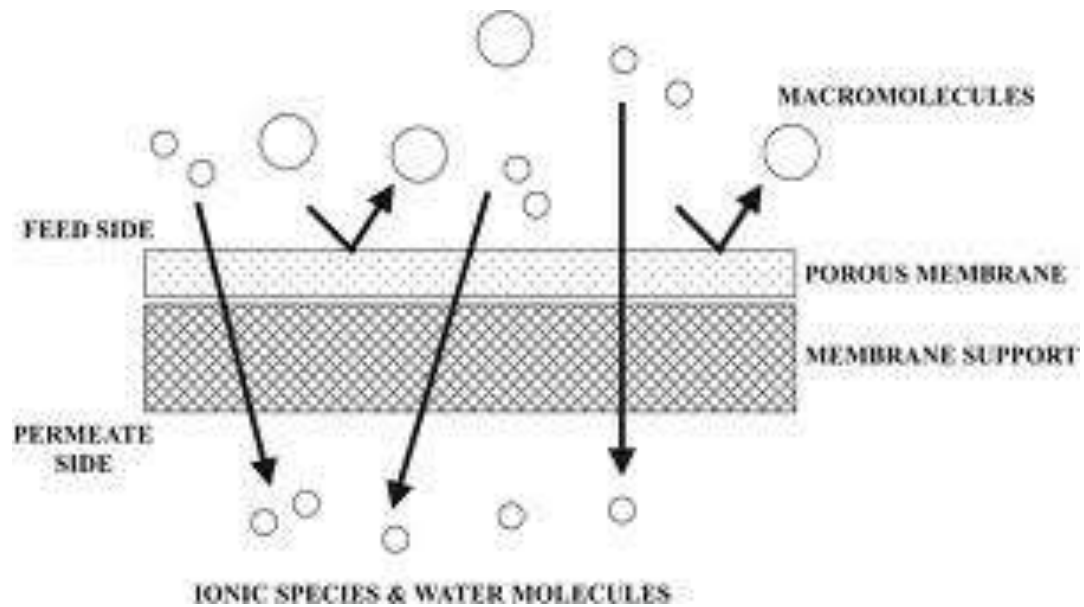
UF is basically a pressure-driven separation process.

The operating pressure is usually between 0.1 and 1 MPa.

# Ultrafiltration

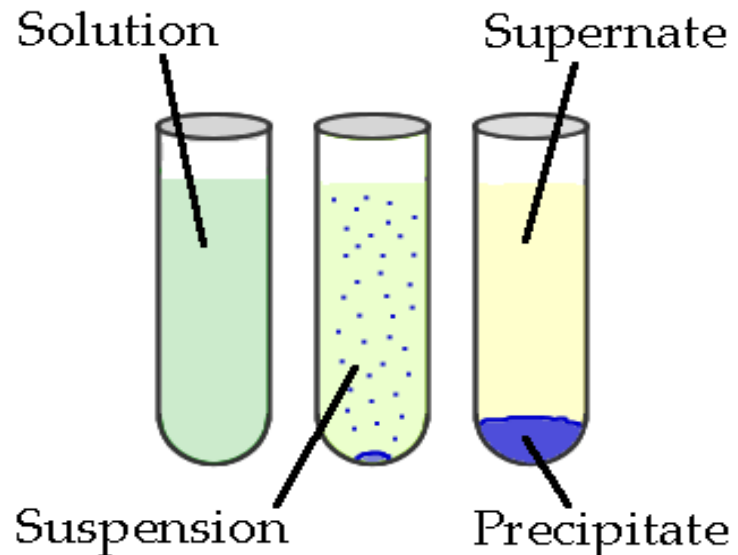
- UF is governed by a screening principle and dependent on particle size.
- UF membranes have a pore size between 1 nm and 100 nm (10 and 2000 Å), thus allowing retention of compounds with a molecular weight of 300 to 500 000 Dalton.
- Typically, the process is suitable for retaining biomolecules, bacteria, viruses, polymers, colloidal particles and sugar molecules.

# Ultrafiltration



# Precipitation

- Formation of a solid in a solution during a chemical reaction.
- Solid formed is called the **precipitate** and the liquid remaining above the solid is called the **supernate**.





# Precipitation

- Salts such as ammonium & sodium sulphate are used for proteins to precipitate.
- Organic solvents methanol used to precipitate dextrans.
- Chilled ethanol and acetone used for protein precipitation.
- Non ionic polymer such as polyethylene glycol used in precipitation.

# Product Purification

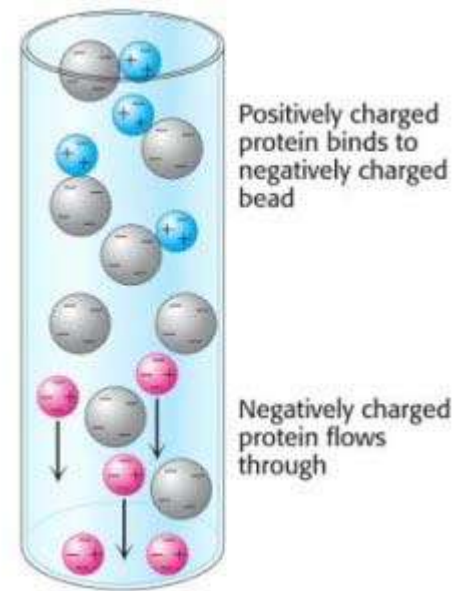
- Done to separate those contaminants that resemble the product very closely in physical and chemical properties.
- Expensive to carry out
- Require sensitive and sophisticated equipment
- Significant fraction of the entire downstream processing expenditure.
- Examples of operations include affinity, size exclusion, reversed phase chromatography, crystallization and fractional precipitation.

# Chromatography

- Separation of mixtures
- Passing a mixture dissolved in a "mobile phase" through a *stationary phase*, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated.

# Ion Exchange Chromatography

- Used charged stationary phase to separate charged compounds
- Resin that carries charged functional groups which interact with oppositely charged groups of the compound to be retained.
- FPLC

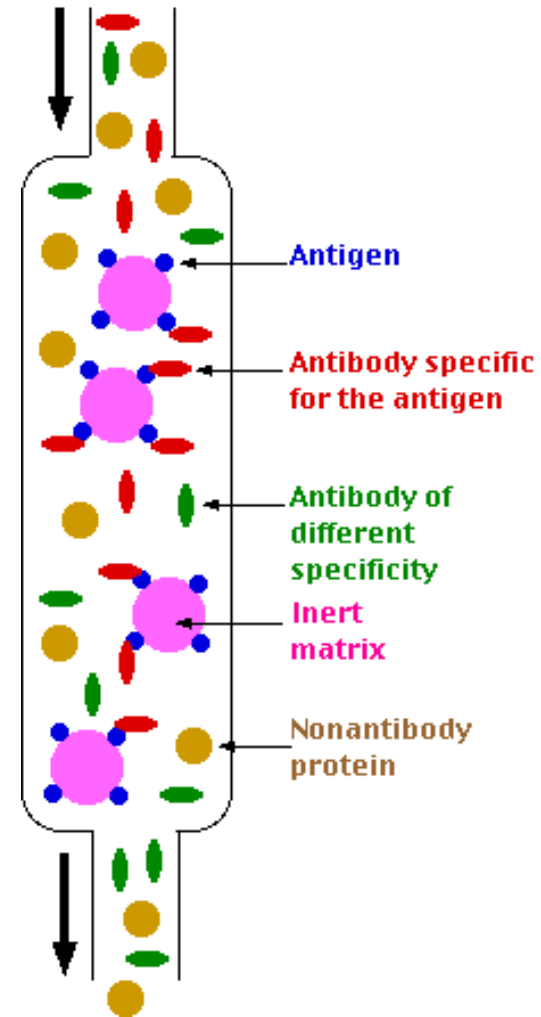


# Definition: Ion

- Ion is an atom or molecule which has lost or gained one or more valence electrons, giving it a positive or negative electrical charge.
- Anions are negatively charged ions, formed when an atom gains electrons in a reaction. Anions are negatively charged because there are more electrons associated with them than there are protons in their nuclei.
- Cations are positively charged ions, formed when an atom loses electrons in a reaction, forming an 'electron hole'.

# Affinity chromatography

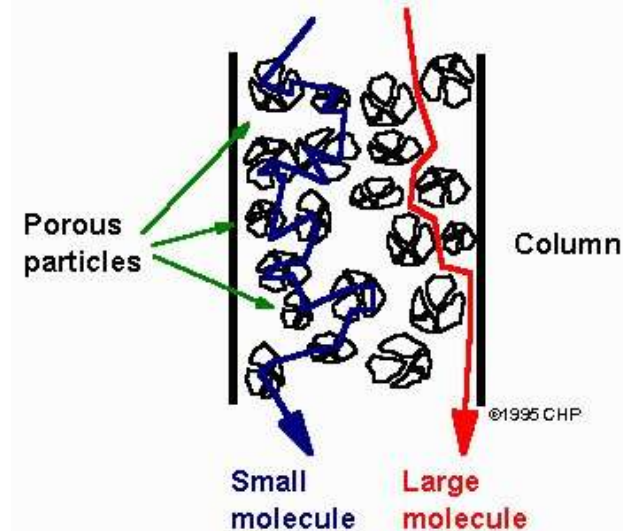
- Affinity chromatography separates the protein of interest on the basis of a reversible interaction between it and its antibody coupled to a chromatography bead (here labeled antigen) .
- With high selectivity, high resolution, and high capacity for the protein of interest, purification levels in the order of several thousand-fold are achievable.
- The protein of interest is collected in a purified, concentrated form. Biological interactions between the antigen and the protein of interest can result from electrostatic interactions, van der Waals' forces and/or hydrogen bonding. To elute the protein of interest from the affinity beads, the interaction can be reversed by changing the pH or ionic strength.
- The concentrating effect enables large volumes to be processed. The protein of interest can be purified from high levels of contaminating substances.
- Making antibodies to the protein of interest is expensive, so affinity chromatography is the least economical choice for production chromatography.





# Size exclusion chromatography

- Gel permeation/filtration chromatography (GPC)
- Separates molecules according to their size
- Low resolution "polishing"
- Tertiary/Quaternary structure(native)



# Reversed phase chromatography

Reversed-phase chromatography is an elution procedure used

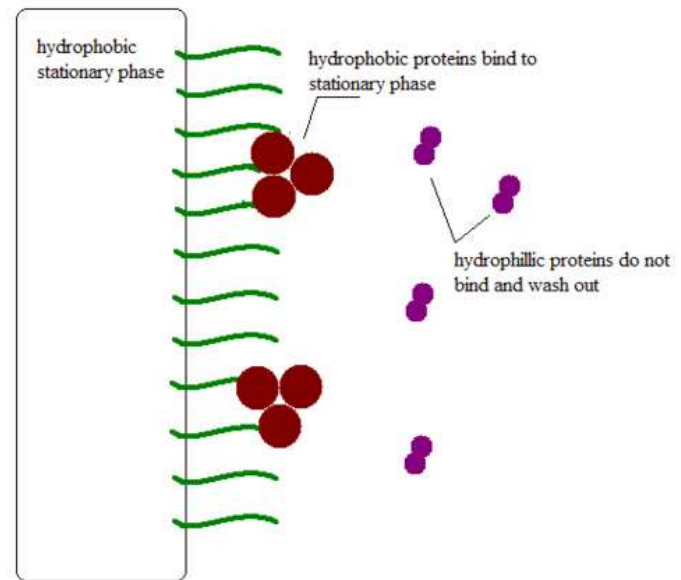
in liquid

chromatography in

which the mobile

phase is significantly

more polar than the stationary phase.



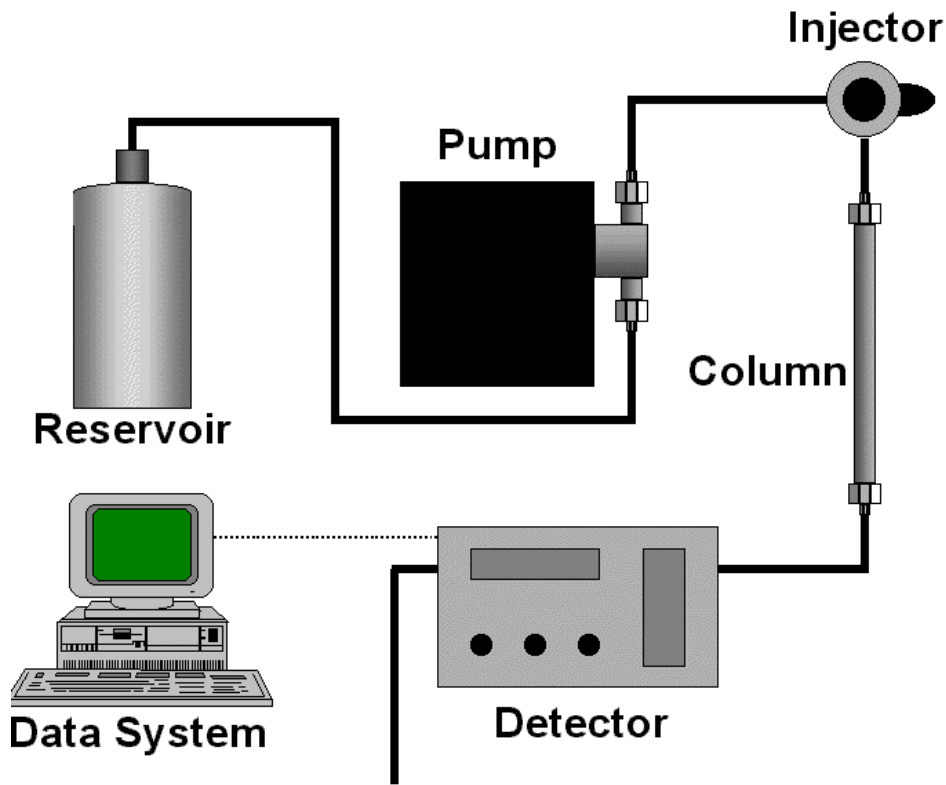
# Definitions: Polarity

- The dipole-dipole intermolecular forces between the slightly positively-charged end of one molecule to the negative end of another or the same molecule.
- Molecular polarity is dependent on the difference in electronegativity between atoms in a compound and the asymmetry of the compound's structure.

# Liquid Chromatography

- Mobile phase is a liquid.
- Carried out either in a column or a plane.
- HPLC
- In the HPLC technique, the sample is forced through a column that is packed with irregularly or spherically shaped particles or a porous monolithic layer (stationary phase) by a liquid (mobile phase) at high pressure.

# HPLC Configuration



# Crystallization

- process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas.
- chemical solid-liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs.



# Product Polishing

- End with packaging of the product in a form that is
- stable, easily transportable and convenient.
- Crystallization
- Desiccation
- Lyophilization
- Spray drying
- May include:
- Sterilization of the product
- Remove or deactivate trace contaminants which might compromise product safety viruses or depyrogenation

# lyophilization

- freezing the material
- reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas.

# Current Research

A Study on Downstream Processing for the production of Pullulan by  
Aureobasidium pullulans-SB-01 from the Fermentation broth

- Pullulan made up of linear  $\alpha$ -D-glucan maltotriose & maltotetraose rinterconnected by  $\alpha$  (1 $\rightarrow$ 6) &  $\alpha$  (1 $\rightarrow$ 4) linkages
- is a water-soluble homopolysaccharide produced extracellularly by Aureobasidium pullulans.
- It is necessary to harvest cells, remove the melanin pigments co-produced during its fermentation followed by its precipitation, concentration and drying.
- Centrifugation of fermentation broth at 8,000 rpm for 20 min gave cell pellets that were discarded & a greenish black supernatant containing melanin pigment .
- It was then subjected to the heat treatment at 80°C for 30 min in order to remove the protein (mainly Pullulanase) in the fermentation broth.
- The supernatant was demelanized by with hydrogen peroxide & activated charcoal, solvent-solvent blends, or by solvent-salt combinations in which hydrogen peroxide treatment shows better result for the removal of melanin pigments.
- For the precipitation of the exopolysachride the cold Isopropanol was used followed by its drying process at 60°C for 40 min. This methodology produced high purity pullulan that was comparable in colour and texture to the commercial samples which was characterized by the HPLC and FT-IR analysis.

# Reference

- Principles of Fermentation Technology by Peter Stanbury, Allan Whiteaker, Stephen Hall, 2<sup>nd</sup> Edition, Chapter 10, Pages : 277-308.
- Biochemical Engineering and Biotechnology By Ghasem Najafpour, 1<sup>st</sup> edition 2007, Chapter 7 , Pages: 170-189.
- <http://www.microbelibrary.org/library/resources/3138-fermentation-process-in-the-stirred-tank-fermenter>
- Current Research :  
[http://www.isca.in/rjrs/archive/special\\_issue2012/4.ISCA-ISC-2012-03BS-46.pdf](http://www.isca.in/rjrs/archive/special_issue2012/4.ISCA-ISC-2012-03BS-46.pdf)
- Research Journal of Recent Sciences Vol. 2(ISC-2012), 16-19 (2013) by Bishwambhar Mishra and Suneetha Vuppu
- Available online at: [www.isca.in](http://www.isca.in) Received 18th October 2012, revised 21st January 2013, accepted 23rd January 2013.

Thank - You