Recovery and Purification of Products

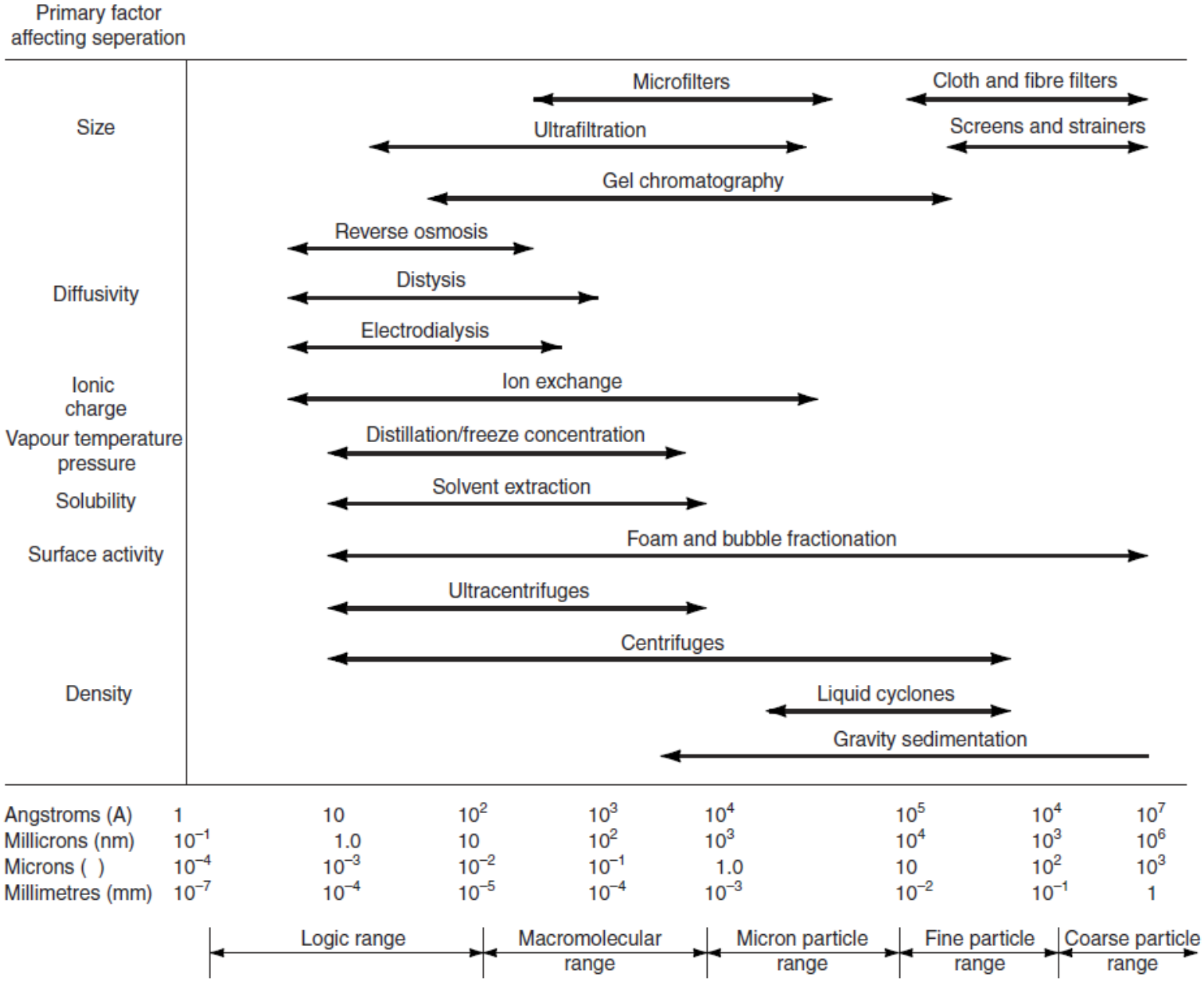
Necessity of Downstream Processing:

- The recovery and purification of product is essential to any commercial process.
- Purity depends on the nature of the product.
- Different products are: biomass, extra or intracellular components
- Since the interested compound is very heat sensitive, traditional separation techniques must be augmented with a more specialized one.
- Recovery and purification comprise over 50% of the total manufacturing cost.

Necessity of Downstream Processing:

- In the chemical and biochemical industries, downstream processing deals with different unit processes.
- It is crucial for the biochemical industry because the cost of chemicals depends on the purity of products. filtration, centrifugation, sedimentation
- It has been observed that the downstream processing cost is inversely proportional to the concentration of products

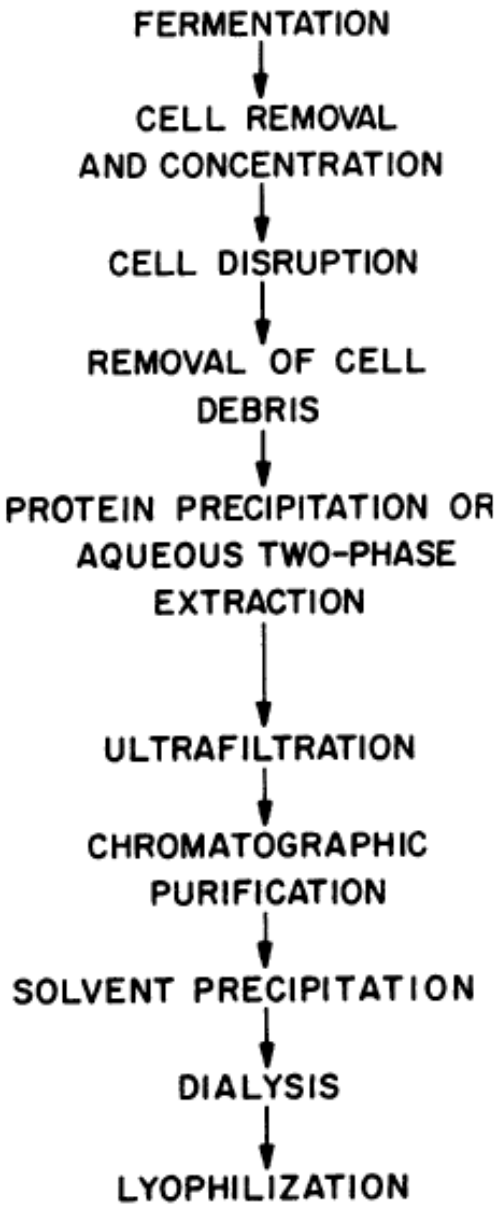
Major unit operations: (depending on size and nature of product)



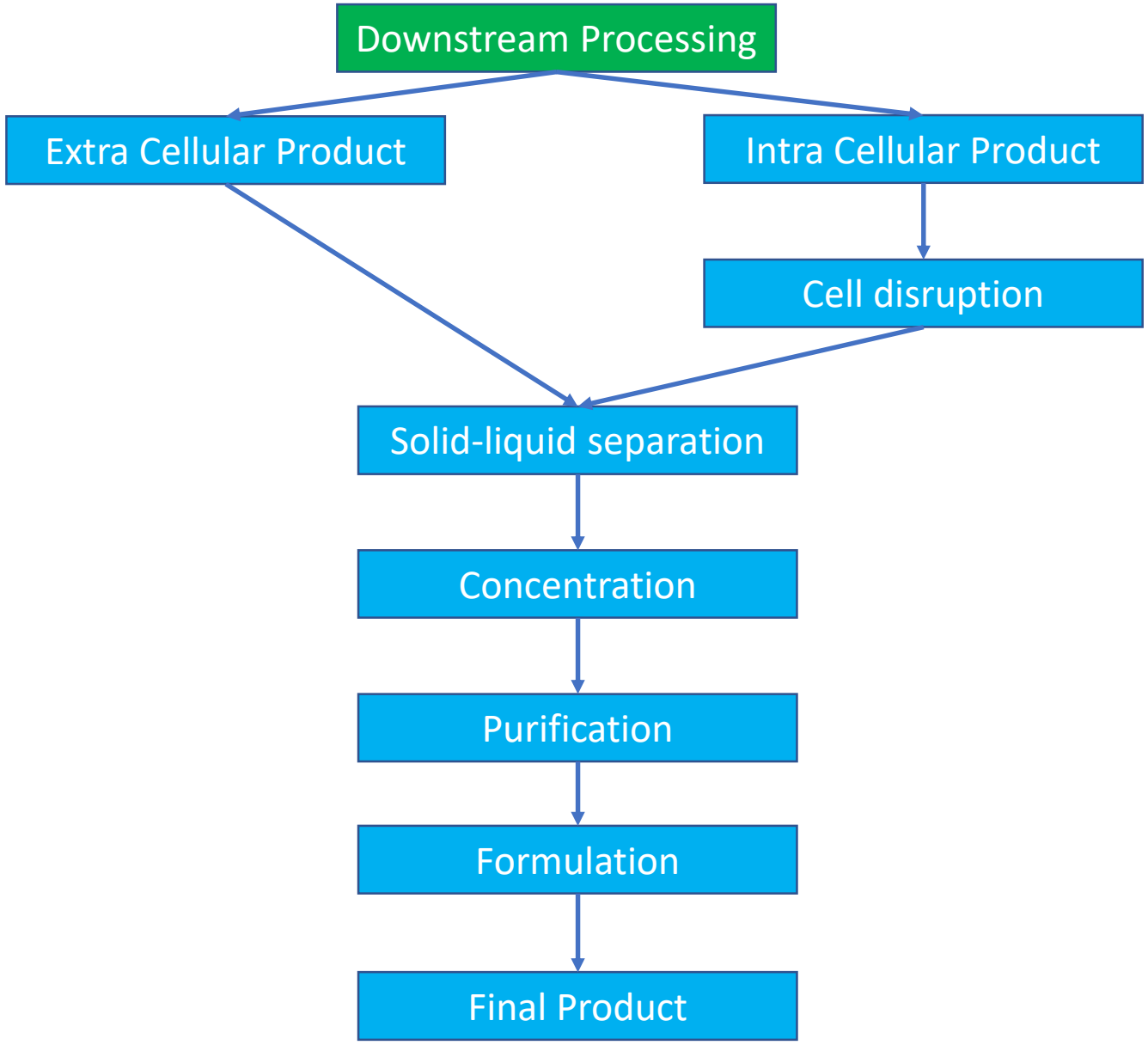
Basis of Separation in Bioprocess:

- **Size**, e.g., centrifugation, filtration, membrane separation
- **Density**, e.g., sedimentation, centrifugation, floatation
- **Shape**, e.g., filtration, centrifugation, sedimentation
- **Polarity**, e.g., chromatography, extraction, adsorption
- **Solubility**, e.g., precipitation, extraction, crystallization
- **Electrostatic charge**, e.g., electrophoresis, adsorption, membrane separation
- **Diffusivity**, e.g., membrane separation
- **Volatility**, e.g., pervaporation, distillation, membrane distillation

Major steps involved in the separation and purification of intracellular enzymes:



Bioprocess overview/ Classification of downstream processes:



Chemical/Enzymatic/Mechanical/Physical

Centrifugation/Sedimentation/Extraction/Filtration

Evaporation/Ultrafiltration/Adsorption/Precipitation

Chromatography

Crystallization/Freeze Drying/ Sterile Filtration

Division of major steps involved:

(1) separation of insoluble products and other solids,

(2) primary isolation or concentration of product and removal of most of the water,

(water must be removed very early in the process train so that the size of the equipment

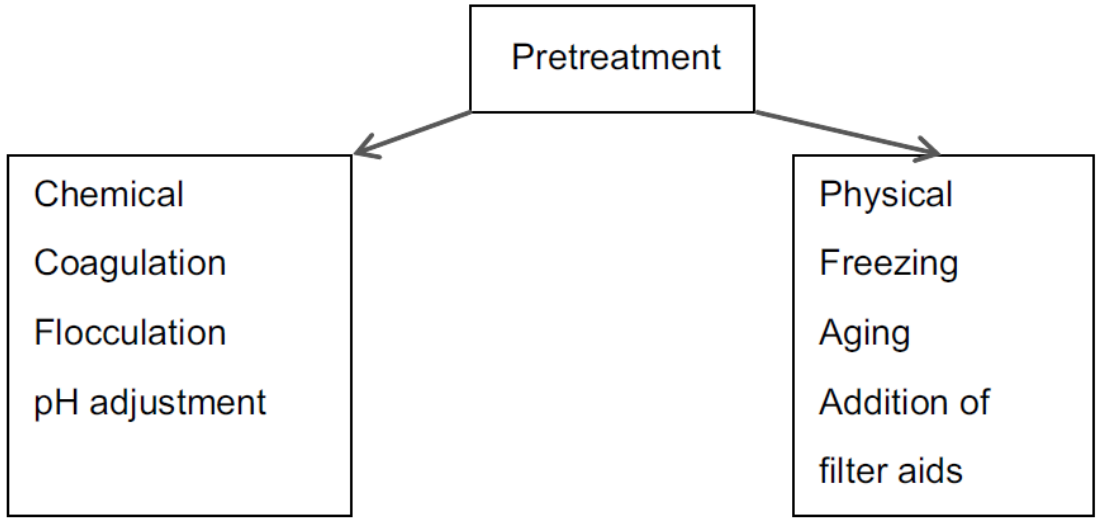
in the following steps will be minimized)

(3) purification or removal of contaminating chemicals, and

(4) product preparation, such as drying.

Pre-treatment:

- Pre-treatment is used primarily with difficult to filter slurries enabling them to filtered more easily.
- Pretreatment is carried out to facilitate separation process
- It usually involves **changing the nature of suspended solids** by either chemical or physical means or by adding solid to the suspension



Pre-treatment:

- Coagulation and flocculation are usually used to form cell aggregates before centrifugation, gravity settling, or filtration to improve the performance of these separation processes.
- **Coagulation** is the formation of small flocs from dispersed colloids using coagulating agents, which are usually simple electrolytes.
- **Flocculation** is the agglomeration of these small flocs into larger settleable particles using flocculating agents, which are usually polyelectrolytes or certain salts, such as $CaCl_2$.

Separation of Insoluble Products:

- Separation of solids such as biomass, insoluble particles, and macromolecules from the fermentation broth is usually the first step in product recovery.

- **The major methods used for the separation of cellular material (biomass) are:**

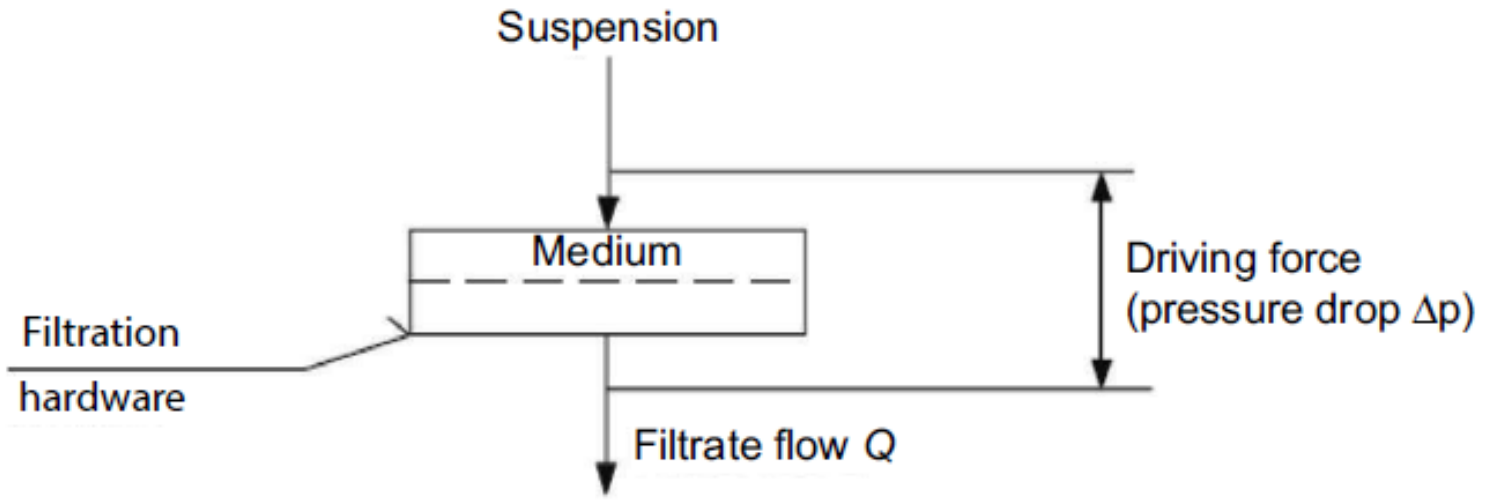
(1) filtration (both rotary vacuum filtration and micro- or ultrafiltration),

(2) centrifugation, and

(3) coagulation and flocculation.

Filtration:

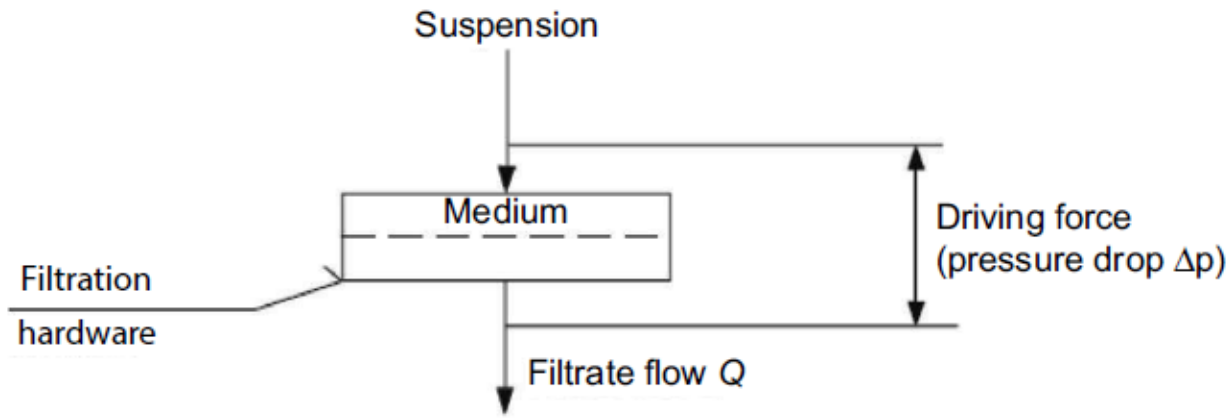
- **A process of separation of solids from a fluid by passing the same through a porous medium that retains the solids but allows the fluid to pass through.**
- When solids are present in very low concentration, i.e. not exceeding 1.0 % w/v the process of its separation from liquid is called clarification.



Filtration:

- **A process of separation of solids from a fluid by passing the same through a porous medium that retains the solids but allows the fluid to pass through.**

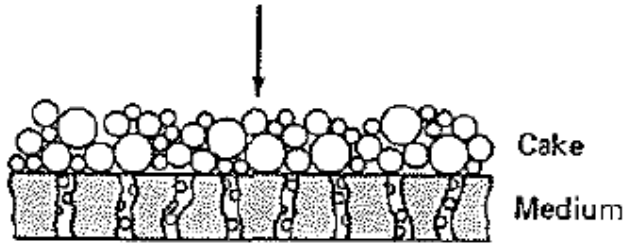
- **Slurry:** Suspension to be filtered.
- **Filter medium:** Porous medium used to retain solid
- **Filter cake:** Accumulated solids on the filter
- **Filtrate:** Clear liquid passing through the filter



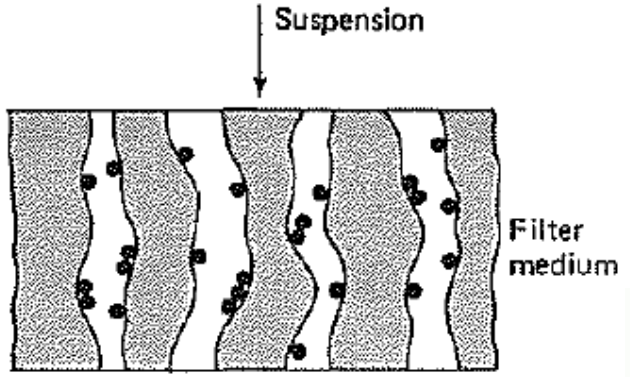
Filter medium:

- The function of filter medium is primarily to act as an **impermeable barrier for particulate matter**.
- The filter medium should have sufficient mechanical strength, should be resistant to the corrosive action of fluids being processed and should offer low resistance to the flow of filtrate.
- The liquid-solid filtration is often called **cake filtration** because of the continuous decomposition of cake on the filter medium.
- **Commonly used filter mediums:** Filter paper, woven material. Non-woven fibre pads, sintered and perforated glass, sintered and perforated material, ceramics, synthetic membrane

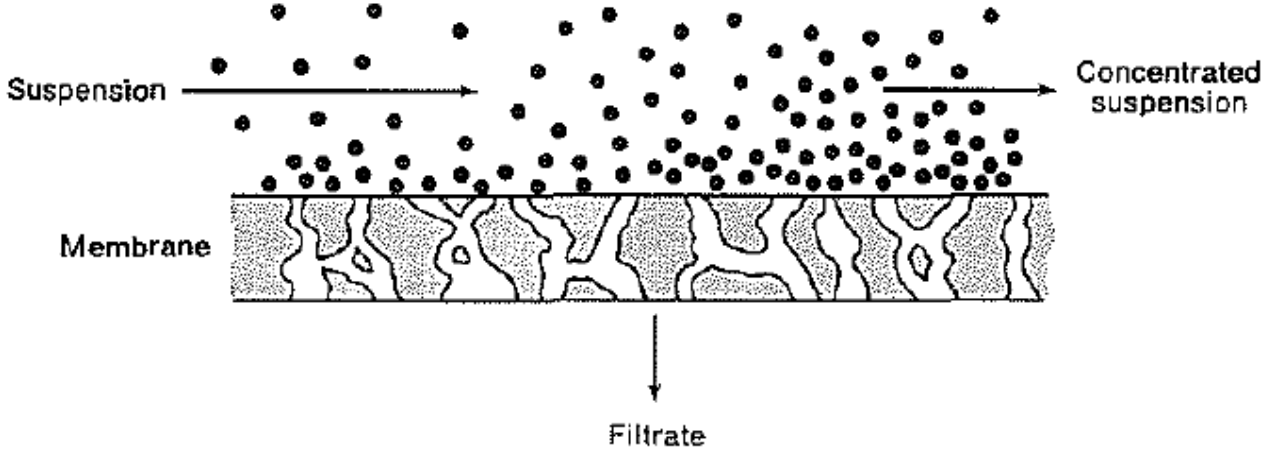
Types of filtration:



Cake filtration



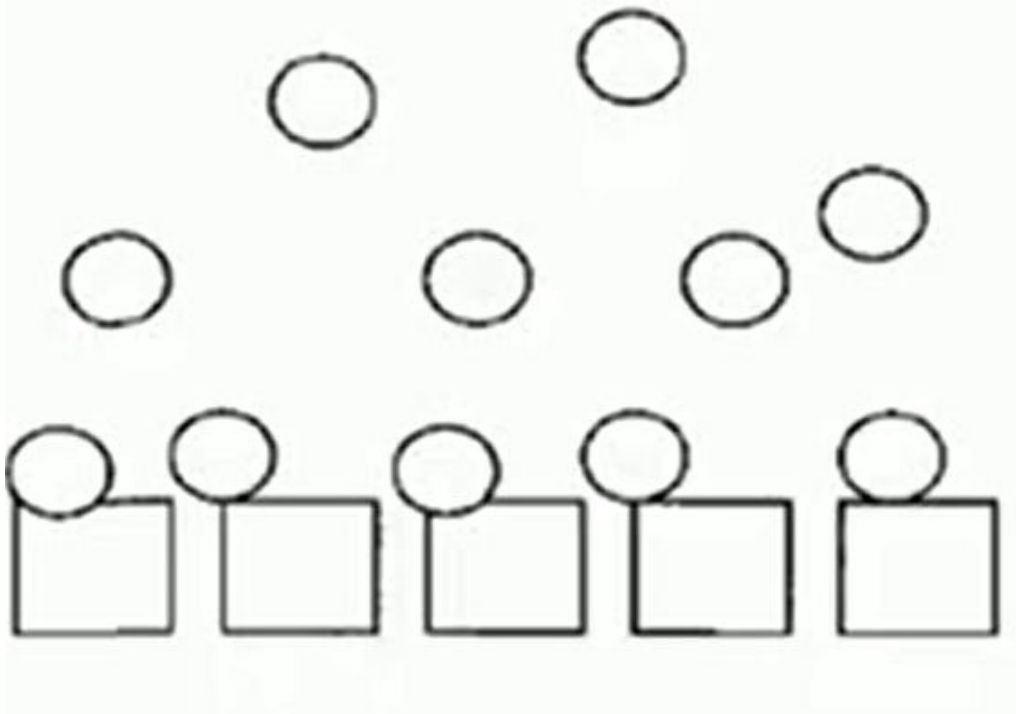
Clarification



Cross-flow filtration

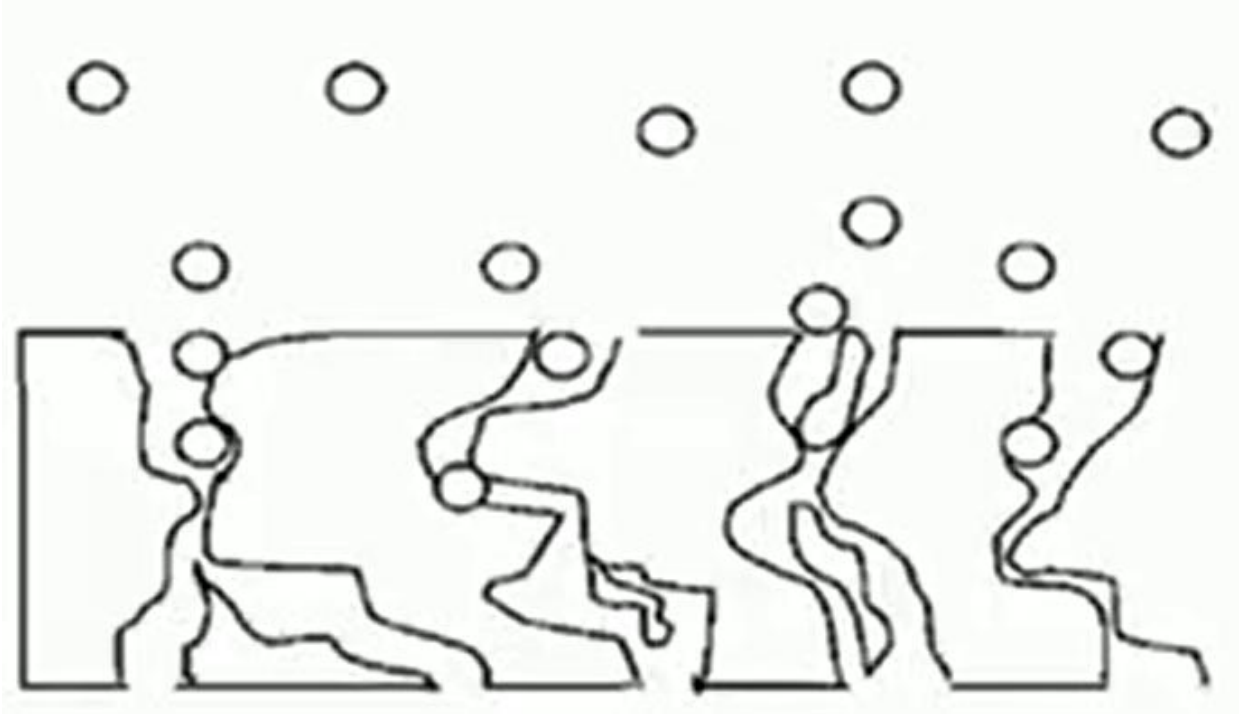
Mechanism of filtration:

- **Surface filtration:** Particles are not allowed to enter filtration medium.
- Cake filtration and particle filtration are based on surface filtration



Mechanism of filtration:

- **Depth filtration:** Particles are not allowed to penetrate the pores and pore networks present in the filtration medium.
- They are retained within the filter by three mechanisms, direct interception, inertial impaction and diffusional interception.



Constant pressure cake filtration:

- A filtration process where the driving force (i.e. the pressure drop across the filter medium) is kept constant.
- leading to a decreasing filtration rate as the filter cake builds up and increases resistance
- The rate of filtration (the flow of filtrate) for a constant-pressure (vacuum) filtration operation is determined primarily by the resistance of the cake and filter medium:

$$\frac{dV}{dt} = \frac{g_c \Delta p A}{(r_m + r_c)\mu} \qquad \textit{eq. A}$$

  - where V is the volume of filtrate,
  - A is the surface area of the filter,
  - Δp is the pressure drop
  - through the cake and filter medium,
  - μ is the viscosity of the filtrate,
  - r_m is the resistance of the filter medium (characteristic of filter medium)
  - rc is the resistance of the cake (increases during filtration)

Constant pressure cake filtration:

- The value of r_c is given by

$$r_c = \alpha \frac{W}{A} = \alpha \frac{CV}{A}$$

  - where W is the total weight of the cake on filter,
  - C is the weight of the cake deposited per unit volume of filtrate, and
  - α is the average specific resistance of the cake.

- eq. A becomes

$$\frac{d(V/A)}{dt} = \frac{g_c \Delta p}{\left(r_m + \alpha \frac{CV}{A}\right)\mu}$$

- Integration of the above eq. from V = 0 to V = V and t = 0 to t = t yields

$$V^2 + 2VV_0 = Kt \qquad \frac{t}{V} = \frac{1}{K}(V + 2V_0)$$

- Where

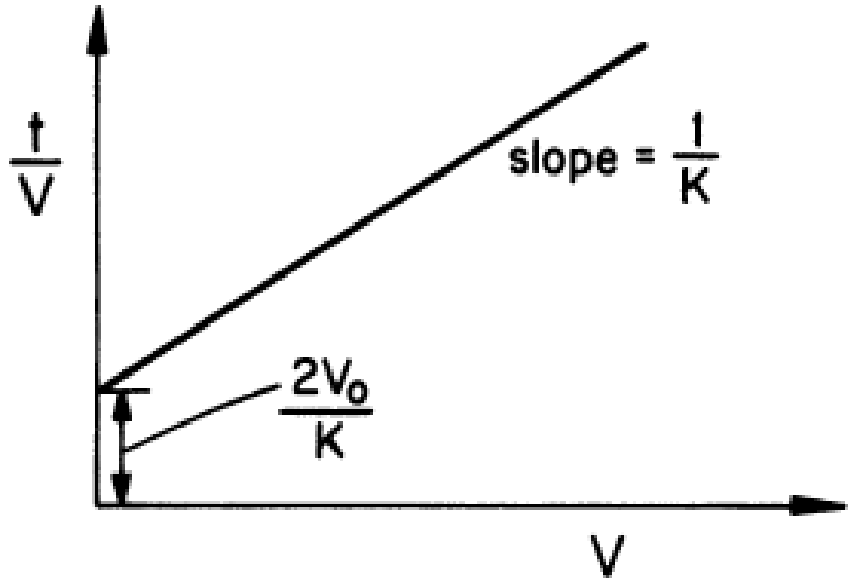
$$V_0 = \frac{r_m}{\alpha C} A \qquad K = \left(\frac{2A^2}{\alpha C \mu}\right) \Delta p \cdot g_c$$

Constant pressure cake filtration:

- Ruth equation for constant-pressure filtration

$$\frac{t}{V} = \frac{1}{K}(V + 2V_0)$$

- A plot of t/V versus V yields a straight line with a slope of 1/K and intercept of $2V_0/K$,
- The values for rm and α are calculated from experimentally determined values of K and V_0.



Problem statement: Constant pressure cake filtration

- The following data were obtained in a constant-pressure filtration unit for filtration of a yeast suspension.

t (min)	4	20	48	76	120
V (l filtrate)	115	365	680	850	1130

- Characteristics of the filter are as follows:

$A = 0.28\ m^2$, $C = 1920\ kg/m^3$, $\mu = 2.9 * 10^{-3}\ kg/m\text{-}s$, $\alpha = 4\ m/kg$

a. Determine the pressure drop across the filter.

b. Determine the filter medium resistance (rm).

c. Determine the size of filter for the same pressure drop to process 4000 l of cell suspension in 20 min.

Solution to problem statement: Constant pressure cake filtration

a. Determine the pressure drop across the filter.

V(l)	115	365	680	850	1130
t/V (min/l)	0.035	0.055	0.07	0.089	0.106

- A plot of t/V versus V results in a straight line with a slope of 0.67 x 10^{-4} (min/l^2) and an intercept of 0.028 (min/l).

$$\text{slope} = \frac{1}{K} = 0.67 \times 10^{-4} \qquad K = 1.5 \times 10^{4}\ \text{l}^2/\text{min}$$

From Kp, we know that

$$\Delta p = \frac{K \alpha C \mu}{2A^2 g_c} \qquad g_c = 9.8 \frac{kg_m}{kg_f \cdot s^2}$$

$$\Delta p = 2.3 \times 10^{-4}\ \text{N/m}^2$$

Solution to problem statement: Constant pressure cake filtration

b. Determine the filter medium resistance (rm).

$$y\ \text{intercept} = \frac{2V_0}{K} = 0.028$$

$$V_0 = 2101$$

$$r_m = \frac{\alpha V_0 C}{A} = 5760\,\text{m}^{-1}$$

Solution to problem statement: Constant pressure cake filtration

c. Determine the size of filter for the same pressure drop to process 4000 l of cell suspension in 20 min.

$$V^2 + 2VV_0 = Kt$$

$$K = (2A^2/\alpha C\mu)\Delta p g_c$$

$$V_0 = (r_m A/\alpha C)$$

By substituting numerical values for V, Δp, and t, we obtain

$$A^2 - 25A - 66.67 = 0$$

Solving, we get

$$A = 27.43 \text{ m}^2$$

Classification of filters:

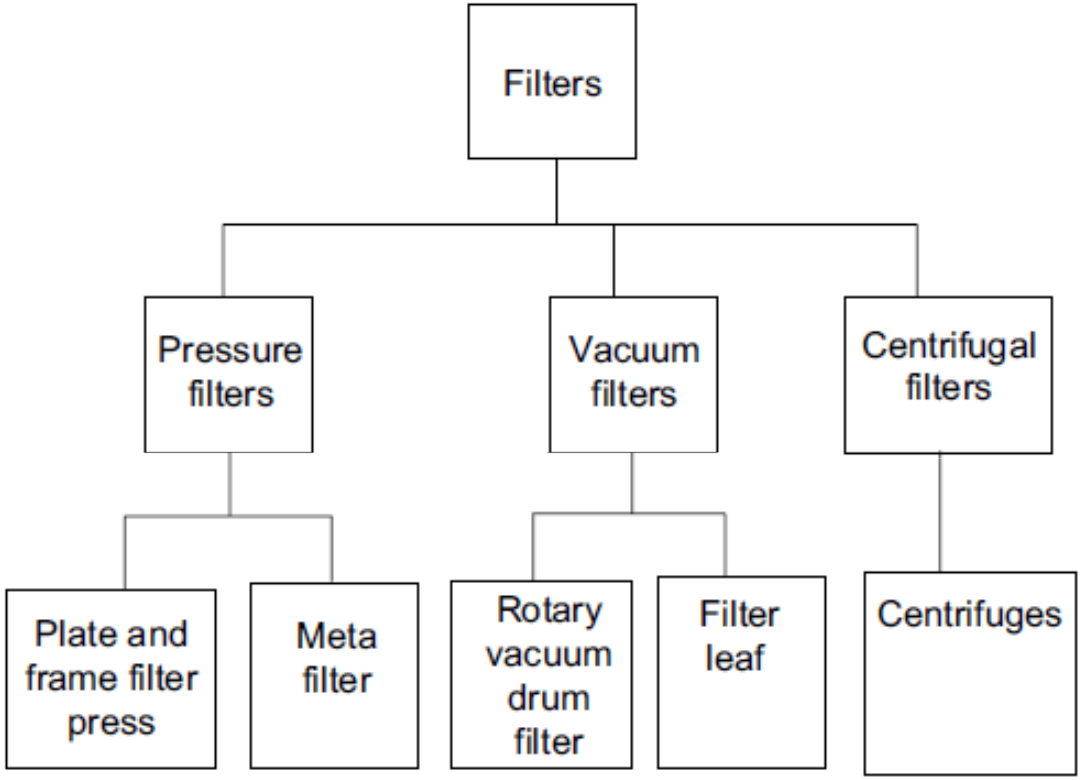
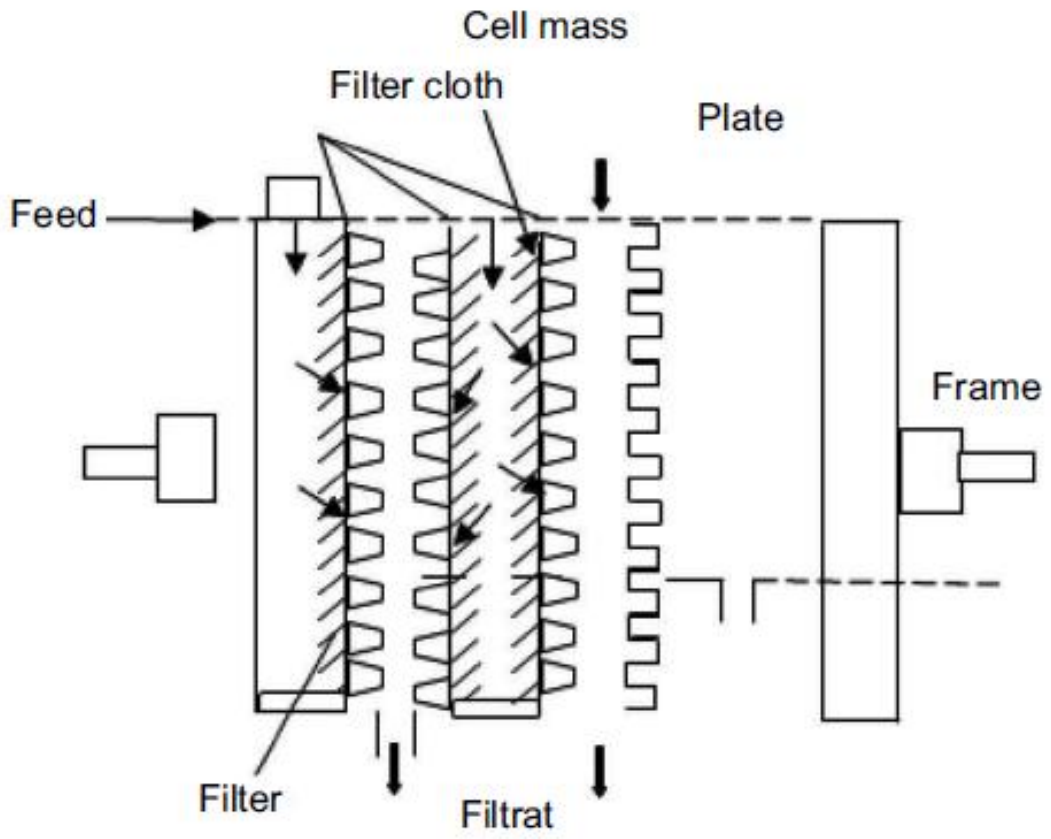


Plate and frame filter press:

- **Principle:** The PFFP is a surface filtration process.
- The suspended cells enter the frame by pressure and flow through the filter medium (e.g., a thick cotton pad).
- The filtrate is taken out from the plates.
- A good number of frames and plates are used to increase the surface area, and consequently, large volumes of cell-suspended liquid can be processed.
- Applications: Baker's yeast producing industry, food industry, mining industry, pharmaceutical industry, chemical industry, wastewater treatment, etc

Plate and frame filter press:



https://www.youtube.com/watch?v=EfTcfQY4kEY&t=90s

Rotary vacuum drum filter:

- RVF consists of a drum **rotating at a very low speed** (1 rpm).
- RVF drum consists of **a drum rotating in a tub of liquid to be filtered.**
- An RVF is suitable for a slurry containing a highly suspended solid, which can clog other forms of filter.
- The diameter of the drum may be **upto 3 meters in diameter** and **3 m to 3.5 m in length**. It gives a surface area of 20 m^2.

Rotary vacuum drum filter:

The operation of an RVF is discussed as follows:

- Rotary drum filters work on the principle of filtering suspended solids through a fine muslin cloth embedded on the rotating drum surface operated under a vacuum.
- The solid cake is washed by sprinkling water on the drum surface. The solid is removed by using a knife touching the surface of the rotating drum.

- **Principle:** Rotary drum filter works on the principle of function of filtering the slurry through sieve like mechanism on a rotating drum surface under the condition of vacuum.
- In addition, compression drying (using hot air) and removing the filter cake (using a knife) are possible.

Rotary vacuum drum filter:

- In a rotating drum filter, the drum rotates at a constant speed (n rps) and only a fraction of drum-surface area is immersed in suspension reservoir (φ).
- The period of time during which filtration is carried out is φ/n per revolution of the drum.
- The equation can be rewritten in this case as

$$V^2 + 2VV_0 = Kt \qquad \left(\frac{V'}{n}\right)^2 + 2\frac{V'}{n}V_0 = K\frac{\varphi}{n}$$

where V’ = filtrate volume per unit time (volume/time) and

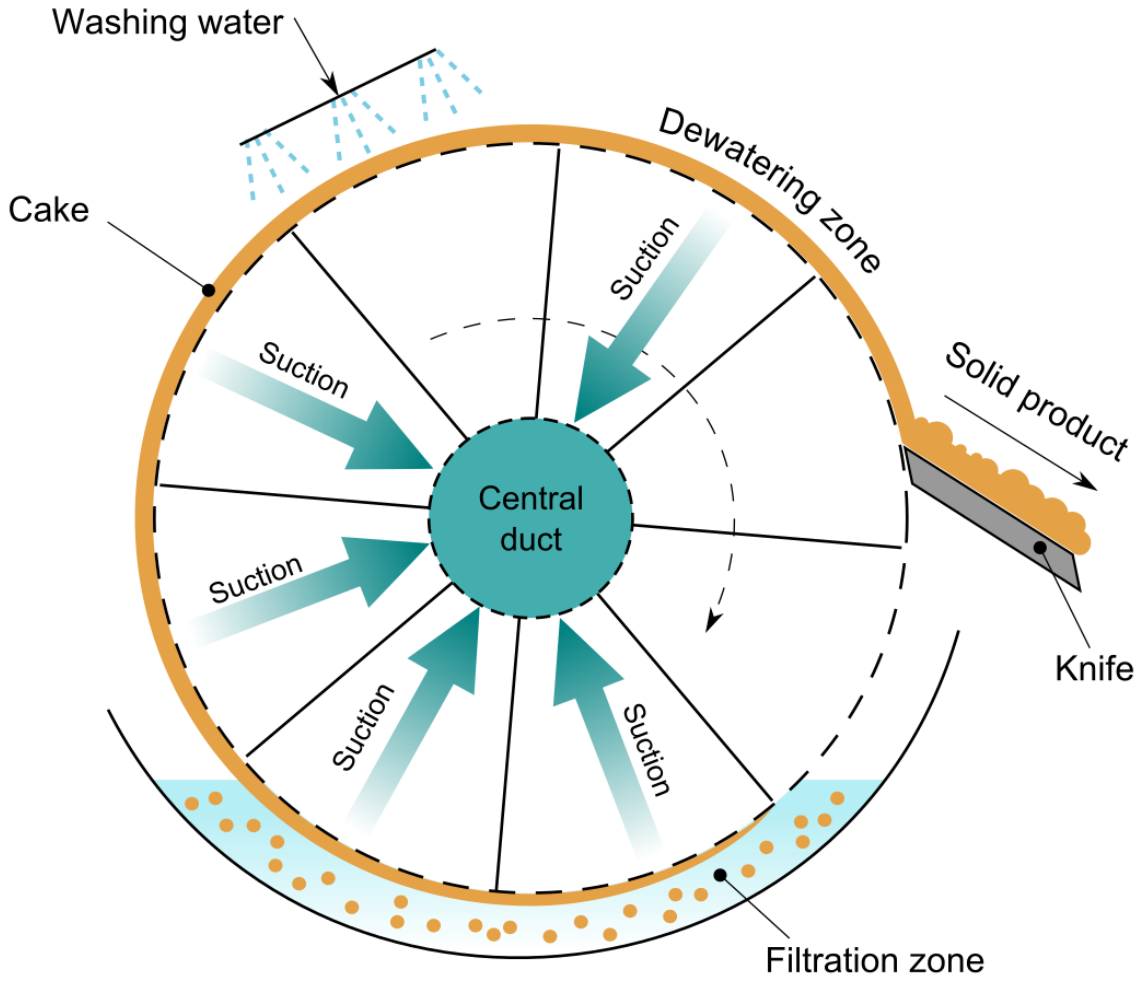
V’/n represents the volume of filtrate filtered for one revolution of the drum.

Rotary vacuum drum filter:

Applications: Dewatering slurries of cell mass,

food, pulp, pharmaceutical products,

and chemicals



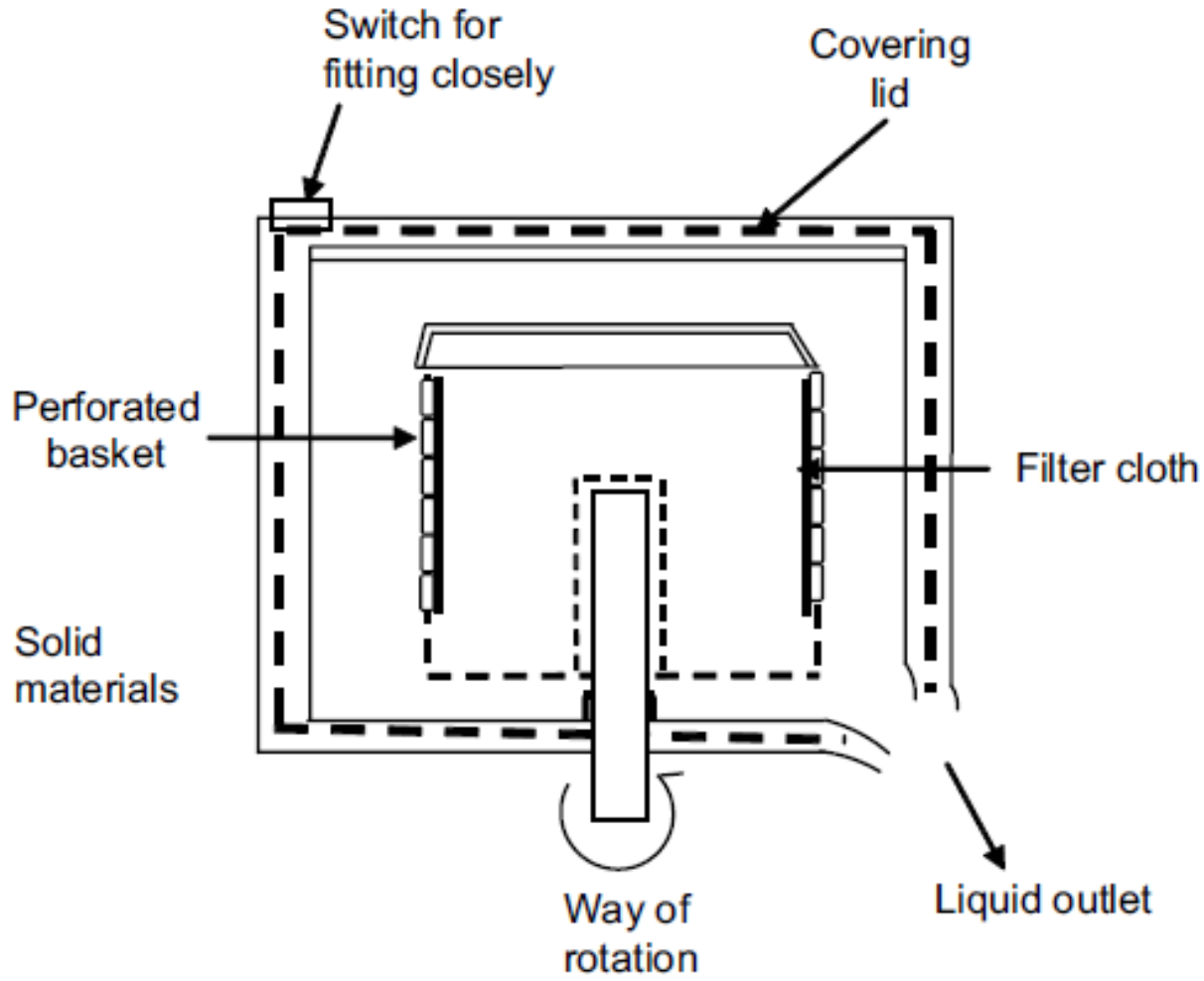
https://www.youtube.com/watch?v=pggFRWM_Zlk&t=83s

https://en.wikipedia.org/wiki/Rotary_vacuum-drum_filter

Centrifugal filter:

- It consists of **a stainless steel perforated basket** (typically 1-2 m in diameter) lined with a filter cloth
- The basket rotates at **a speed which is typically < 25 s^{-1}**, higher speed tending to stress the basket excessively
- The product enters centrally and is **thrown outward by centrifugal force and held against the filter cloth**
- **The filtrate is forced through the cloth and removed via the liquid outlet; the solid material is retained on the cloth**
- **Applications:** It can be used in the preparation of aspirin and for removing precipitated proteins from insulin to handle concentrated slurries that might block other filters.

Centrifugal filter:



https://www.youtube.com/watch?v=ZZKhxcEJc9Q&t=1s

Centrifugal filter:

- Centrifugation is used to separate particles of size between 100 and 0.1 mm from liquid by centrifugal forces.
- The major forces acting on a solid particle settling in a liquid by gravitational forces are gravitational force (F_G), drag force (F_D), and buoyant force (F_B).
- When the particles reach a terminal settling velocity, forces acting on a particle balance each other, resulting in a zero net force. That is,

$$F_G = F_D + F_B$$

eq A

$$F_G = \frac{\pi}{6} D_p^3 \rho_p \frac{g}{g_c} \qquad F_B = \frac{\pi}{6} D_p^3 \rho_f \frac{g}{g_c} \qquad F_D = \frac{C_D}{2g_c} \rho_f U_0^2 A$$

Centrifugal filter:

- F_D is the drag force exerted by the fluid on solid particles,
- C_D is the drag coefficient,
- ρ_f is fluid density,
- U_0 is the relative velocity between the fluid and particle or the terminal velocity of a particle, and
- A is the cross-sectional area of the particles perpendicular to the direction of fluid flow

- For spherical particles, $A = (\pi/4)D_p^2$
- when $Re_p < 0.3$,
- The drag force, FD, is given by the Stokes equation:

$$F_D = 3\pi\mu D_p U_0 \frac{1}{g_c}$$

Centrifugal filter:

- The relationship between C_D and Re_p:

$$C_D = \frac{24}{\mathrm{Re}_p}$$

- Equation A becomes:

$$3\pi\mu D_p U_0 = \frac{\pi}{6} D_p^3 (\rho_p - \rho_f) g$$

$$U_0 = \frac{g D_p^2 (\rho_p - \rho_f)}{18\mu}$$

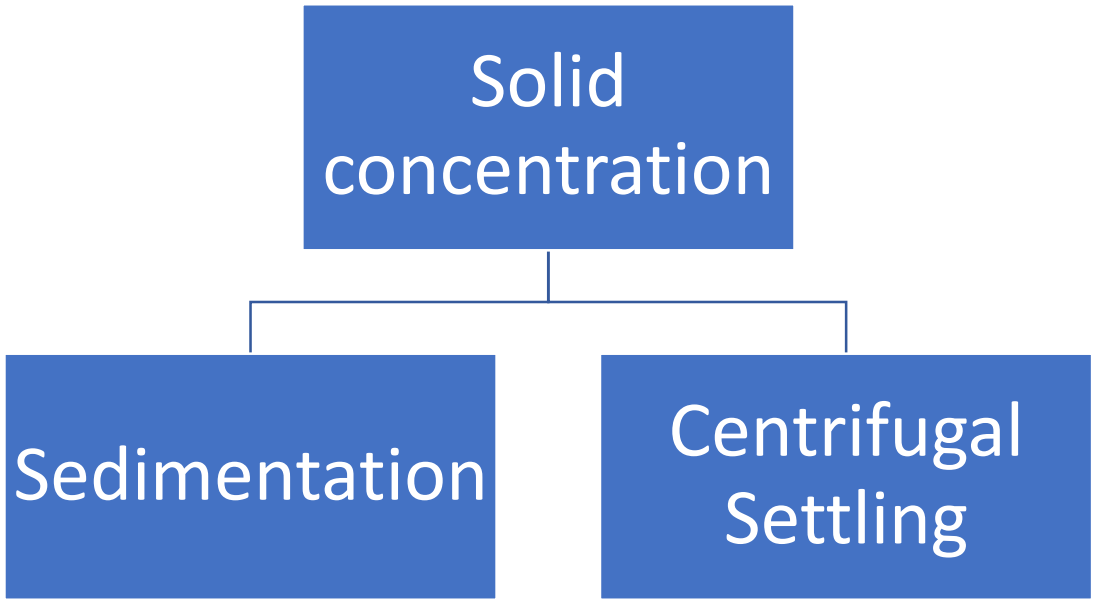
- where Dp and ρ_p are particle diameter and density, respectively
- In a centrifugal field, the terminal separation velocity of particles, U_{0c}, is,

$$U_{0c} = \frac{r\omega^2 \cdot D_p^2 (\rho_p - \rho_f)}{18\mu}$$

- where the centrifugal acceleration is substituted for the gravitational acceleration:

Solid concentration:

- In solid concentration, part of the liquid may be removed by (gravity or centrifugal) thickening or hydrocycloning to reduce liquid volume throughput load on the filter.



Sedimentation:

- Sedimentation is the process of letting suspended material settle by natural gravity force
- In sedimentation, the particles that are denser than the liquid medium would settle and form a zone with very high particulate concentration
- The settling velocity of solids in a liquid can be given by Stokes law,

$$V_g = \frac{g(\rho_s - \rho_l)d^2}{18\mu}$$

- where V_g is the settling velocity of the solid,
- P_s is the mass density of solid,
- ρ_1 is the mass density of liquid, and
- d is particle diameter (assuming spherical)
- μ is viscosity

Centrifugal settling:

- Process which separates particles from a solution according to their density, viscosity of the medium and rotor speed. (where a force greater than gravity is desired)
- When the mixture is introduced at a location within a liquid medium which is then subjected to an artificially induced gravitation field, this process utilizes density difference between particles/ macromolecules and the medium.

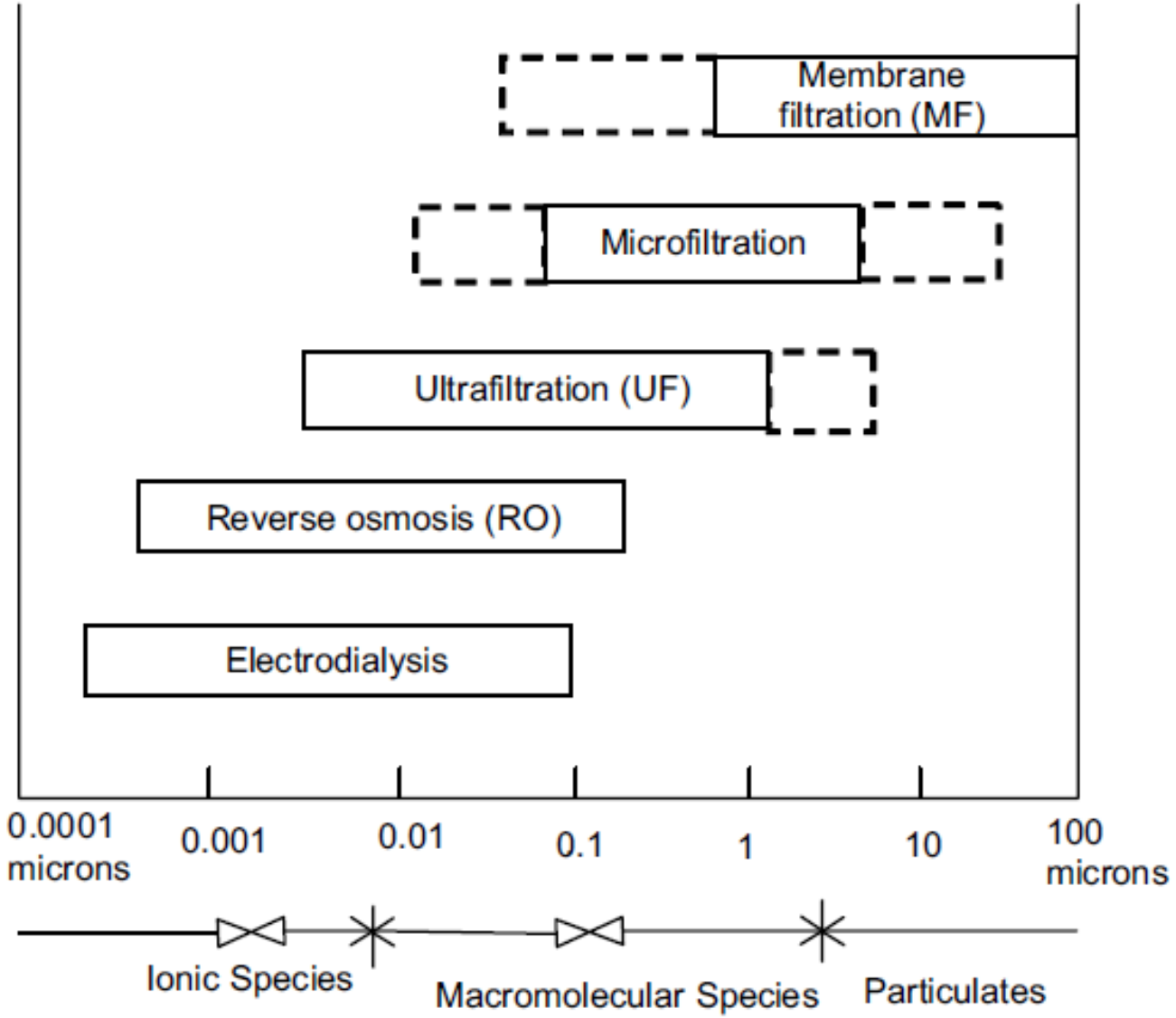
- The settling velocity can be given as,
  - where ω is the angular velocity (radians/s) and
  - r is the radial direction.

$$V_c = \frac{\omega^2 r(\rho_s - \rho_l)d^2}{18\mu}$$

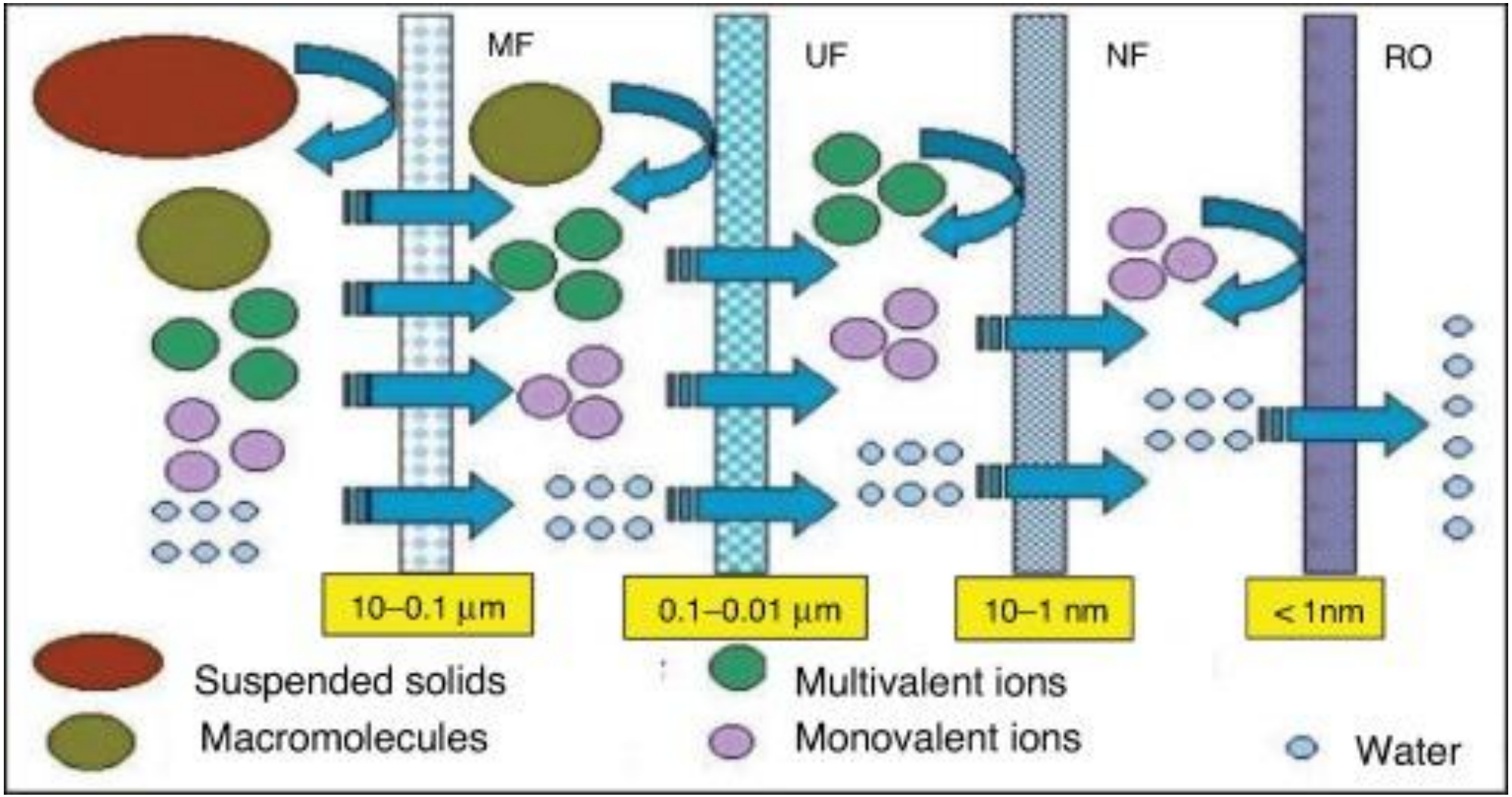
Ultracentrifugation:

- A special type of centrifuge in which the rotor rotates at a **much higher speed than a standard centrifuge.**
- Typical rotation speed: **30000 rpm – 50000 rpm**
- An analytical centrifuge (AUC) is mainly used for studying the properties of macromolecules as well as for **analyzing complex mixtures of macromolecules.**
- Preparative ultracentrifuges are **used to purify macromolecules** such as proteins and nucleic acids on their physical properties such as size, molecular weight, density and mobility.
- Cooling arrangements are required in these devices.

Classification of filtration techniques on the basis of pore size:



Separation of different sizes of particles using different membranes:

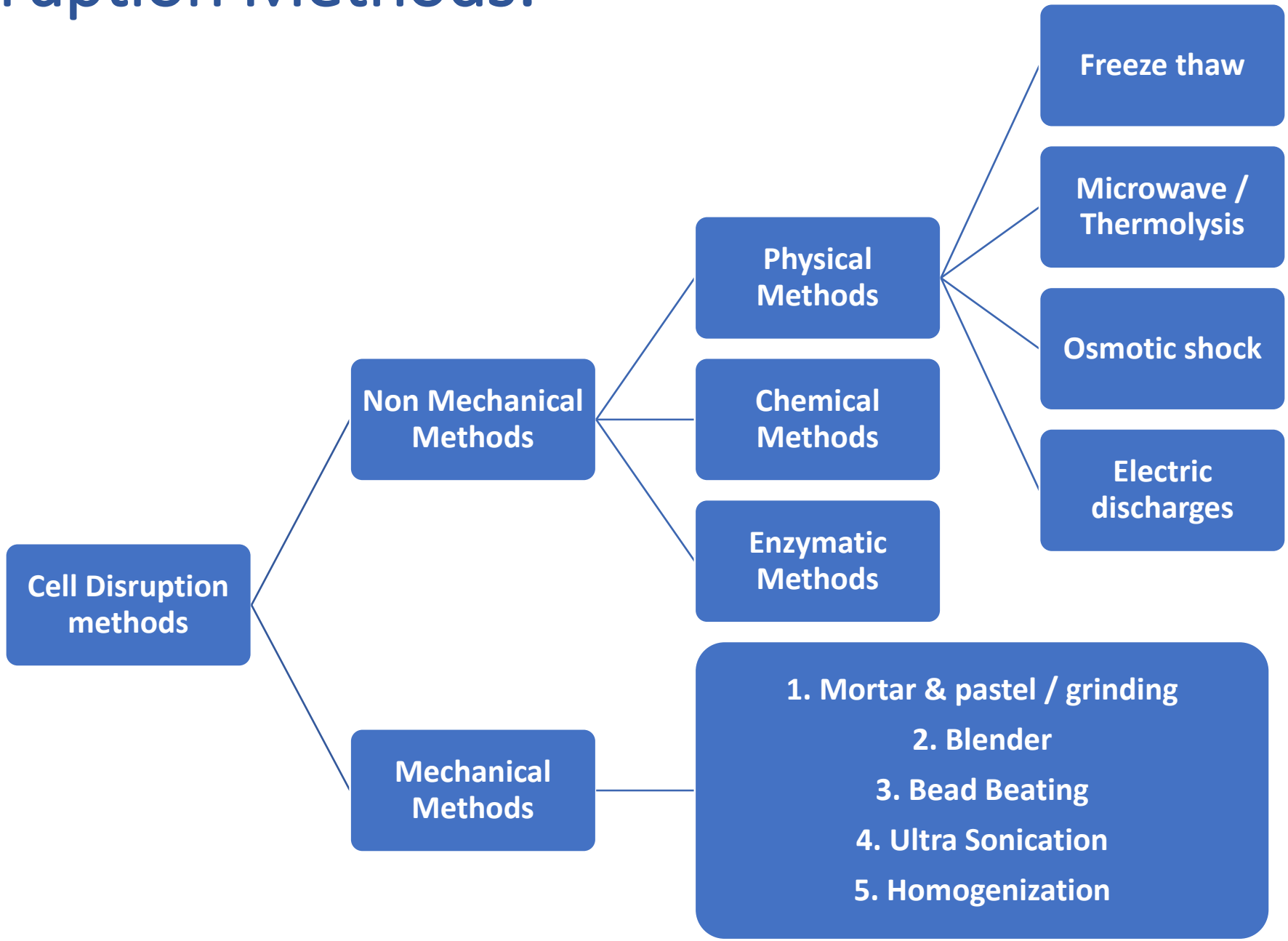


https://doi.org/10.1016/B978-0-12-822880-7.00013-3

Cell Disruption:

- After cells are separated from the liquid broth, if the desired product is intracellular, then the cells need to be disrupted to release the intracellular products.
- The method of disruption varies with the type of cells and the nature of intracellular products.
- **Cell disruption** is the process of obtaining intracellular fluid via methods that open the **cell** wall.
- The overall goal in **cell disruption** is to obtain the intracellular fluid without **disrupting** any of its components

Cell Disruption Methods:



Separation of soluble products:

- Most microbial products, such as antibiotics, organic acids, solvents, amino acids, and extracellular enzymes, are soluble.

- **Various methods** have been developed to recover such soluble products, including
- extraction,
- adsorption,
- ultrafiltration, and
- chromatography.

Liquid–Liquid Extraction: When?

- The compounds have very similar boiling points,
- One or more components are heat-sensitive,
- or distillation requires extremely low pressure or
- A high distillate/feed ratio,
- Azeotropes,
- Dilute solutions.

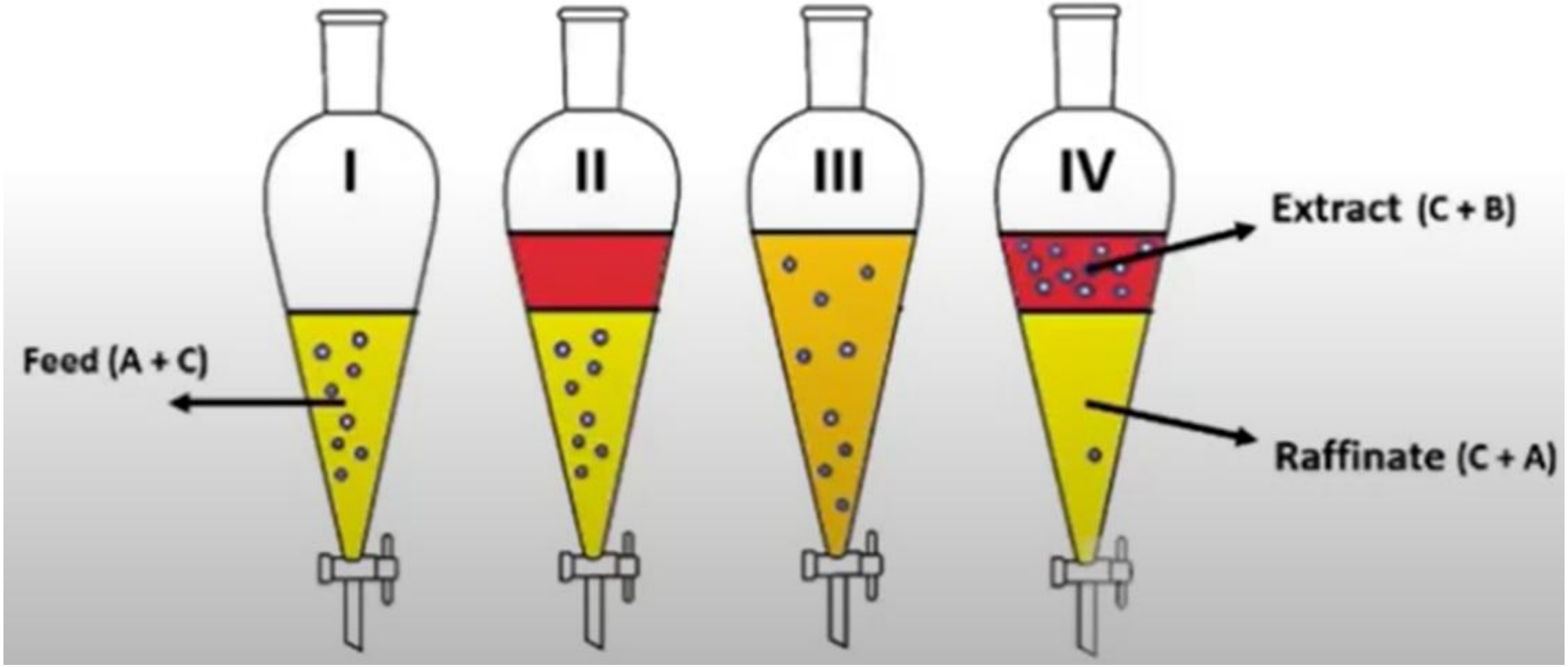
- It is usually considered when distillation is not economically viable (since the solvent usually has to be removed by distillation).

Liquid–Liquid Extraction:

- Separation of two components of a liquid (the feed) by contact with a second immiscible liquid (the solvent)
- Separates components (solute) **based on their relative solubilities** in two immiscible liquids.

- **For example,**
- Extraction of **penicillin** from the fermentation broth by contact with amyl or butyl acetate.
- Recovery of **acetic acid** from dilute aqueous solution by using ethyl acetate or ethyl ether.
- Separation of **high-MW fatty acids** from vegetable oil by using liquid propane.

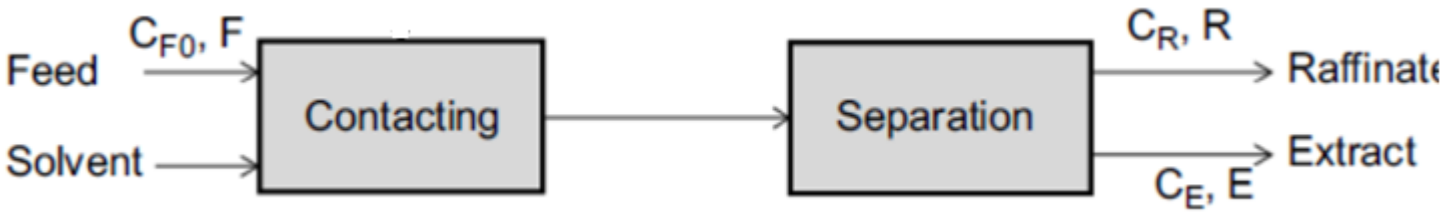
Liquid–Liquid Extraction:



https://www.youtube.com/watch?v=HolH_ZT02DE

Liquid–Liquid Extraction:

- **The principles of the liquid–liquid extraction process are as follows:**
- The liquid extraction method utilizes the differences in the **solubility of the components of a liquid mixture**.
- In the extraction operation, the liquid mixture to be extracted is called the **feed**.
- Solute extraction takes place by the liquid which is in contact with feed is known as **solvent**.
- The **extract** is the solvent-rich product of operation containing the extracted solute. It is also called the extract phase.



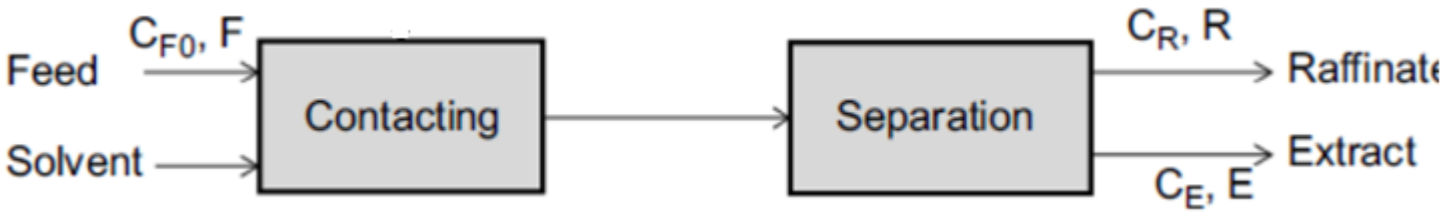
Liquid–Liquid Extraction:

- The **raffinate** is the spent feed, while the extract is the enriched extracting solvent
- The distribution of solute between the raffinate and the extract can be expressed in terms of the **partition coefficient K**:

$$K = \frac{C_E}{C_R}$$

- where C_E is the equilibrium solute concentration in the extracting solvent (kg/m^3) and
- C_R is the equilibrium solute concentration in raffinate (kg/m^3).

- At low solute concentration, the value of K is independent of the solute concentration.



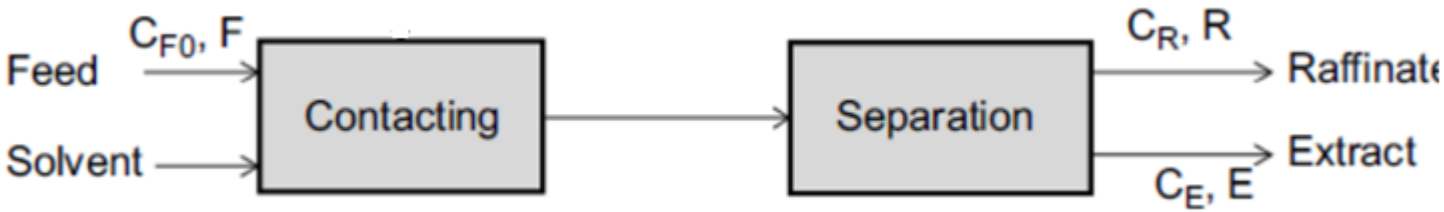
Theory of Liquid–Liquid Extraction:

- Solute material balance (batch extraction): $FC_{F0} = RC_R + EC_E$

- The extraction factor λ is defined as $\lambda = \frac{KE}{R}$

- The fraction extracted is given by $p = \frac{EC_E}{FC_{F0}} = \frac{\lambda}{1+\lambda}$

- where E is the rate of volume extract, C_E is the concentration of solute in the extract, F is the rate of the feed, C_{F0} is the concentration of solute, and p is the fraction of solute extracted.



Problem statement: Liquid–Liquid Extraction

100 L/h of citric acid solution (1 g/L) is contacted with 10 L/h of an organic solvent. The equilibrium relationship between raffinate and extract is

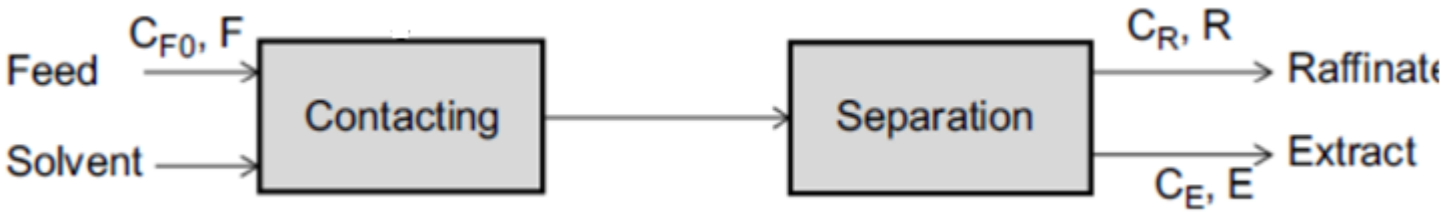
$$C_E = 100\, C_R^2$$

where C_E and C_R are the citric acid concentrations in the extract and raffinate, respectively, and are expressed in g/L. Calculate the following:

(a) The citric acid concentration in the extract and the raffinate.

(b) The citric acid fraction extracted.

Assume, flow rate of feed (F) = flow rate of raffinate (R)



Solution to problem statement: Liquid–Liquid Extraction

Given data

C_{F0} = 1 g/L, E = 10 L/h, R = F = 100 L/h

In the given problem, CE = 100 CR^2

(a) The citric acid concentration in the extract and the raffinate.

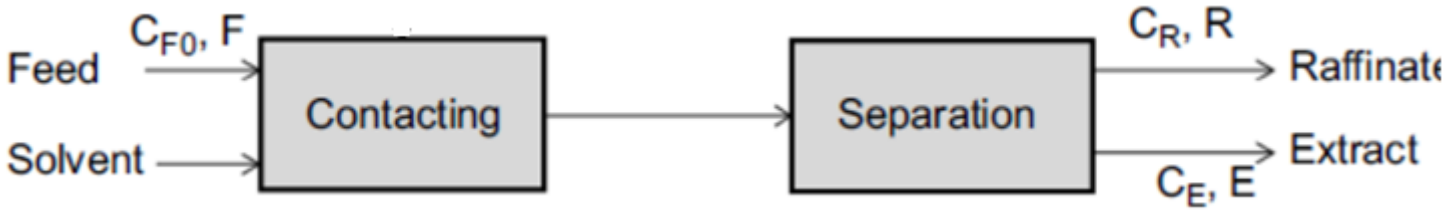
Solution: We know that

$$FC_{F0} = RC_R + EC_E$$

$$FC_{F0} = RC_R + E \times 100\ C_R^2$$

Putting all the known values gives,

$$100 \times 1 = 100\ C_R + 10 \times 100\ C_R^2 \qquad C_R = 0.27 \frac{g}{L}$$



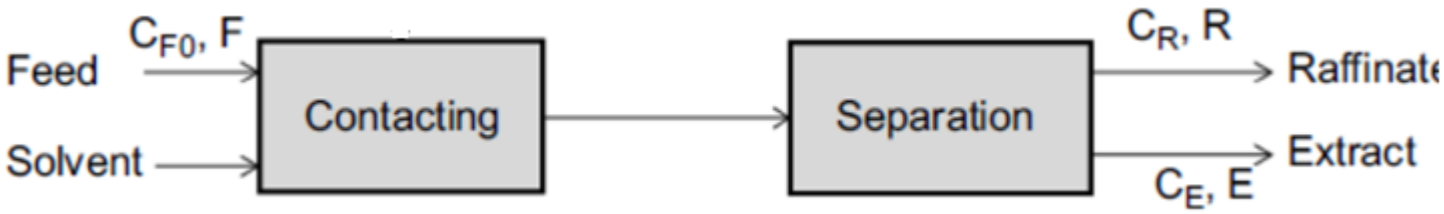
$$C_E = 100 \times C_R^{\ 2} = 100 \times 0.27^2 = 7.29 \frac{g}{L}$$

Solution to problem statement: Liquid–Liquid Extraction

(b) The citric acid fraction extracted.

The citric acid fraction extracted in the continuous extraction process is

$$p = \frac{EC_E}{FC_{F0}} = \frac{10 \times 7.29}{100 \times 1} = 0.729$$



Adsorption:

- Adsorption is known as a **surface phenomenon**.
- Adsorption of solutes from liquid media onto solids is a common practice in separating soluble materials from fermentation broth.
- A gas or liquid is concentrated on the surface of solid particles or at the fluid interface.
- Adsorption has the same purpose as extraction in isolating products from dilute fermentation liquors.
- Adsorption takes place due to the van der Waals, electrostatic, reactive, or other binding forces between individual atoms, ions, or molecules.
- There are four types of adsorption: ion exchange, physical, chemical, and non-specific.

Applications of Adsorption:

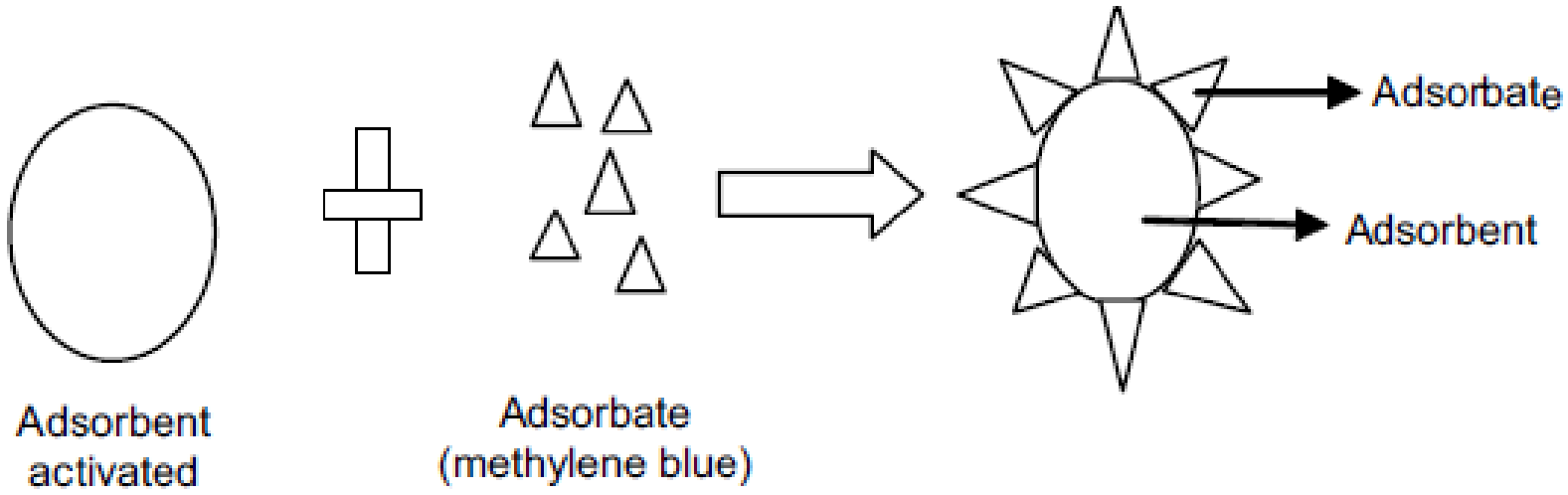
- Various mechanisms involved in adsorption.
- **In physical adsorption,** weak forces, such as van der Waals forces, are dominant;
- Physical adsorption (van der Waals) using activated charcoal is mostly used for the purification of chemicals such as citric acid. It is used as a decoloring agent.

- **In ion-exchange adsorption,** strong ionic bonds are utilized.
- Ion-exchange adsorption (electrostatic) is used for the recovery of amino acids, proteins, antibiotics, and vitamins.

- **Chemical adsorption** is used in wastewater treatment.
- The widely used adsorbent for waste-water treatment applications is activated carbon since it has a large internal surface area per unit weight.

Adsorption:

- **Adsorbate:** is the material being adsorbed
- **Adsorbent:** is the material to which the adsorbate binds. The surface area per unit volume for an ideal adsorbent material is high, e.g., ion-exchange resin, activated carbon, etc.



Representation of adsorption of methylene dye on the surface of activated charcoal.

Adsorption:

- Adsorption capacity varies depending on:
  - adsorbent,
  - adsorbate,
  - physicochemical conditions, and
  - the surface properties of the adsorbent and adsorbate.

Adsorption:

- The adsorption process is usually studied by using graphs known as adsorption isotherms.

- The graph between the equilibrium concentration or loading of solute on the adsorbent and equilibrium concentration of solute in the fluid phase.

- The equilibrium relationship between the adsorbate and the adsorbent may be represented by

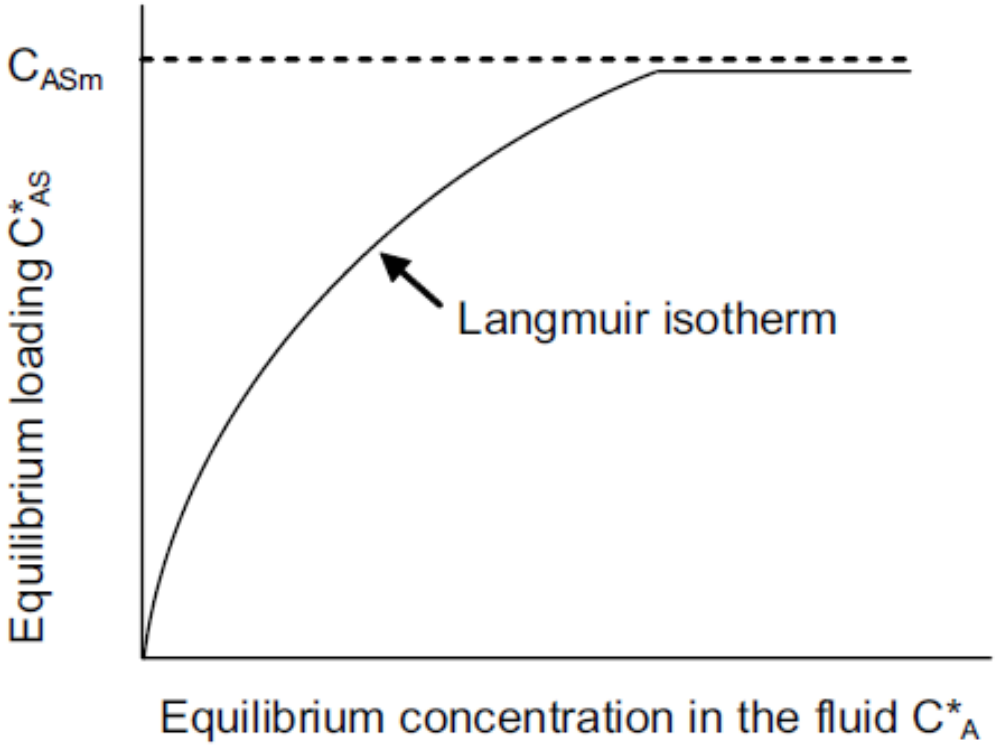
$$\text{Adsorbate} + \text{Adsorbent} \leftrightarrows \text{Adsorption}$$

$$A + B \leftrightarrows AB$$

Adsorption isotherm:

- Solute (adsorbate) extraction by the solid material (adsorbent) follows adsorption isotherms.
- **The Langmuir isotherm**

$$C^*_{AS} = \frac{C_{ASm} K_A C^*_A}{1 + K_A C^*_A}$$



- where C^*_{AS} is the equilibrium concentration of the solute on the solid surface (kg/kg or kg/m^3), C_{ASm} is the maximum solute adsorption capacity of the solid (kg/kg or kg/m^3), K_A is the adsorption constant (m^3/kg), and C_A* is the equilibrium concentration of the solute in the fluid phase.

Adsorption isotherm:

The Langmuir isotherm is applicable in the following conditions:

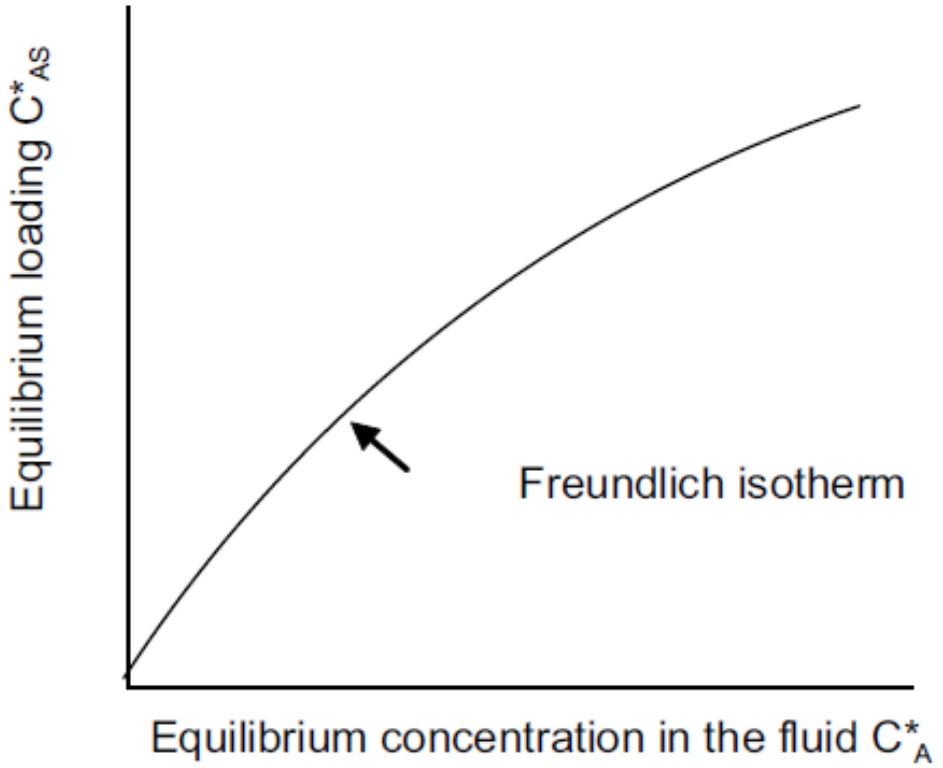
- A monolayer formation on the surface by the adsorbed molecules.
- Adjacent adsorbed molecules are assumed to have no interactions.
- Adsorption energy is equivalent in each adsorption site.

Adsorption isotherm:

- **The Freundlich isotherm may be represented by the following equation:**

$$C_{AS}^{*} = K_F \left(C_A^{*}\right)^{1/n}$$

$$C_{AS}^{*} = \frac{C_{ASm} K_A C_A^{*}}{1 + K_A C_A^{*}}$$



- where K_F and n are constants depending on the characteristics of the adsorption system.
- This isotherm is particularly applicable to the adsorption of hormones, antibiotics, and steroids.

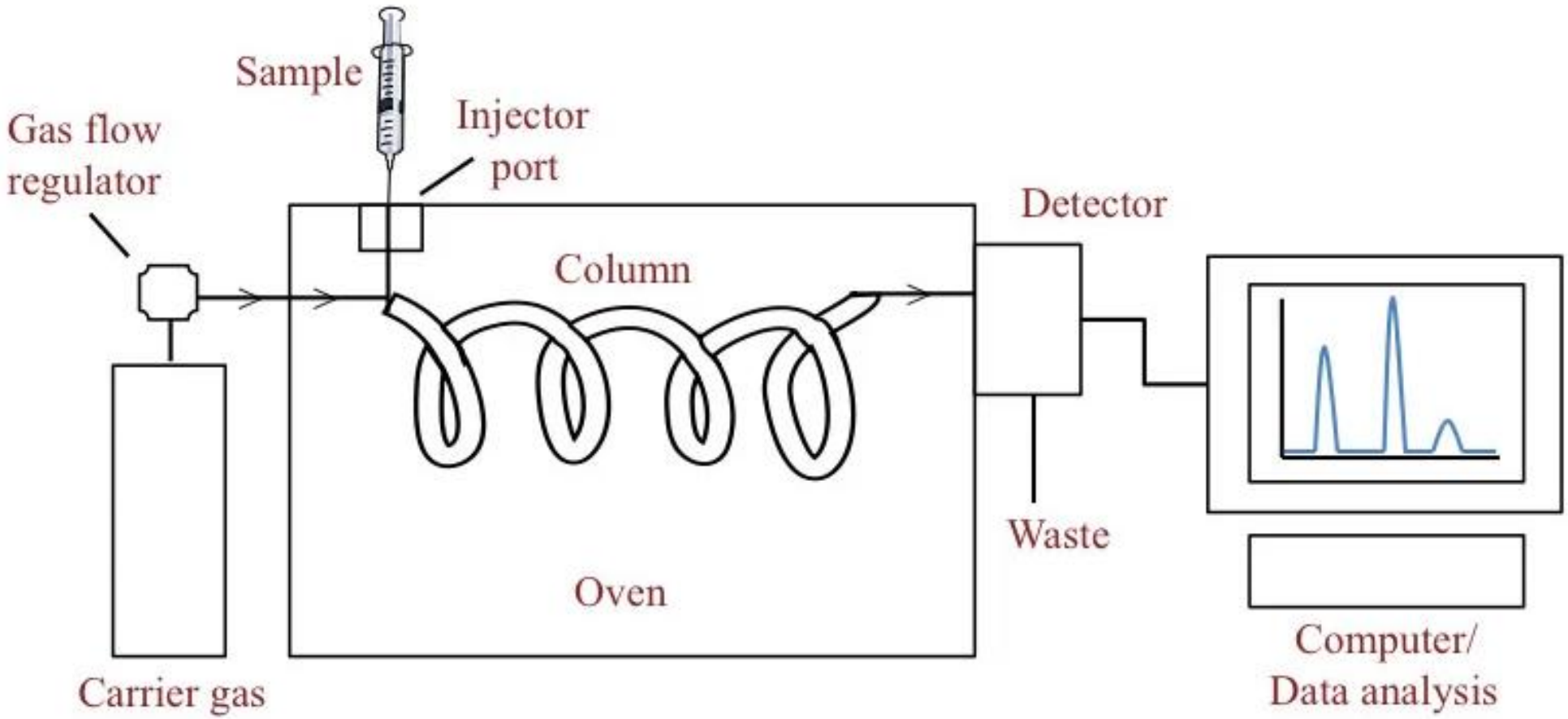
Chromatography:

- Chromatography separates mixtures into components by passing a fluid mixture through a bed of adsorbent material.
- Chromatography may be defined as the **solute fractionation technique**.
- Two phases are used: **a stationary (or binding) phase and a mobile (or carrier) phase**.
- Based on **differential distribution between two phases**, substances are separated.
- The distribution coefficient may be expressed as

$$\text{Distribution coefficient} = \frac{\text{Concentration of component in the stationary phase}}{\text{Concentration of component in the mobile phase}}$$

- **Applications:** Biopharmaceutical production, Biopharmaceutical and biomedical analysis, Environmental analysis, Diagnostics, Process monitoring

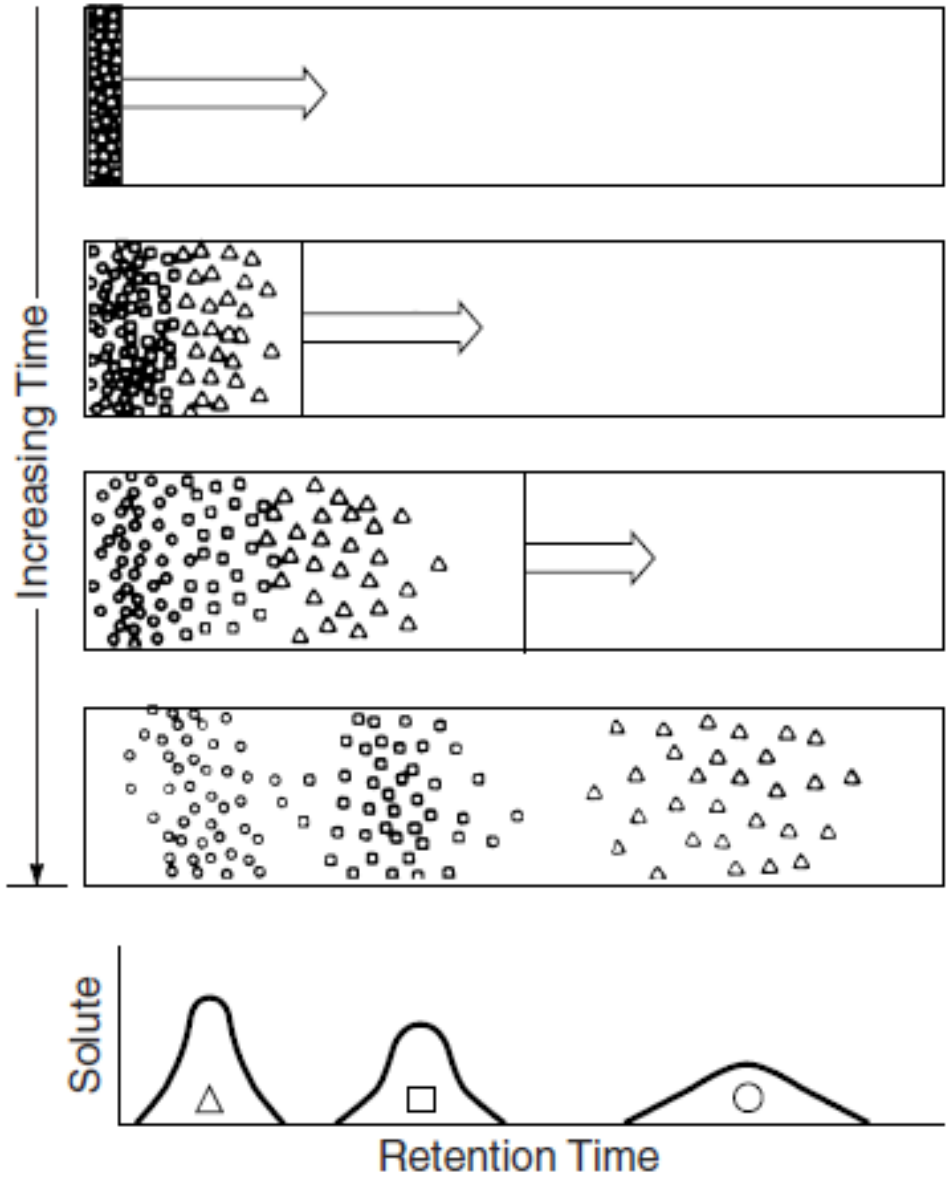
Chromatography:



https://www.sciencephoto.com/media/775881/view/gas-chromatography-animation

https://www.youtube.com/watch?v=0m8bWKHmRMM&t=25s

Chromatography terms:



Concentrations in elution chromatography.

Chromatography terms:

- **Chromatography:** Equipment that enables a sophisticated separation.
  - Ex: Gas chromatography, liquid chromatography
- **Eluent:** Fluid entering column/solvent that carries the analyte
- **Eluate:** Mobile phase leaving the column
- **Stationary phase – Immobilized phase**
- Immobilized on the support particles or on the inner wall of the column tubing
- Silica layer – Thin layer chromatography, silica gel, alumina, or cellulose on a flat
- **Chromatography:** The mobile phase of gas chromatography is gas, which carries the sample through the column, e.g., nitrogen, hydrogen, helium, etc.
- Similarly, in liquid chromatography, liquid is used as the mobile phase.

Chromatography terms:

- The **mobile phase** passes through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- **Retention time** is the time taken by an analyte to pass through the system (from the column inlet to the detector) under the operating conditions.
- **Sample:** Substance analyzed in chromatogram
- **Solvent:** Any substance capable of solubilizing another substance
- A **chromatogram** is the visual output of the chromatograph.
- Different peaks on the chromatogram correspond to the different components present in the sample.
- It is basically a plot of detector signal versus retention time.
- The signal is proportional to the concentration of the specific analyte separated.

Important chromatographic methods:

- The type of chromatography depends on the nature of the solutes and process goals.

- **Adsorption chromatography (ADC)** is based on the adsorption of solute molecules onto solid particles, such as alumina and silica gel, by weak van der Waals forces and steric interactions.
- **Liquid–liquid partition chromatography (LLC)** is based on the different partition coefficients (solubility) of solute molecules between an adsorbed liquid phase and a passing solution. Often, the adsorbed liquid is nonpolar.
- **Ion-exchange chromatography (IEC)** is based on the adsorption of ions (or electrically charged compounds) on ion-exchange resin particles by electrostatic forces.
- **Gel-filtration (molecular sieving) chromatography** is based on the penetration of solute molecules into small pores of packing particles on the basis of molecular size and the shape of the solute molecules. It is also known as size exclusion chromatography.

Important chromatographic methods:

- **Affinity chromatography (AFC)** is based on the specific chemical interactions between solute molecules and ligands (a functional molecule covalently linked to a support particle). Ligand–solute interaction is very specific, such as enzyme– substrate interaction, which may depend on covalent, ionic forces or hydrogen-bond formation. Affinity binding may be molecular size and shape specific.
- **Hydrophobic chromatography (HC)** is based on hydrophobic interactions between solute molecules (e.g., proteins) and functional groups (e.g., alkyl residues) on support particles. This method is a type of reverse phase chromatography which requires that the stationary phase is less polar than the mobile phase.
- **High-pressure liquid chromatography (HPLC)** is based on the general principles of chromatography, the only difference being high liquid pressure applied to the packed column. Owing to high-pressure liquid (high liquid flow rate) and dense column packing, HPLC provides fast and high resolution of solute molecules.

Precipitation:

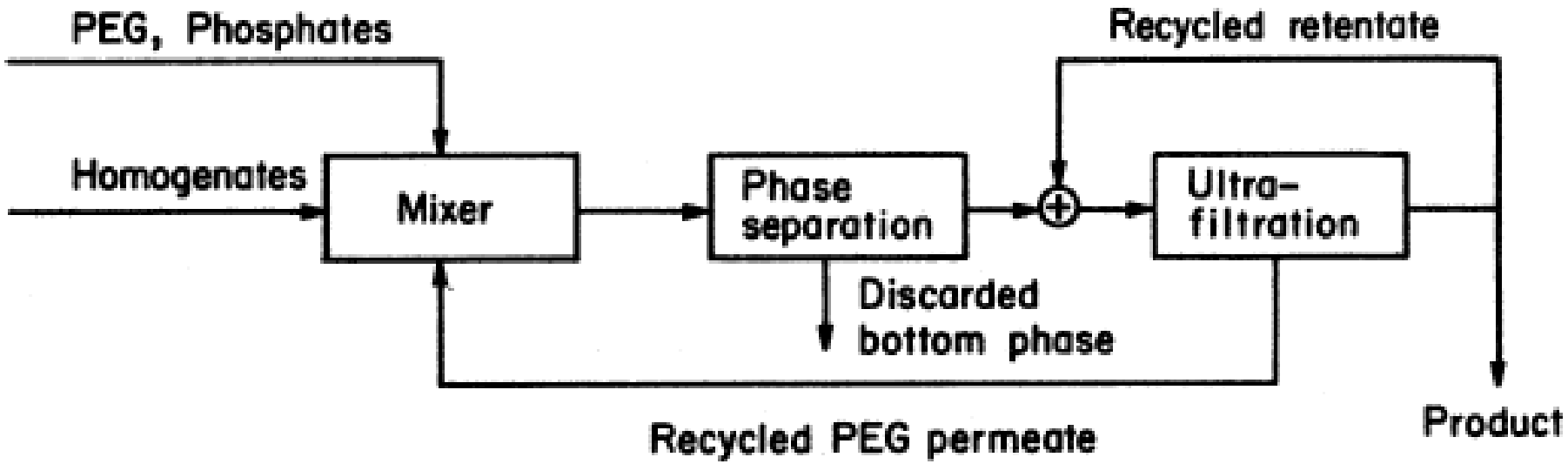
- The first step in the purification of intracellular proteins after cell disruption is usually precipitation.
- Proteins in a fermentation broth (before or after cell lysis) can be separated from other components by precipitation using certain salts.

- Examples include streptomycin sulfate and ammonium sulfate.

- The two major methods used for protein precipitation are as follows:
  - Salting-out by adding inorganic salts such as $(NH_4)_2SO_4$ at high ionic strength.
  - Solubility reduction at low temperatures by adding organic solvents (T < -5°).

Precipitation:

- Salting-out of proteins is achieved by increasing the ionic strength of a protein-containing solution
- The solution by adding salts such as $(NH_4)_2SO_4$ or Na_2SO_4. The added ions interact with water more strongly, causing protein molecules to precipitate.



Two-phase extraction process with PEG recovery

https://www.youtube.com/watch?v=wKbk3S0N4P8&t=34s

Evaporation:

- Concentrating a solution containing a non-volatile solute by boiling away the solvent (usually water)
- Removal of a part of the solvent from a solution of a non-volatile solute by vaporization

- **Example:**
- Concentration of milk and fruit juice
- Production of sugar
- Citric acid industry

https://www.youtube.com/watch?v=N7iIzKA5xh8&t=116s

Evaporation:

- The performance of a steam heated is evaluated by
- **Capacity**
- **Economy**

- **Capacity:** The number of kg of **water vaporized/evaporated per hour**
- **Economy:** The numbers of **kg of water evaporated per kg of steam fed** to the evaporator
- The ratio of capacity to economy gives the steam consumption of the evaporator per hour

Evaporation:

- The types of evaporators:
- **Long tube vertical evaporators**
  - **Upward flow (climbing film)**
  - **Downward flow (falling film)**
  - **Forced circulation**
- **Agitated film evaporators**

Dialysis:

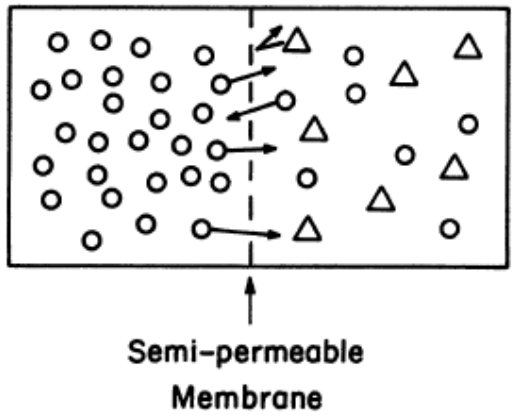
- Dialysis is a membrane separation operation used for **the removal of low-MW solutes,** such as organic acids ($100 < MW < 500$) and inorganic ions ($10 < MW < 100$), from a solution.
- A well-known example is the use of dialysis membranes to remove urea ($MW = 60$) from urine in artificial kidney (dialysis) devices.
- In biotechnology, dialysis can be used to remove salts from a protein solution.
- Note that the membrane is selective.
- The dialysis membrane separates two phases containing low-MW and high-MW solutions.
- Low-MW solutes move from a high to a low-concentration region.

Dialysis:

- At equilibrium, the chemical potentials of diffusing compounds on both sides of a membrane are equal.

$$\mu_1^{\alpha} = \mu_1^{\beta}$$

- where μ is the chemical potential of the diffusing compound.



A typical dialysis membrane separation. Low-MW component 1 (o) diffuses through the membrane from a high to low concentration region. The high-MW component (Δ) cannot pass.

https://www.youtube.com/watch?v=NKAJTmaP7Jg&t=1s

Reverse Osmosis:

- For fermentation broths, osmosis is the transport of water molecules from a high to a low-concentration region separated by a selective membrane.
- The water passes the membrane easily, while the salt does not.
- At equilibrium, the chemical potential of water must be the same on both sides of the membrane. As the water passes into the salt solution, its pressure increases.

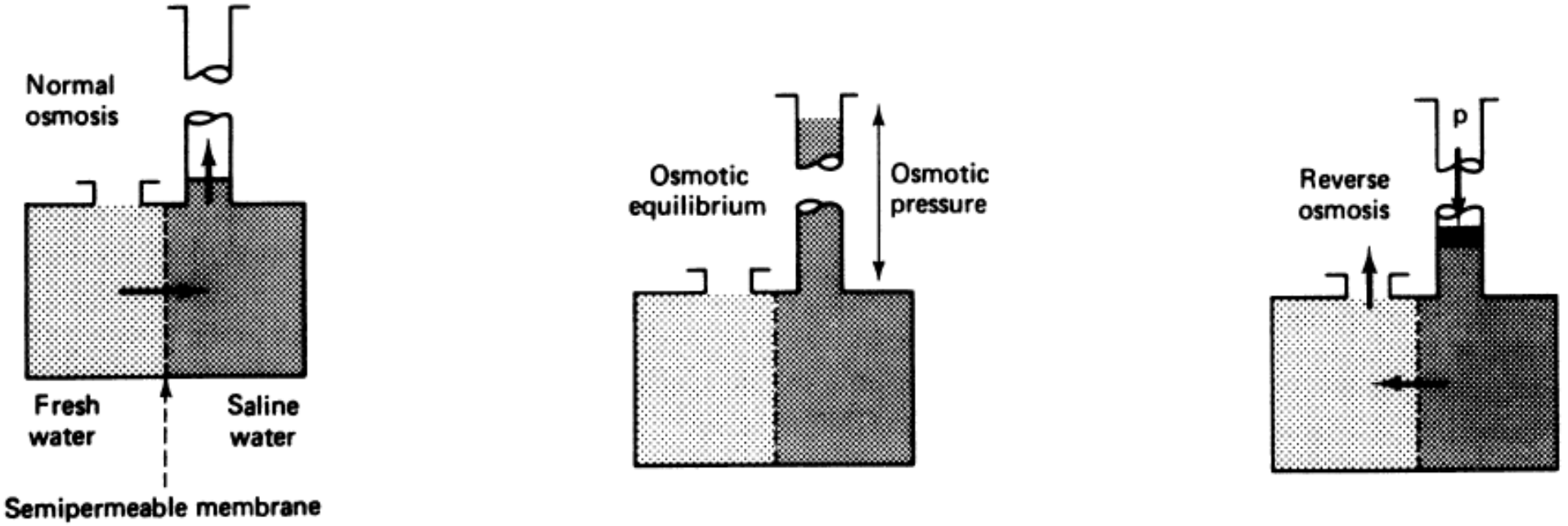
$$\pi = CRT(1 + B_2C + B_3C^2 + \cdots)$$

- where C is the concentration of the solute, T is temperature, R is the gas constant, and B_2 and B_3 are the virial coefficients for the solute. For very dilute, ideal solutions, $B_2 = B_3 = 0$.

$$\pi = CRT$$

Reverse Osmosis:

- In reverse osmosis (RO), a pressure is applied onto a salt-containing phase,
- which drives water (solvent) molecules from a low- to a high-concentration region and results in the concentration of solute (salt) molecules on one side of the membrane.



Osmotic flows across a membrane separating fresh water and saline water.

https://www.youtube.com/watch?v=4RDA_B_dRQ0

Ultrafiltration and Microfiltration:

- **Microfiltration** or microporous filtration (MF) separates species, such as bacteria and yeast, that range from **0.1 to 10 μm** in width.
- **Ultrafilters** are used for macromolecules with a molecule-weight range of 2000 to 500,000.
- All these membrane sieving methods (microfiltration, ultrafiltration, reverse osmosis) are based on the same **driving force**, namely **pressure**, but have some minor differences.
- **Anisotropic structure:** contains different pore size
- **Isotropic structure:** well-defined pores of uniform size

- **Applications:** pharmaceutical, chemical, and food industries for the separation of vaccines, fermentation products, enzymes, and other proteins.

Ultrafiltration and Microfiltration:

- **Factors affecting** choice of membrane materials are
  - Interactions with proteins,
  - mechanical stability,
  - chemical stability (especially to cleaning agents),
  - biocompatibility,
  - flux rates,
  - ease of sterilization (e.g., thermal stability), and
  - Cost
  - Application
  - Feed stream composition
  - Characteristics of product required

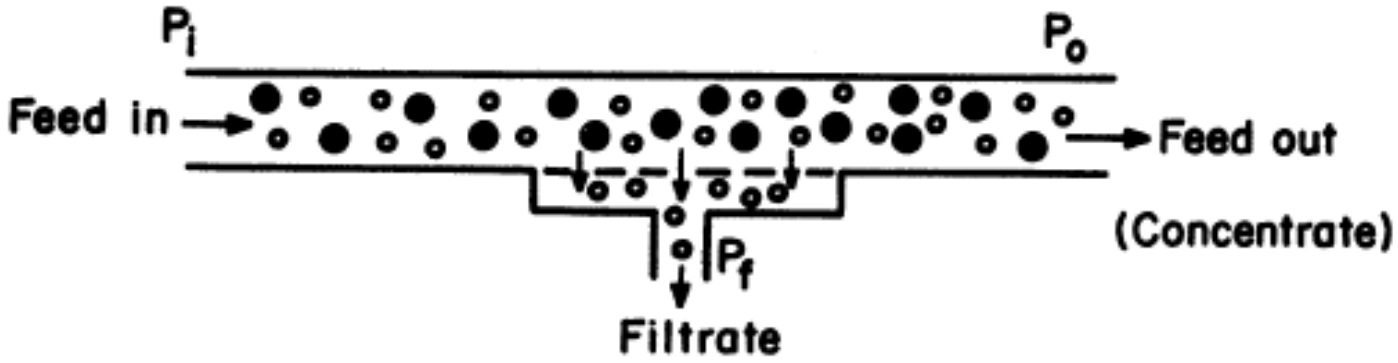
https://www.youtube.com/watch?v=WWIzoyAwccA&t=1s

Ultrafiltration:

- Three major applications of ultrafiltration are
- **Concentration:** water is removed from the aqueous solution of solute using an UF membrane and solute is concentrated
- **Purification:** Solvent and low-MW products are separated from high-MW compounds using a UF membrane
- **Diafiltration:** low-MW solutes such as salts, sugars, and alcohols pass through the filter, and the retained stream contains the product

Cross-flow Ultrafiltration and Microfiltration:

- Pressure is not applied directly perpendicular to the membrane but parallel to the membrane surface. This process is also called tangential flow filtration.
- That is, fluid flows parallel to the membrane surface and passes through the membrane, leaving solutes in a liquid phase above the membrane.
- Mechanical agitation or vibration of the membrane surface can also be used to alleviate gel formation.



https://www.youtube.com/watch?v=nG64bYVt7vk

Different membrane configurations:

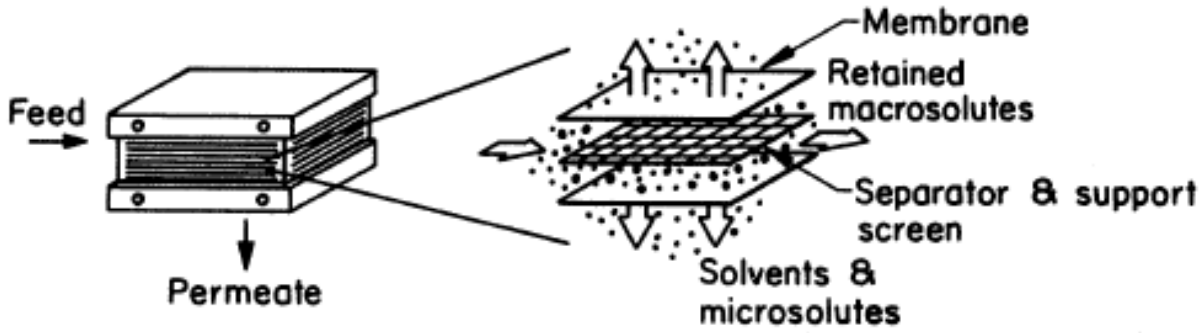
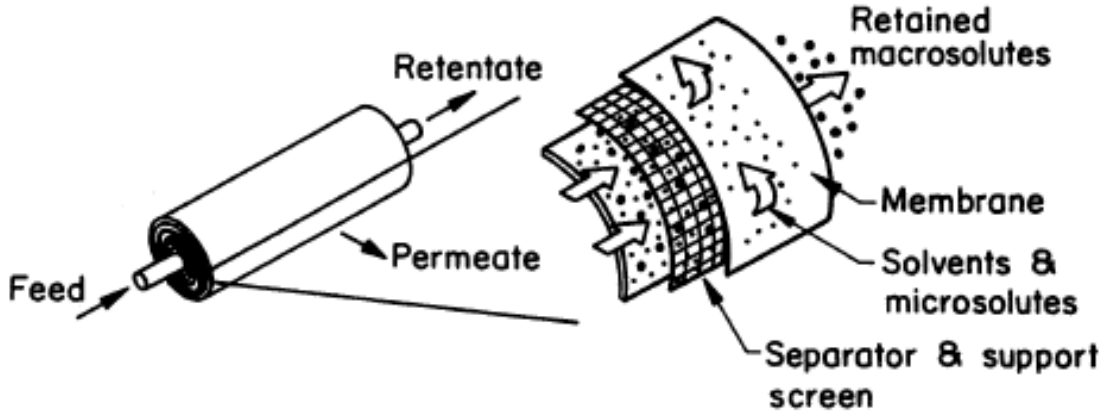
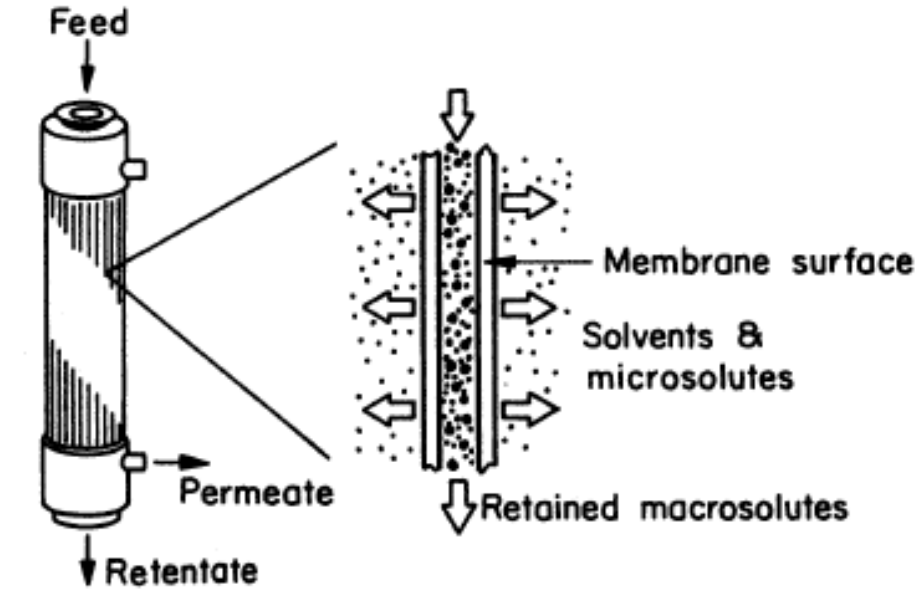


PLATE & FRAME DEVICE



SPIRAL CARTRIDGE



HOLLOW FIBER CARTRIDGE

configured to increase the surface-to-volume ratio

Electrophoresis:

- Electrophoresis is used for the separation of charged biomolecules according to their size and charge in an electric field.
- In an electric field, the drag force on a charged particle is balanced by electrostatic forces when the particle is moving with a constant terminal velocity.

Finishing Steps for Purification:

- The major finishing steps in fermentation-product purification are
- Crystallization and
- Drying

Crystallization:

- Crystallization is the process of formation of **solid crystals precipitating from a solution**, melt or rarely deposited directly from a gas.
- Crystallization is also a chemical solid-liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid.
- Crystallization operates at low temperatures, which minimizes the thermal degradation of heat-sensitive materials.
- Operations are conducted at high concentrations and, therefore, unit costs are low, and separation factors are high.

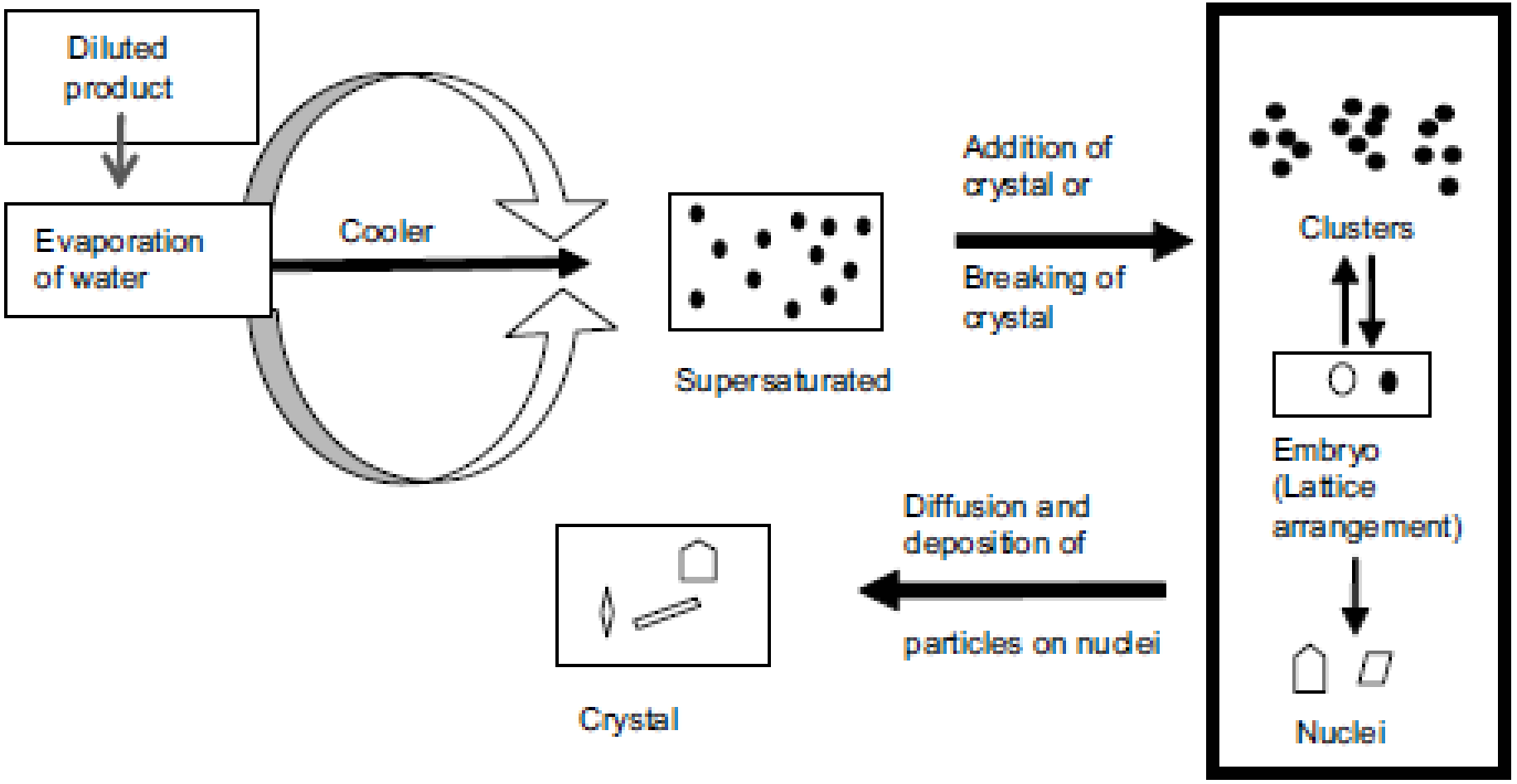
- **Applications:**
  - Production of sugar
  - Purification of drug

Theory of Crystallization:

- Three major stages in the process of crystallization are -
- **Supersaturation of the solution (heating, cooling, salting)**
- **Nucleation**
- **Crystal growth**

https://www.youtube.com/watch?v=3Nlnj0gBJhk&t=91s

Theory of Crystallization:



Drying:

- The removal of solvent from purified wet product (crystal or dissolved solute) is usually achieved by drying.

- Properties considered in selecting drying conditions,
  - the physical properties of the product,
  - its heat sensitivity, and
  - The desirable final moisture content must be considered.

- The parameters affecting drying
  - physical properties of the solid–liquid system,
  - intrinsic properties of the solute,
  - conditions of the drying environment, and
  - heat-transfer parameters.

Drying:

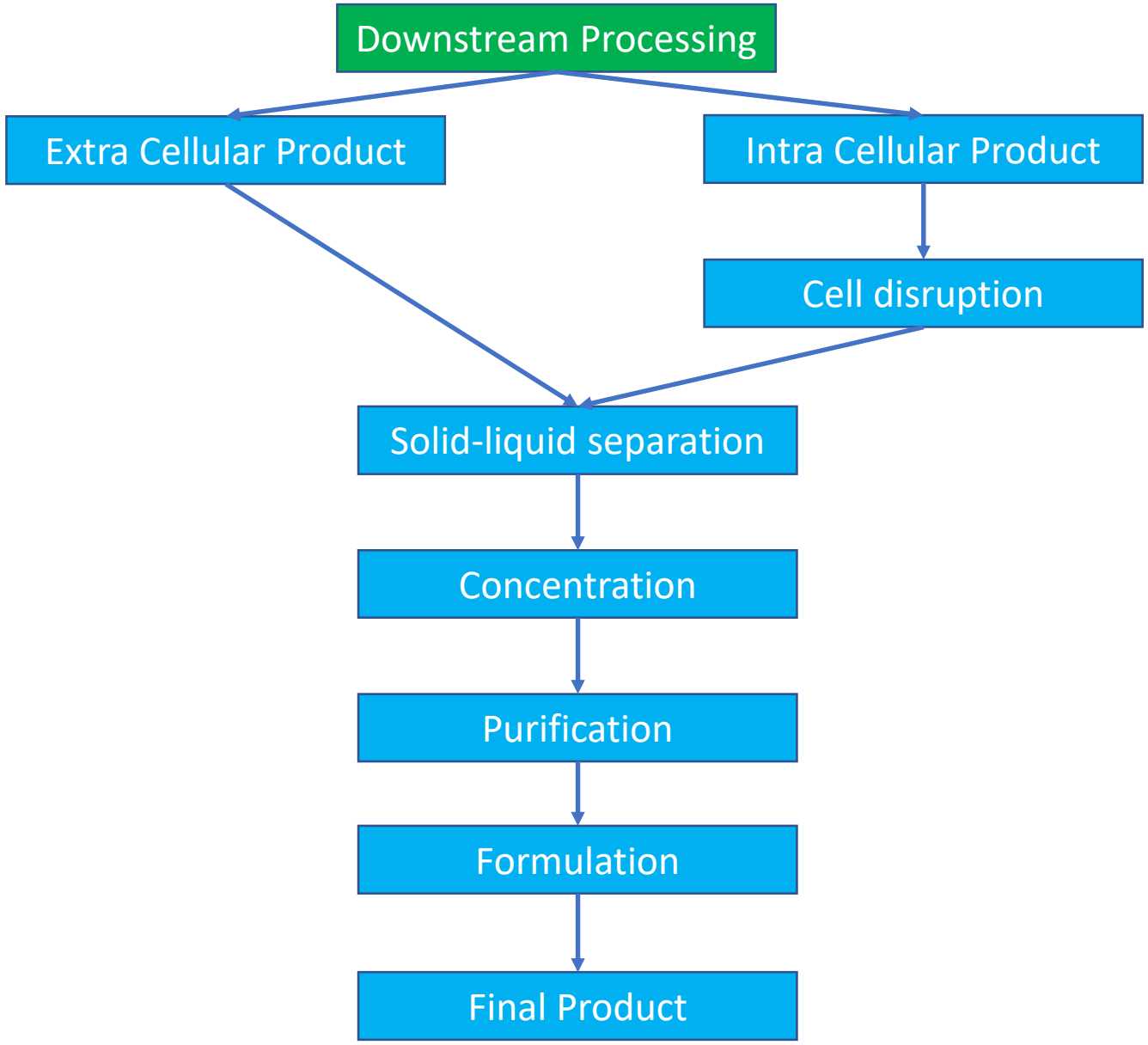
- The major types of driers used for drying fermentation products are the following:
- **A vacuum-tray drier** consists of heated shelves in a single chamber and is usually used for pharmaceutical products. This is a good method for small batches of expensive materials where product loss and heat damage must be minimized.
- **Freeze drying (lyophilization)** is a method where water is removed by sublimation (from solid ice to vapor) from the frozen solution. The freezing can be accomplished either outside or inside the vacuum chamber prior to drying. This method is used for antibiotics, enzyme solutions, and bacterial suspensions.
- **Rotary-drum driers** are not good for crystal solutions. Water is removed by heat conduction over a thin film of solution on the steam-heated surface of the rotating drum. The dried product is scraped from the drum with the aid of a knife at the discharge point.

Drying:

- **Spray dryers** employ atomization and spraying of product solution into a heated chamber through a nozzle. Hot gas inside the chamber provides the necessary heat for the evaporation of the liquid. Dried particles are separated from hot gases using cyclones. Spray dryers are expensive to purchase but are the preferred method for heat-sensitive materials.

- **Pneumatic conveyor driers** use a hot air stream to suspend and transport particles. The retention time of a particle in the gas stream is short, usually a few seconds. Such systems work well when surface drying is critical but do not provide sufficient exposure times to dry large, porous particles where water removal is diffusion-controlled. Pneumatic conveyor systems are well suited for heat-sensitive and easily oxidized materials.

Bioprocess overview/ Classification of downstream processes:



Chemical/Enzymatic/Mechanical/Physical

Centrifugation/Sedimentation/Extraction/Filtration

Evaporation/Ultrafiltration/Adsorption/Precipitation

Chromatography

Crystallization/Freeze Drying/ Sterile Filtration

Division of major steps involved:

(1) separation of insoluble products and other solids,

(2) primary isolation or concentration of product and removal of most of the water,

(water must be removed very early in the process train so that the size of the equipment

in the following steps will be minimized)

(3) purification or removal of contaminating chemicals, and

(4) product preparation, such as drying.


THANK YOU!