Bioprocess Engineering Lab: Separations in Bioprocessing

ABE 304

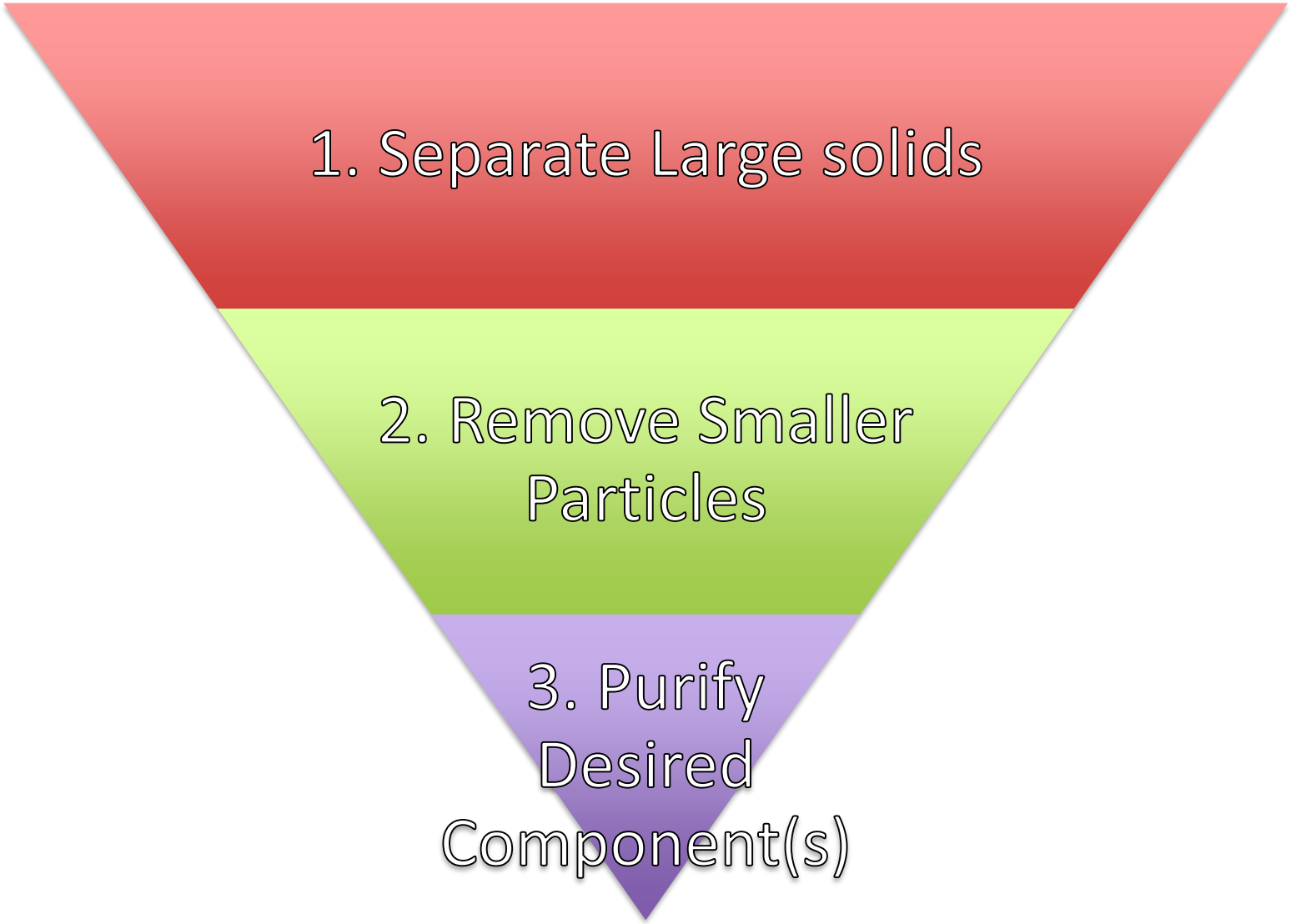
Why are Separations important?

- Biological components are rarely found alone

Why are Separations important?

- Biological components are rarely found alone
  - Biomass components
  - Pharmaceuticals
  - Proteins
  - Bacteria
  - Yeast

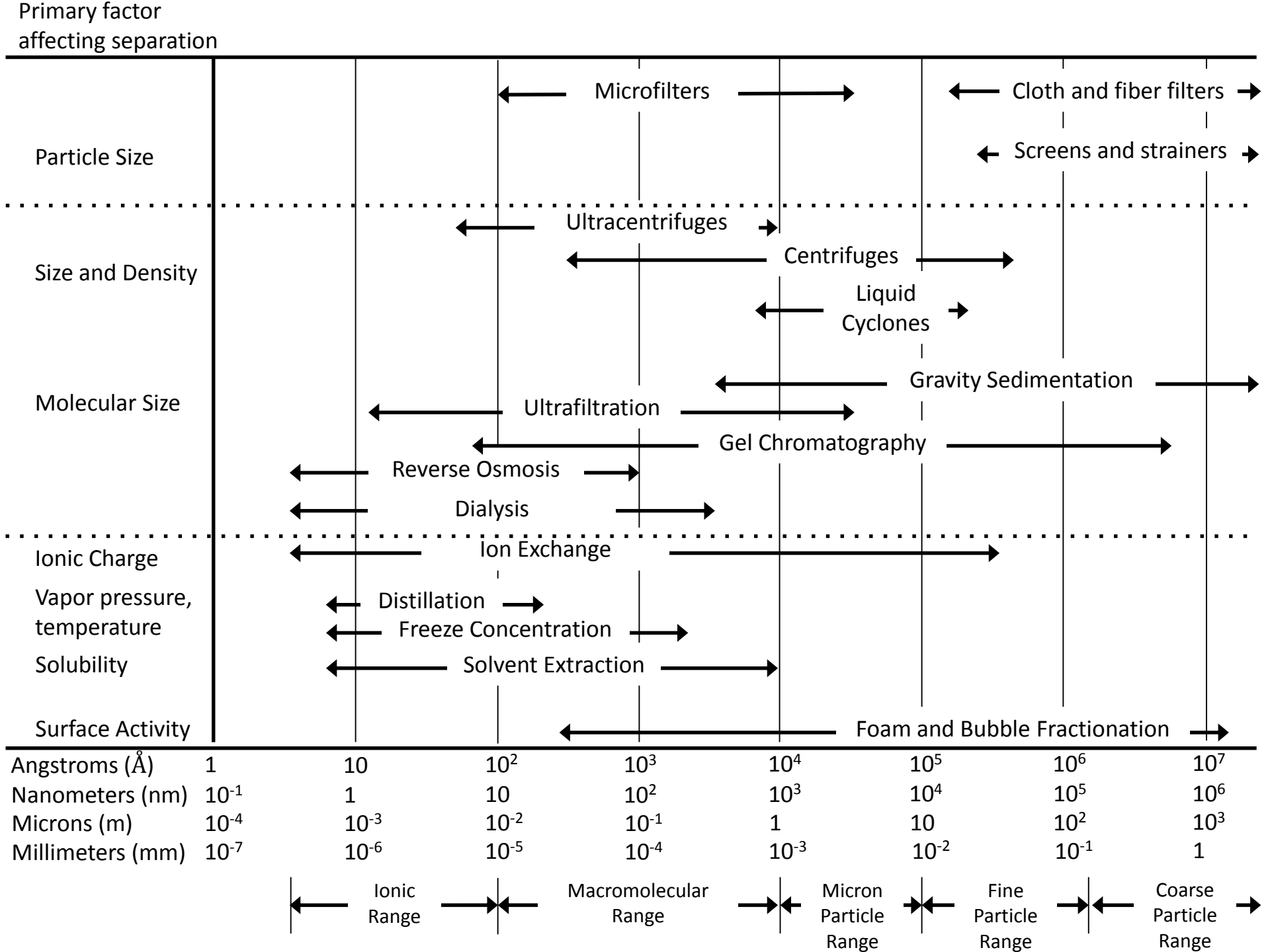
BioSeparations… It's a process



BioSeparations… It's a process

- Solid/Liquid
  - Sedimentation
  - Centrifugation
  - Filtration
- Recovery
  - Membrane
  - Precipitation
  - Extraction
- Purification
  - Crystallization
  - Chromatography

Unit Operation	Level at which separation occurs
Centrifugation	Macroscopic
Filtration	Macroscopic
Microfiltration	Macroscopic
Ultrafiltration	Molecular
Reverse Osmosis	Molecular
Crystallization	Macroscopic
Extraction	Molecular
Adsorption Desorption	Molecular Molecular
Chromatography	Molecular
Affinity Chromatography	Molecular


Primary factor affecting separation
Particle Size
Size and Density
Molecular Size
Ionic Charge
Vapor pressure, temperature
Solubility
Surface Activity
Microfilters
Cloth and fiber filters
Screens and strainers
Ultracentrifuges
Centrifuges
Liquid Cyclones
Gravity Sedimentation
Ultrafiltration
Gel Chromatography
Reverse Osmosis
Dialysis
Ion Exchange
Distillation
Freeze Concentration
Solvent Extraction
Foam and Bubble Fractionation
Angstroms (Å) 1 10 10^2 10^3 10^4 10^5 10^6 10^7
Nanometers (nm) 10^{-1} 1 10 10^2 10^3 10^4 10^5 10^6
Microns (m) 10^{-4} 10^{-3} 10^{-2} 10^{-1} 1 10 10^2 10^3
Millimeters (mm) 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 1
Ionic Range
Macromolecular Range
Micron Particle Range
Fine Particle Range
Coarse Particle Range

Sedimentation

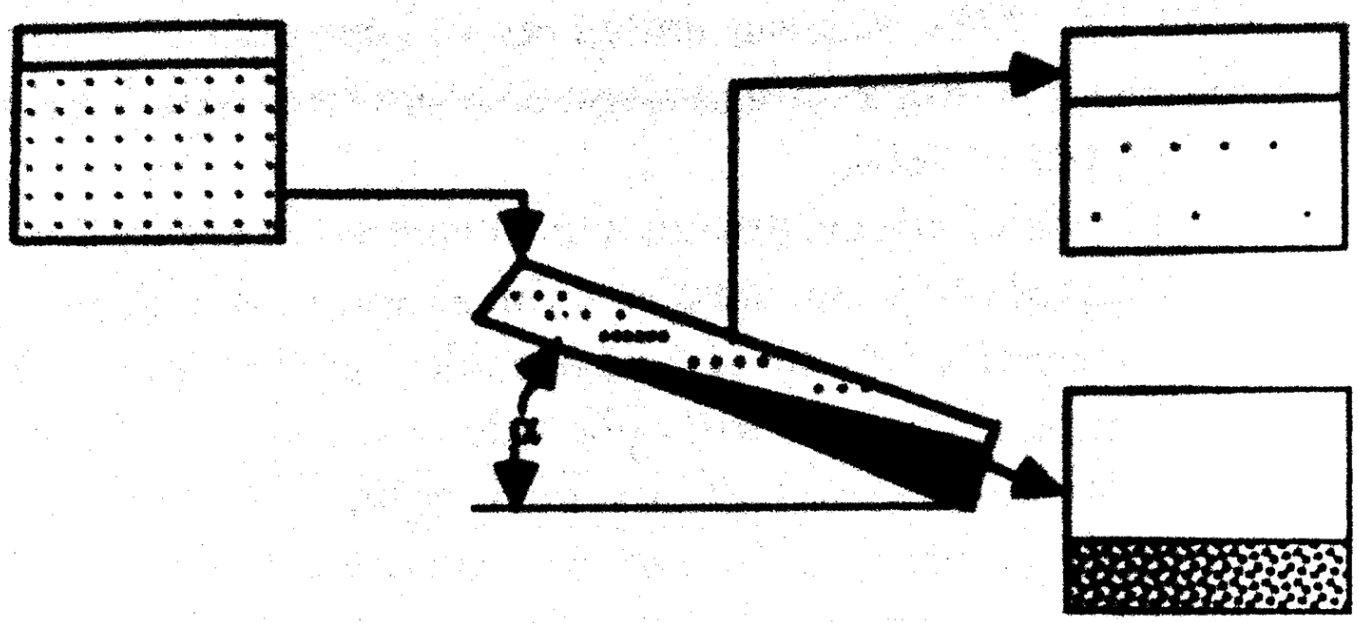
- Gravity driven separation
  - Large particle size
  - Difference in density of solid from the liquid
- Common in wastewater applications

Sedimentation

- Gravity driven separation
  - Large particle size
  - Difference in density of solid from the liquid
- Common in wastewater applications
- Also used to separate Brewers yeast
  - CO_2 created during fermentation can complicate the settling
  - Cells settle out once fermentation is finished

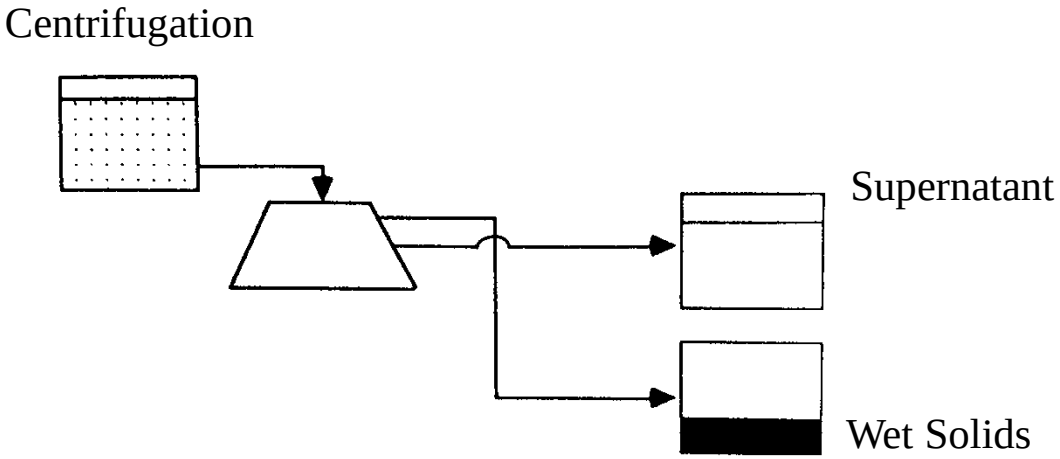
Sedimentation

- Can manipulate gravity to decrease separation time



Centrifugation

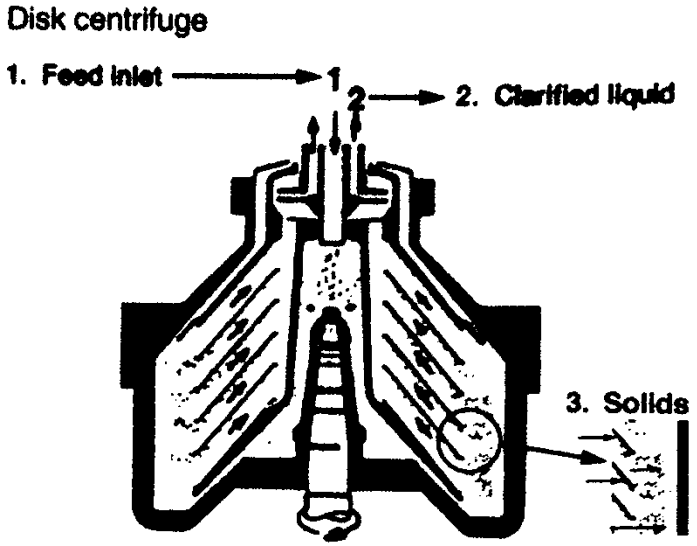
- Enhanced sedimentation
  - Multiply the force of gravity by rotating the container
  - Centrifugal force function of diameter and rotational speed
  - Results in two streams
    - Clarified liquid (supernatant)
    - Wet solids



Centrifugation

- Batch process

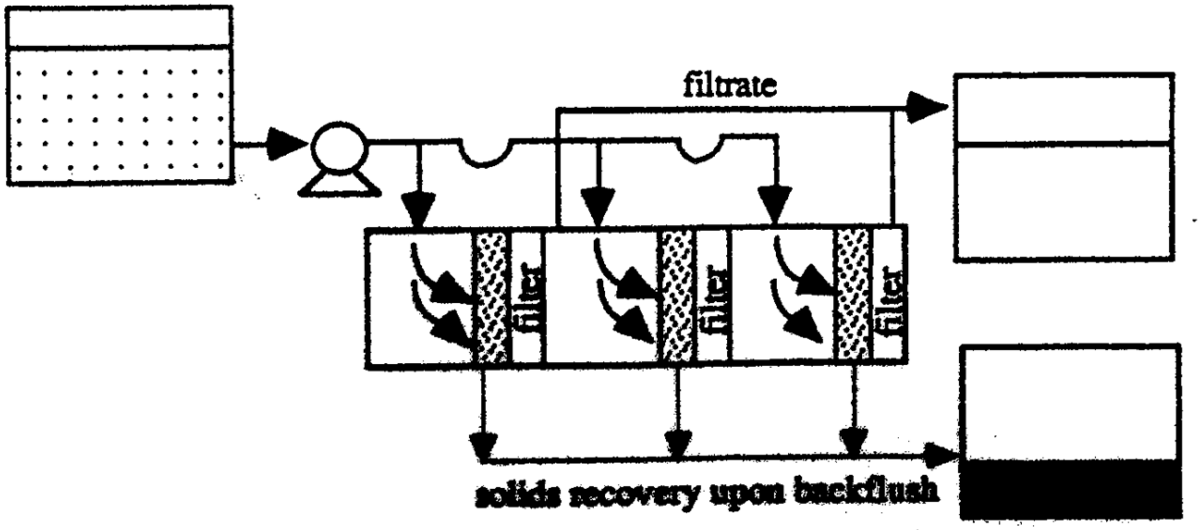
- Continuous Process



https://www.youtube.com/watch?v=zSL_-DcsjR4

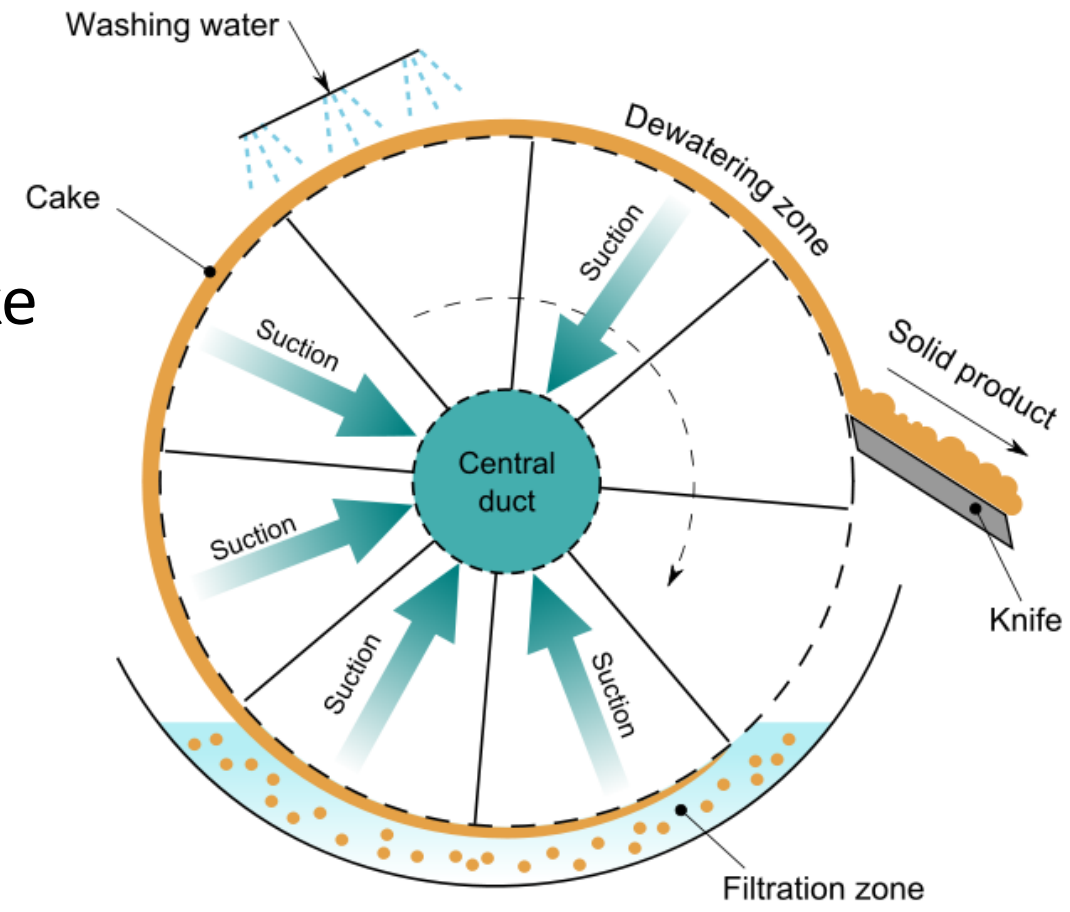
Filtration

- Used when solids are large enough to be stopped by a filter cake
  - Either pumped



Filtration

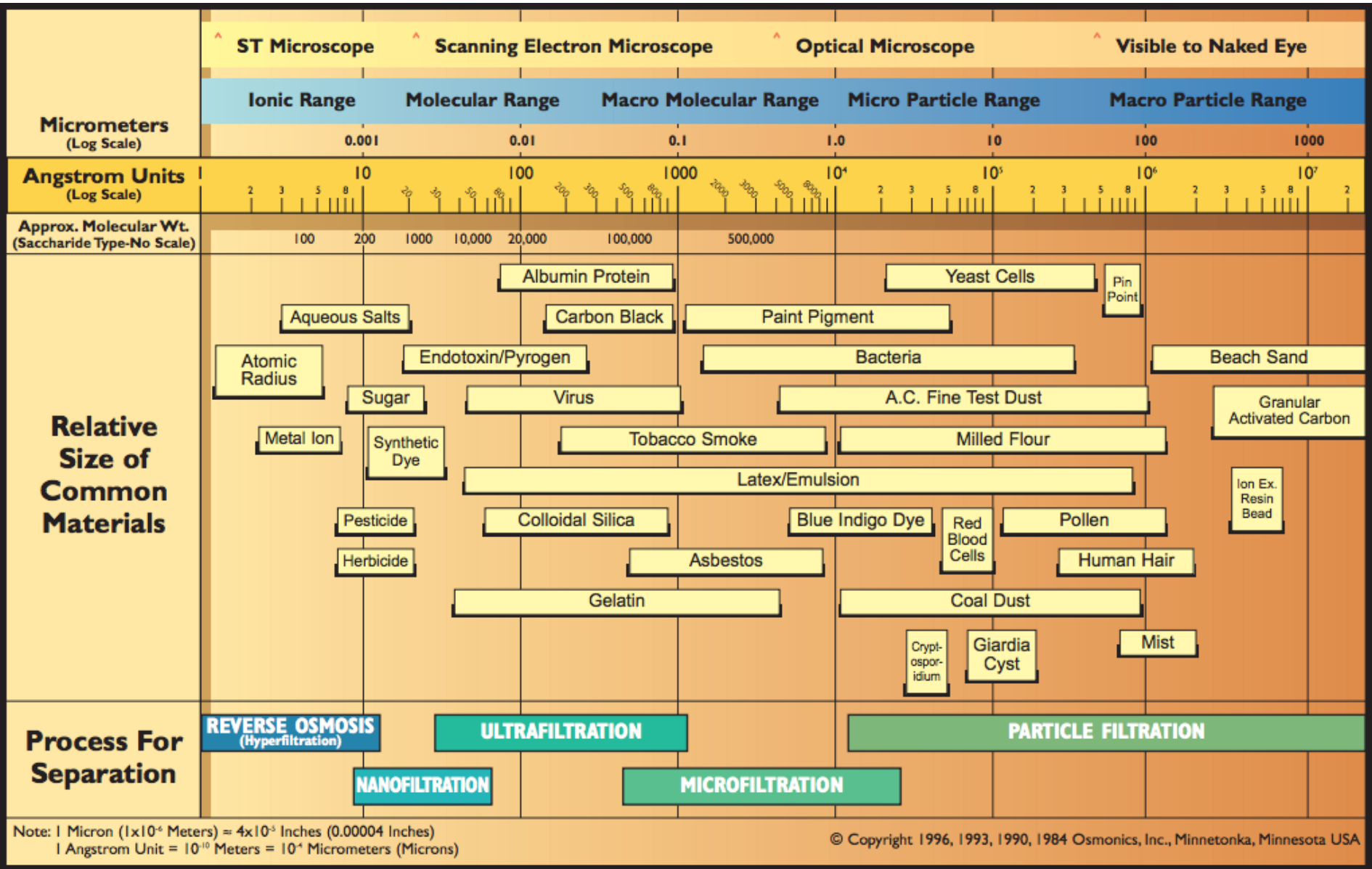
- Used when solids are large enough to be stopped by a filter cake
  - Either pumped or pulled through



Membrane Separations

- Membranes enable filtration of
  - Colloids
  - Cells
  - Molecules
- By means of
  - Microfiltration – remove microorganisms to a 0.45 or 0.22 μm cut off
  - Ultrafiltration – cut off of 5 to 5000nm
  - Reverse Osmosis – semipermeable with a pore size of 0.5 to 5 nm

Membranes

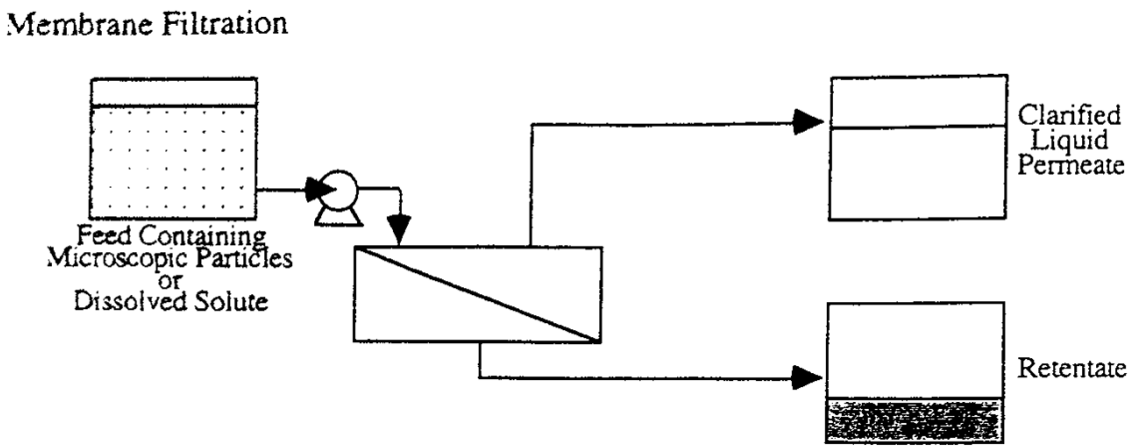

ST Microscope
Scanning Electron Microscope
Optical Microscope
Visible to Naked Eye
Ionic Range
Molecular Range
Macro Molecular Range
Micro Particle Range
Macro Particle Range
Micrometers (Log Scale)
0.001
0.01
0.1
1.0
10
100
1000
Angstrom Units (Log Scale)
10
100
1000
10⁴
10⁵
10⁶
10⁷
Approx. Molecular Wt. (Saccharide Type-No Scale)
100
200
1000
10,000
20,000
100,000
500,000
Relative Size of Common Materials
Albumin Protein
Yeast Cells
Pin Point
Aqueous Salts
Carbon Black
Paint Pigment
Atomic Radius
Endotoxin/Pyrogen
Bacteria
Beach Sand
Sugar
Virus
A.C. Fine Test Dust
Granular Activated Carbon
Metal Ion
Synthetic Dye
Tobacco Smoke
Milled Flour
Latex/Emulsion
Ion Ex. Resin Bead
Pesticide
Colloidal Silica
Blue Indigo Dye
Red Blood Cells
Pollen
Herbicide
Asbestos
Human Hair
Gelatin
Coal Dust
Crypt-ospor-idium
Giardia Cyst
Mist
Process For Separation
REVERSE OSMOSIS (Hyperfiltration)
ULTRAFILTRATION
PARTICLE FILTRATION
NANOFILTRATION
MICROFILTRATION
Note: 1 Micron (1x10⁻⁶ Meters) = 4x10⁻⁵ Inches (0.00004 Inches)
1 Angstrom Unit = 10⁻¹⁰ Meters = 10⁻⁴ Micrometers (Microns)
© Copyright 1996, 1993, 1990, 1984 Osmonics, Inc., Minnetonka, Minnesota USA

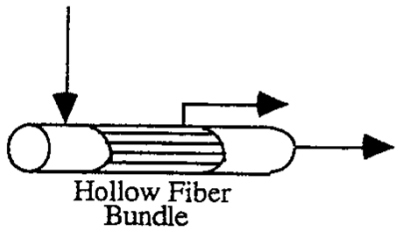
Membranes

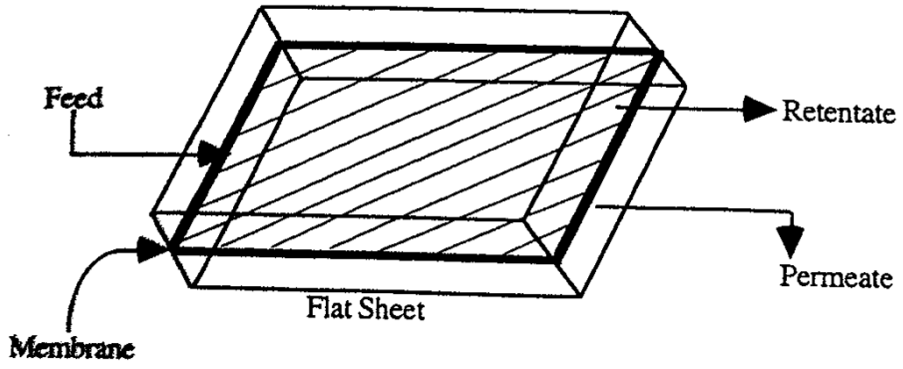

Water
Monovalent ions
Multivalent ions
Viruses
Bacteria
Suspended solids
Microfiltration
Ultrafiltration
Nanofiltration
Reverse osmosis
www.zena-membranes.cz

Microfiltration

- Organic or inorganic membranes
  - Pores 0.1 to 10 microns
- Membrane structure
  - Flat, pleated sheet
  - Hollow fiber

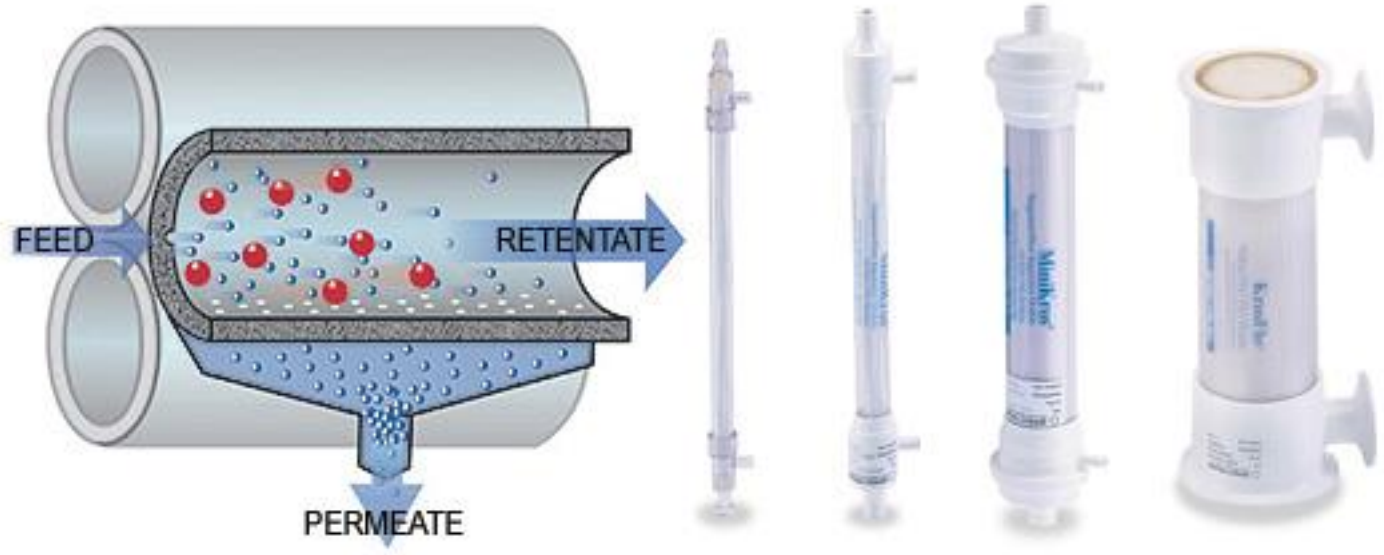




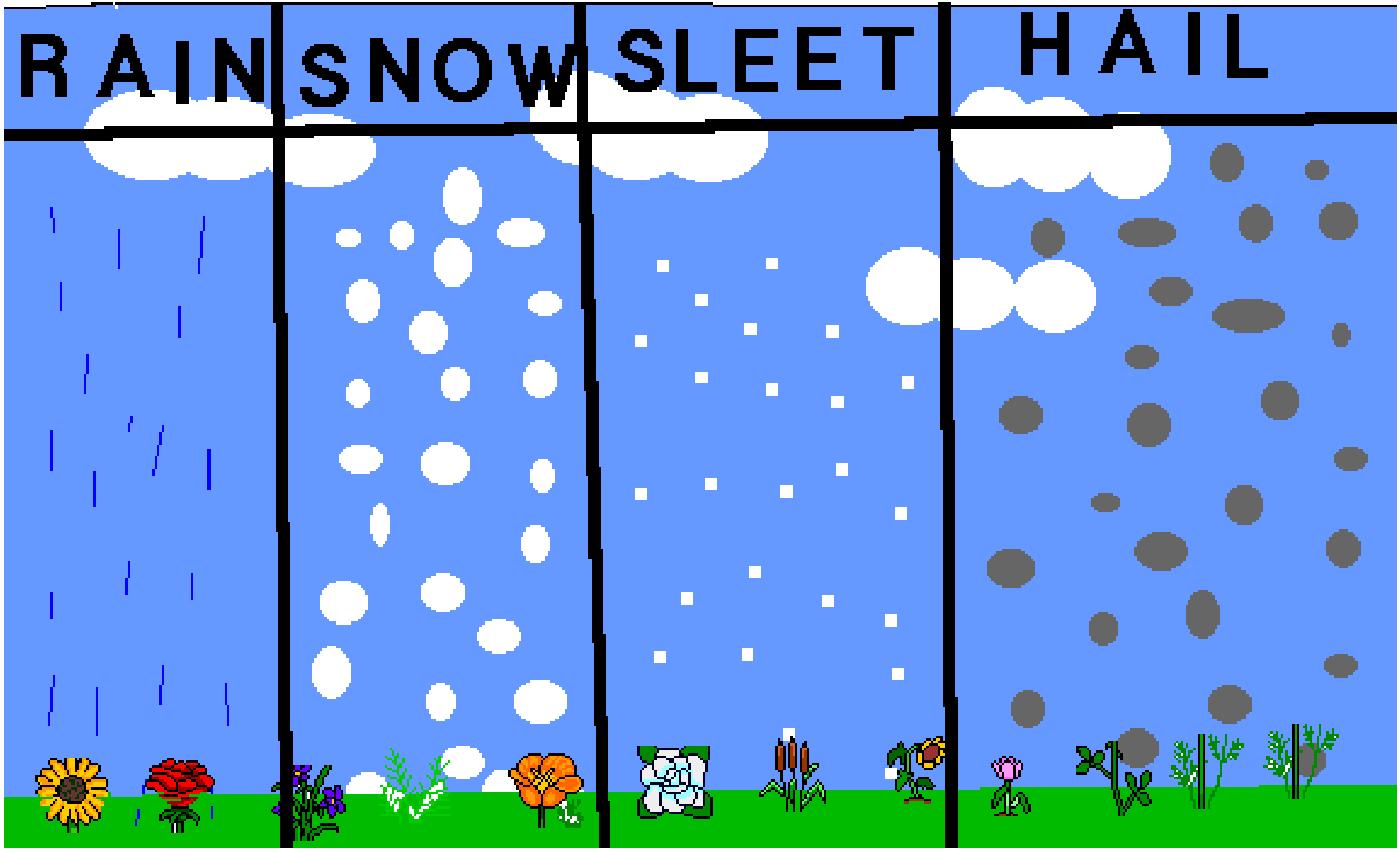


Microfiltration

- Membrane structure
  - Hollow fiber
    - Individual fibers are 1 to 2 mm in diameter
      - Bundled together for more surface area



Precipitation

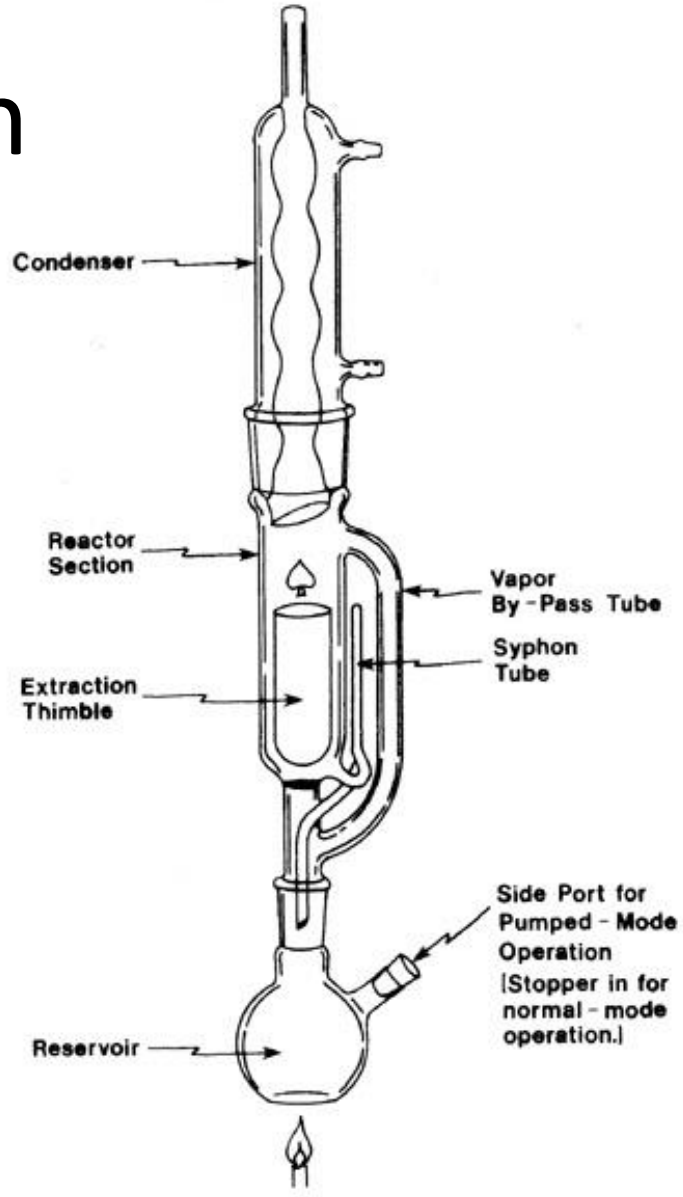


The other kind of precipitation

- Salting-out
  - Hydrophobic
    - Decreases the solubility of the solute in solution
- Salting-in
  - Electrostatic
    - Increase solubility with increasing salt
- Example: Proteins in milk

Extraction

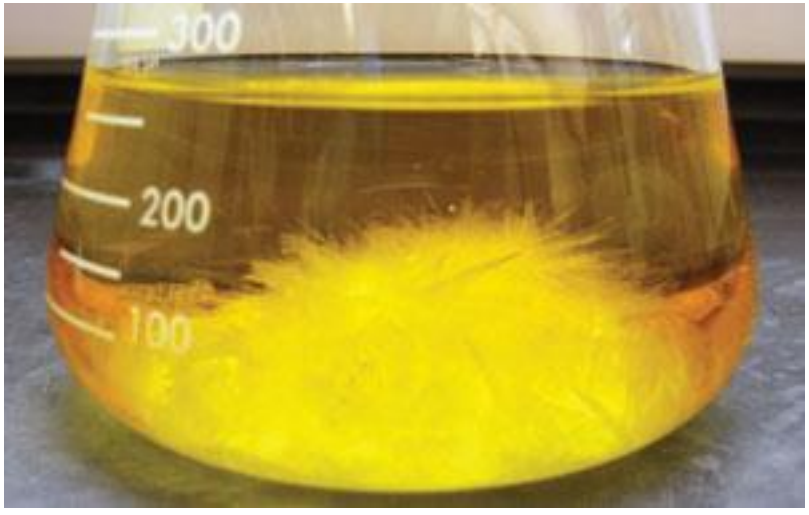
- Uses solubility differences of biological molecules between
  - Two different liquid phases
  - A solid and a liquid



Soxhlet Extractor

Crystallization

- Nucleation process that occurs in a concentrated solution
- Nucleation occurs when
  - The solute concentration exceeds the saturation AND
  - Solute molecules are present in large enough clusters to serve as nucleation sites



Supersaturation

- For crystals to form, the solution must be super saturated
- How to get a super-saturated solution?
  - Cool a solution
    - Works if the solubility of the salt in the solution decreases with temperature
  - Evaporate a portion of the solution
  - Add a third component
    - Salting-out

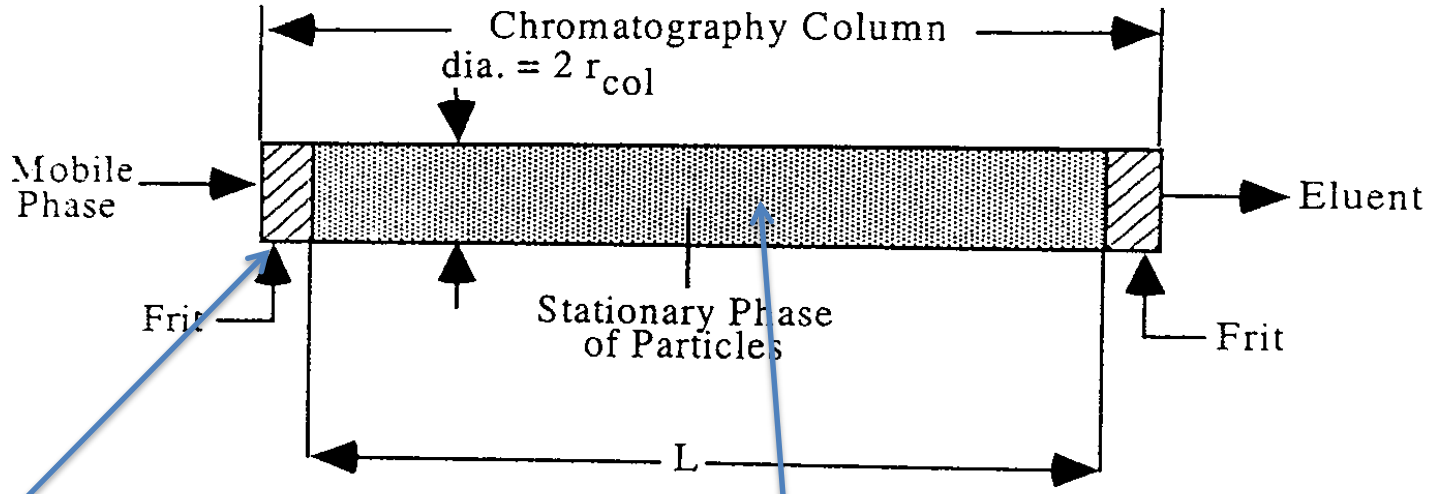
Chromatography

- Partitioning effects based on
  - Differential binding
  - Molecular sieving
- Partitioning of the target molecule relative to other molecules in the solution
- Stationary phase binding
  - Ion exchange
  - Reversed and normal phase
  - Hydrophobic interaction
  - affinity

Purification Types

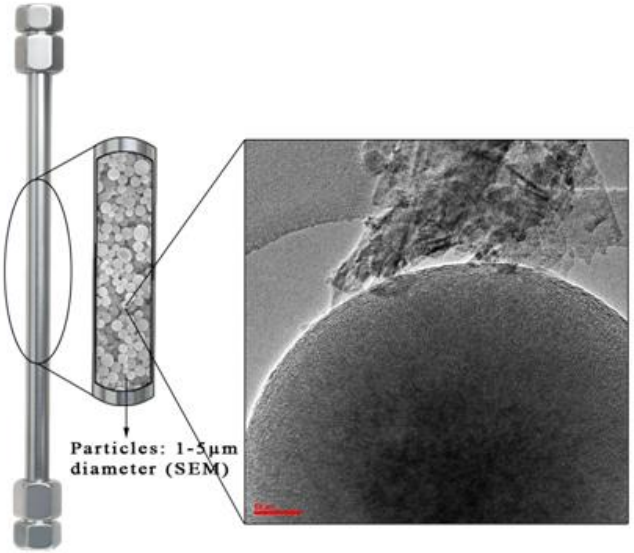
- Stationary phase binding
  - Ion exchange
  - Reversed and normal phase
  - Hydrophobic interaction
  - Affinity
- Size Exclusion
  - Gel permeation
    - Partitions with a sieving effect
      - Small molecules penetrate the stationary phase
      - Larger molecules are excluded and elute first

Chromatography Nomenclature



Frits are sintered metal or polymeric filters with particle cut-off from 2 to 20 microns

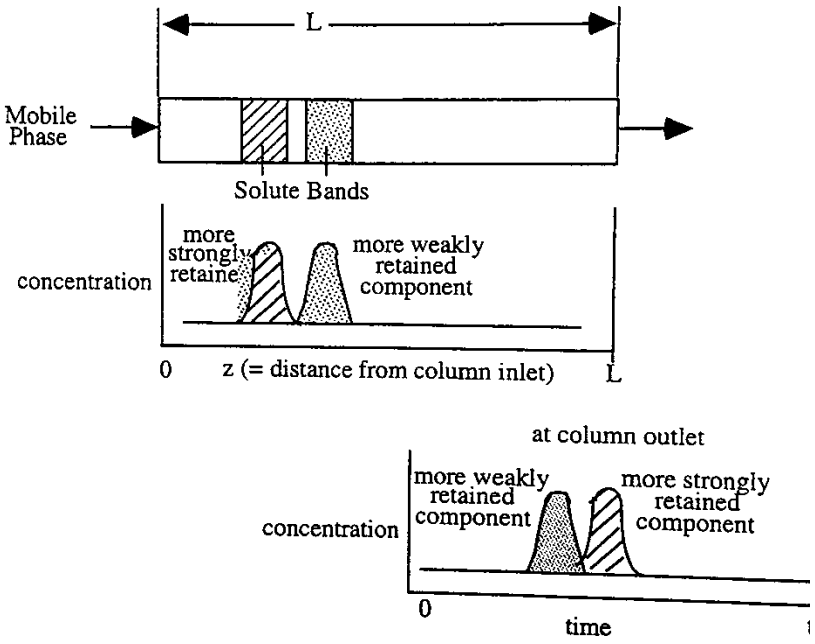
Column is packed with spherical particles (5 – 300 micron)



Chromatography Nomenclature

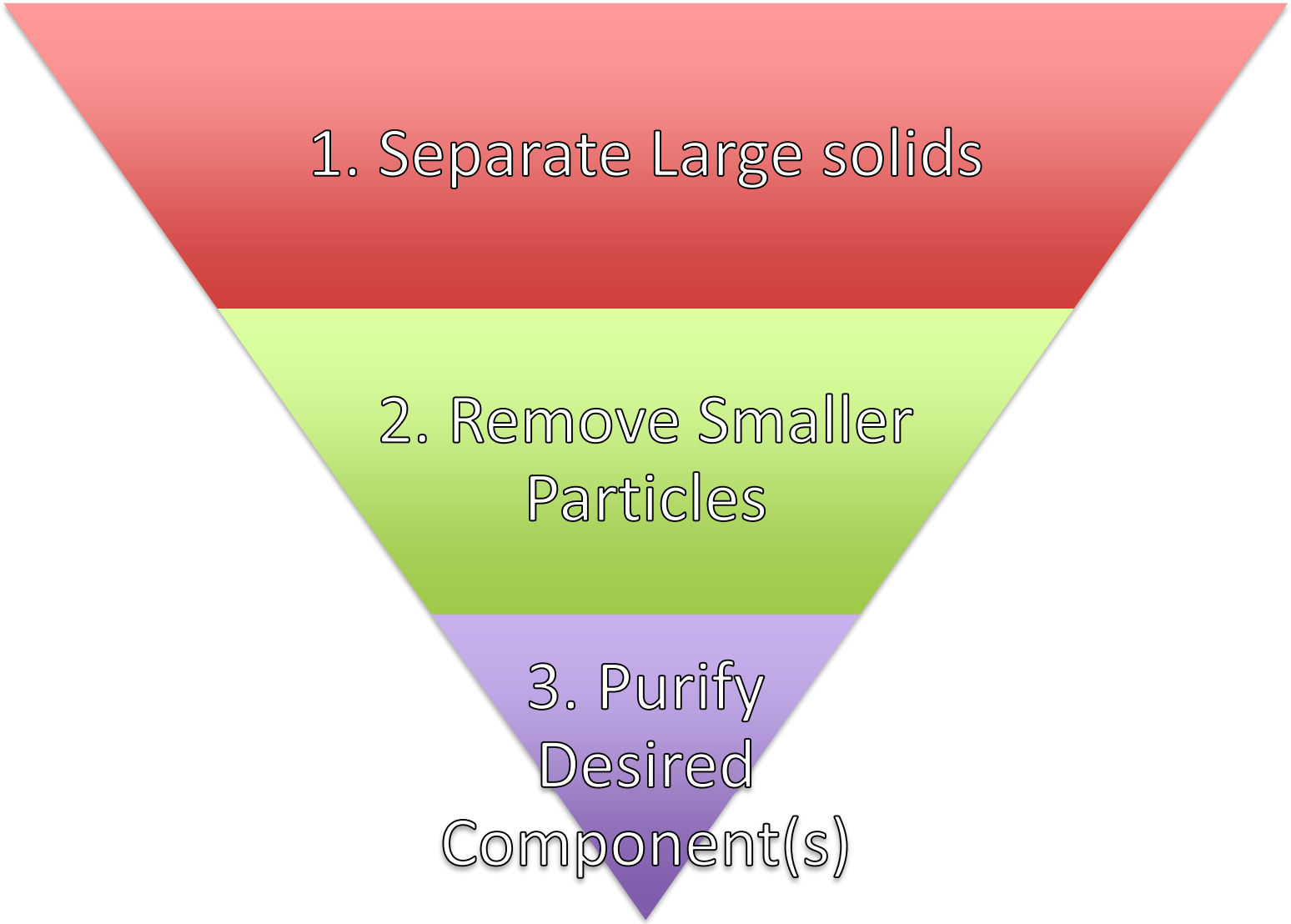
Analytical scale are operated either incline, vertical or horizontal

Process scale columns are usually vertical



Less strongly retained solute elutes before the more strongly retained solute.

BioSeparations… It's a process

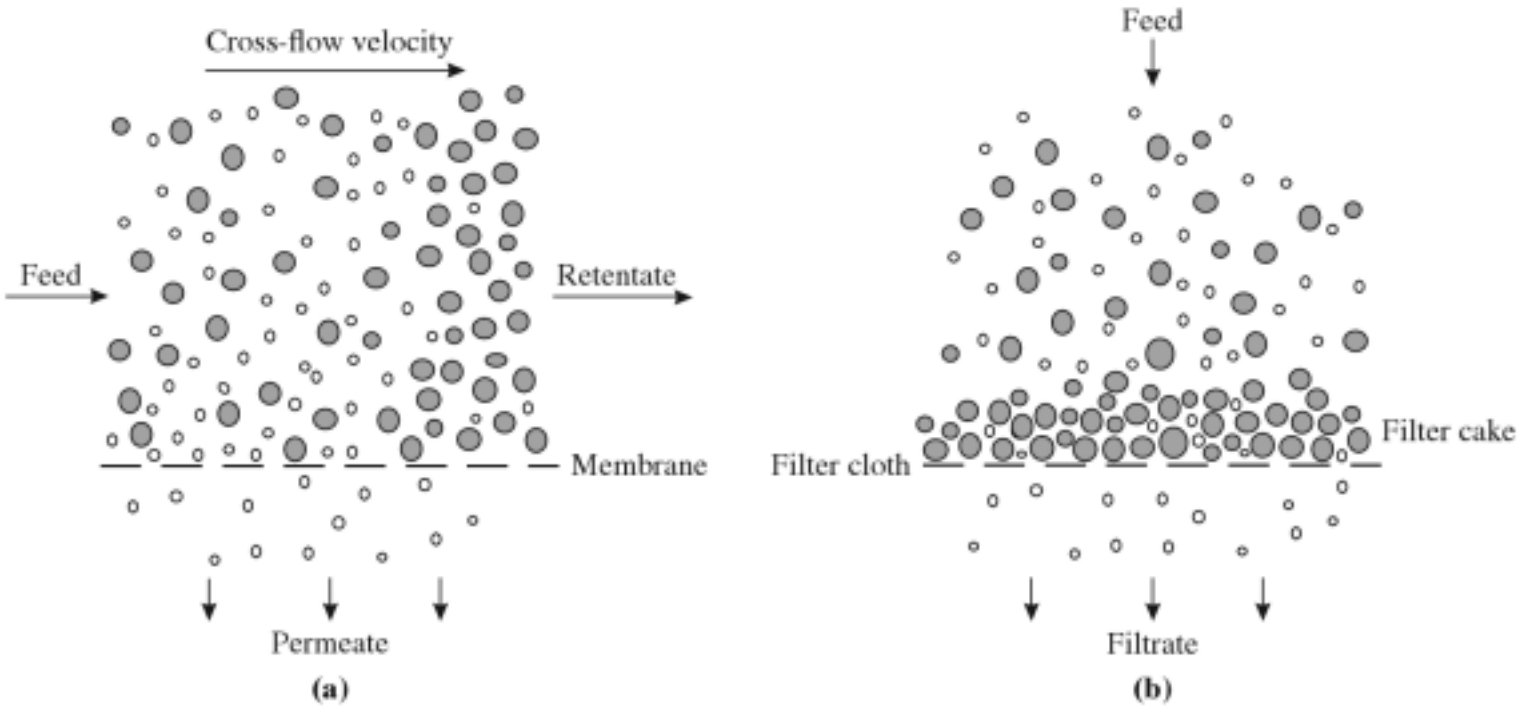


Xanthan Gum purification and concentration

BIOSEPARATIONS LAB

Read through Chapter 11 in *Bioprocess Engineering Principles* for additional help

Types of Membrane Filtration

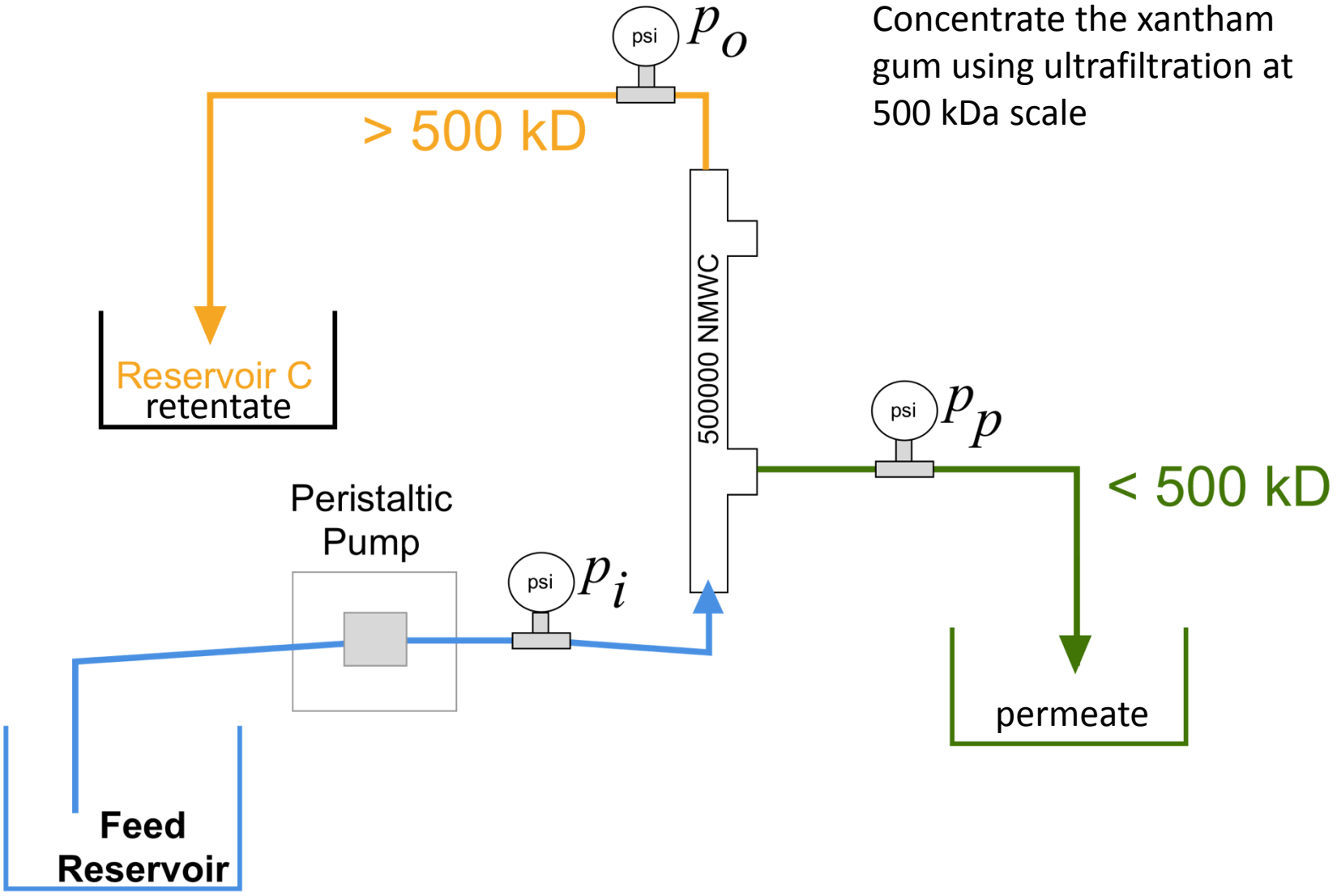


Cross-flow

Conventional dead end

Figure 11.23 Doran

Process for Lab



Concentrate the xantham gum using ultrafiltration at 500 kDa scale

Phenomena to Explore

- Retention coefficient $$R = \frac{C_R - C_P}{C_R}$$
- Permeate Flux $$J = \frac{F_p}{A}$$
- Mass Transfer Coefficient $$J = k \ln \frac{C_F}{C_B}$$

Control Regime

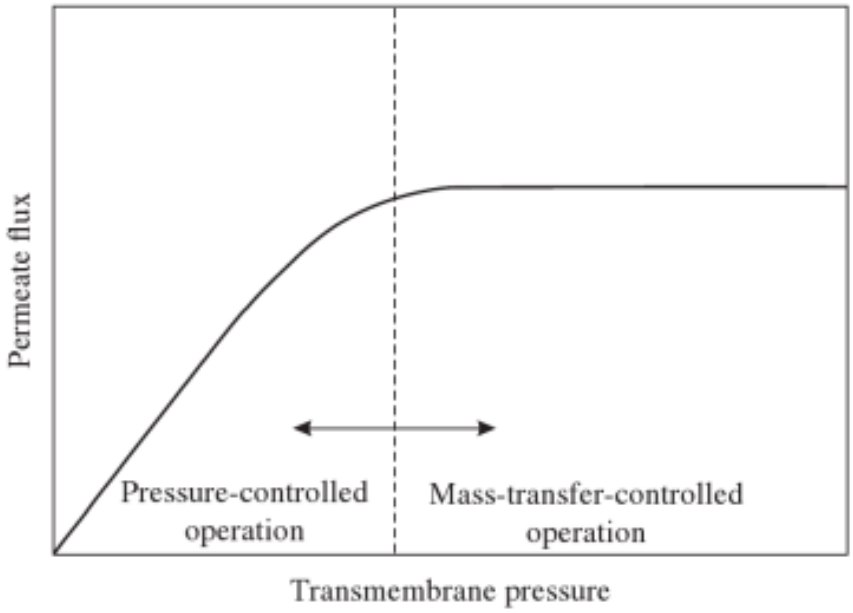


Figure 11.2 - Doran

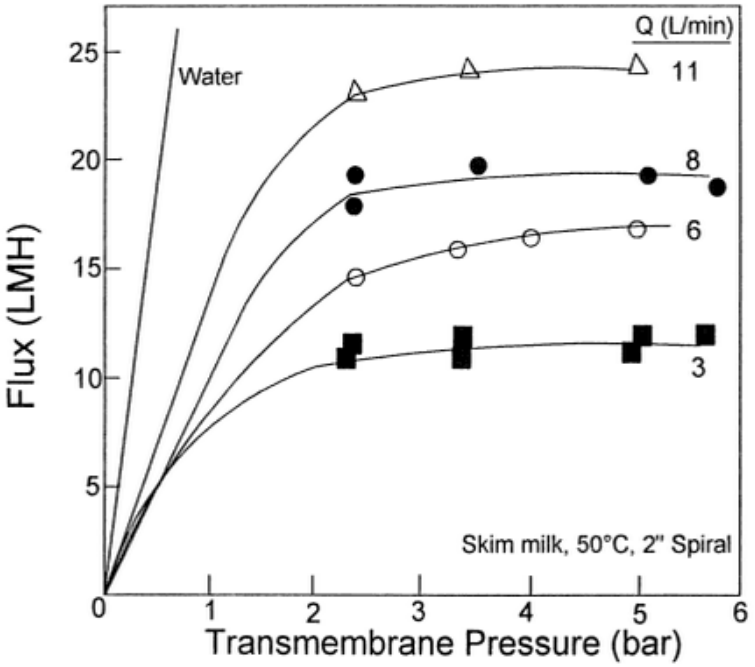


Figure 4.11 - Cheryan, 1998
Ultrafiltration and Microfiltration Handbook

Use your data to determine if filtration is pressure-controlled or mass-transfer controlled.

Similar to Figure 11.30 - Doran

Basic Lab Procedures

- Calibrate the spectrophotometer
- Calibrate the peristaltic pump
- Test 4 flow rates at 5 transmembrane pressures (20 pairs of conditions)
- At each setting, collect samples from the permeate and retentate (40 samples total)
- Determine xanthan concentrations using spectrophotometer