Module 4. MEMBRANE SEPARATION & ENRICHMENT OPERATIONS:

Syllabus

A. Membrane separation

1. Membrane based separations theory;
2. Design and configuration of membrane separation equipment;
3. Solute polarization and cake formation in membrane ultra filtration – causes, consequences and control techniques;
4. Applications:
    i. Use of membrane diffusion as a tool for separating and characterizing naturally occurring polymers;
    ii. enzyme processing using ultra filtration membranes;
    iii. separation by solvent membranes;
    iv. reverse osmosis.

B. **Precipitation** methods with salts, organic solvents, and polymers,

C. **Extractive separations**. Aqueous two-phase extraction, supercritical extraction;

D. **In situ product removal / integrated bioprocessing.**

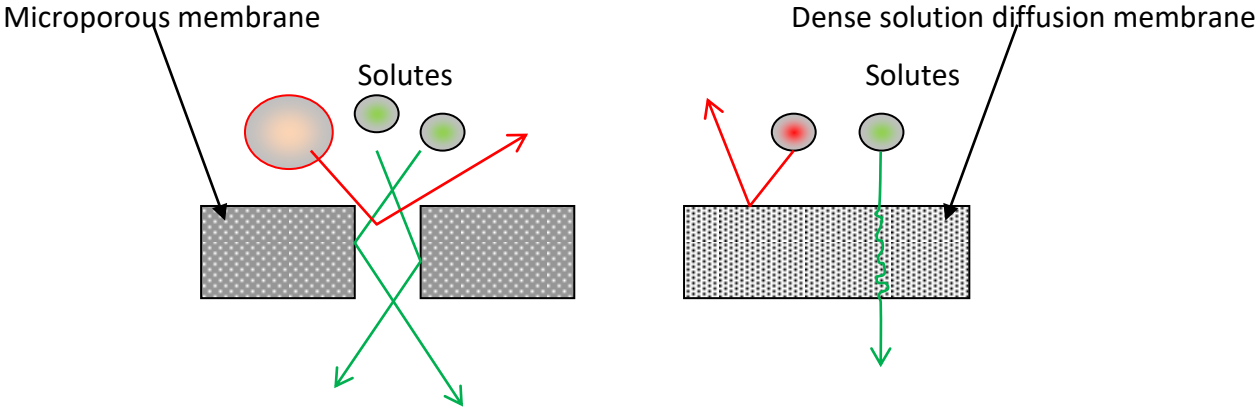
Course outcome 3: Identify and summarize the effect of change in unit's operations and its impact on the process.

Course outcome 4: Illustrate how emerging technologies would benefit the bio chemical product recovery and show the likely benefits it would have over the traditional operations.

Course outcome 6: Outline the processes involving large-scale, high-purity protein production

Question 1: How knowledge of **membrane-based separation theory** can be used to product purification in bioprocess industry. 6 marks

- The membrane is a barrier that separates two phases and restricts the transport of various chemicals in a selective manner.
- A membrane based separation system separates an influent stream into two effluent streams as permeate and retentate.
- Membrane base separation process is very important because of its cost effectiveness, environmental friendliness, simplicity, flexibility and convenience, compared to classical methods such as filtration, precipitation, centrifugation and distillation.
- Membrane based separation achieves all of these three objectives of DSP such as Isolation, concentration and purification efficiently in a single step, compared to multistage process including distillation, extraction, precipitation, ion-exchange adsorption and crystallization.
- The four well-established industrially important membrane separation processes are microfiltration, ultrafiltration, reverse osmosis, and electrodialysis.
- Two basic models, **capillary flow model** and **solution diffusion model** explain the selectivity exhibited by membrane separation



a. Capillary flow model. b. Solution diffusion model.

- Figure 18.6 Schematic representation of molecular flow through membrane.

1. Solution-diffusion model

- The solution-diffusion model is suitable to understand the mechanism of separation in reverse osmotic pervaporation and gas-permeation membranes.
- All the above three separation processes involve diffusion of molecules in a dense polymer or membrane.

- The temperature, pressure, and ingredients of the solution on either side of the dense polymer determine the quantum of the species diffuse at the membrane surface that is in equilibrium with the fluid.
- After dissolving in the membrane, individual diffusing species transport by the same random process of molecular diffusion regardless of type of membrane separation process.
- According to the **solution-diffusion model**, permeants dissolve in the membrane material to diffuse through the membrane down a concentration gradient.
- Differences in the solubility of the materials in the membrane and the differences in the rates at which the materials diffuse through the membrane separate the molecules.

Fick recognized this concept theoretically and experimentally formulated his results as the equation now known as **Fick's law of diffusion**, which states that

$$J_i = - D \frac{dC_i}{dx} (dc_s / dx) \quad \text{... (18.5)}$$

where dC_i / dx *is* the concentration gradient of component *i,* J_i is the rate of transfer of component *i* in g/cm^2/s and *D* is the diffusion coefficient in cm^2/s that is a measure of the mobility of the individual molecules.

- Membrane based solution-diffusion model and that follows Fick's law consisting of tiny spaces between polymer chains formed by thermal motion of the polymer molecules, and these pores appears and disappears on about the same timescale as the motions of the permeants traversing the membrane.
- Due to Fluctuations in the volumes between polymer chains due to thermal motion, the molecule bounces around in the narrow cavity and jumps to an adjacent cavity where it remains until another movement of the polymer chains allows it to jump to another cavity

2. Capillary-flow model

- This model is used to understand the use of Microporous ultrafiltration and microfiltration membranes.
- According to the **capillary-flow model**, pressure-driven convective flow drives permeants through tiny pores that is otherwise impermeable membrane.
- Separation occurs because of the discrimination of the molecules in solution by the pores based on the shape and the size by pores.
- Capillary flow model explains the pressure-driven convective flow in a capillary or porous medium, and the basic equation of Darcy's law explaining this type of transport, which written as follows.

$$J_i = -K'C_i \frac{dP}{dx} \quad \ldots (18.6)$$

where C_i is the concentration of component *i* in the medium, *dP/dx* is the pressure gradient existing in the porous medium and *K'* is a coefficient reflecting the nature of the medium.

- Membrane based on capillary-flow model and follows Darcy's law, pores are comparatively larger and fixed, and these pores are connected to one another and do not fluctuate in position or volume on the timescale of permeant motion.
- Larger the pores size of the membranes, it more likely that these membranes allow convective flow of the permeants across the membranes, and the demarcation between or the transition between the transient or solution diffusion and permanent or pore-flow pores is in the range 5–10˚A diameter.

Question 2. Analyse Design and configuration of membrane separation equipment approprioate for bioprocessing 6 marks

- Proper design of the filtration unit and suitable configuration between membrane module, separation methodology and feed is very much important for optimum membrane separation efficiency in an economic manner.
- Typical membrane separation plant is schematically represented in the figure 18.26.
- Membrane separation unit consists of reservoir for feed, permeate and reject solution. High pressure pumps of piston, diaphragm or centrifugal type pumps feed across the membrane.
- Membrane modules of tangential submerged or dead end configuration separates product of interest from the feed.
- High pressure regulator and turbidity regulators are incorporated to avoid fouling of the membrane.
- Different membrane modules have been designed based on different criteria such as surface-volume ratio, membrane support, pressure drop, turbulence and back flushing.
- A high membrane surface to feed volume ration is important for reducing space requirement and cutting capital cost.
- An adequate structural support, supports thin membrane at required operating pressure is important for the shelf life of the membrane.
- A low pressure drop on the retentate side of the membrane is critical for maintaining the driving force for permeation.
- Provisions for turbulence on the retentate side to drive away concentration polarization that minimizes the membrane fouling.
- Provisions for back flushing and replacement of members are very important criteria.

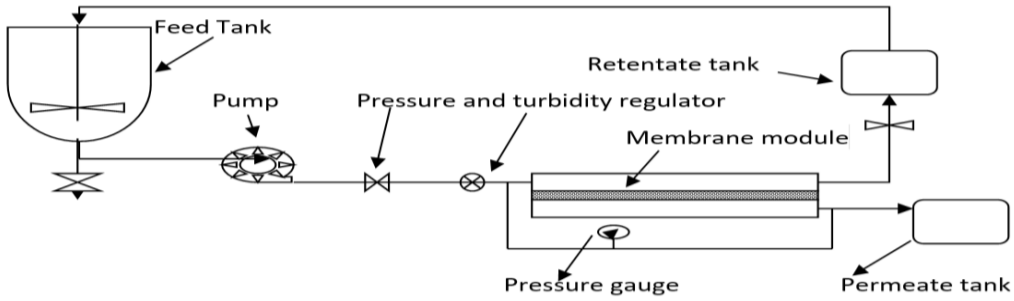


Figure 18.26 Schematic representation of typical cross flow batch membrane separation plant.

- On industrial scale, membranes are available in the form of modules that can contain up to 50 m^3 of membrane area.

- These modules are available in hollow fiber, spiral wound, flat plate and tubular forms.
- Each of the membrane designs available is suited for particular application on large scale.
- Process economics are critically important on deciding between different types of the modules.
- There are several different ways to constrict membrane modules by the arrangement of inlet and outlet streams.
- There are two systems**: Dead end membrane system** and **Crossflow membrane system.**
- Batch system is suited for separating the product of interest in small scale, and commonly used in biotechnology and pharmaceutical industries.
- In **cross flow membrane system,** there are many designs such as cassettes, hollow fiber membrane cartridges, and spiral cartridges are available.
- Cross flow membrane system may operate in batch mode or continuous mode.
- There are three types of continuous systems such as **one-, two-, and three-stage feed and bleed systems are in us**e.
- In most of large scale industrial process, multi stage feed and bleed membrane separation systems of between three to five stages are used.
- Two stage membrane filtration process using coarse filter and fine filter in series extends the life span of the membrane.
- A typical brackish water reverse osmosis plant contains seven pressure vessels each containing six membrane modules. The pressure vessels are in a 'Christmas tree' or 'tapered module' design to maintain high average feed solution velocity through the module.
- Submerged membranes such as hollow fibers and flat sheet panels in the bioreactor are very much useful in large scale membrane separation process by extending the membrane shelf life.

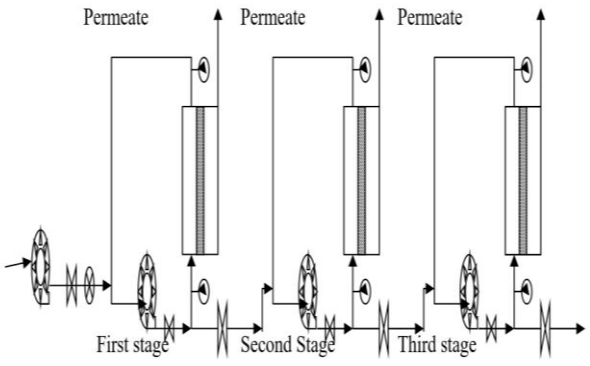


Figure 18.29 Schematic representation of three-stage feed and bleed system

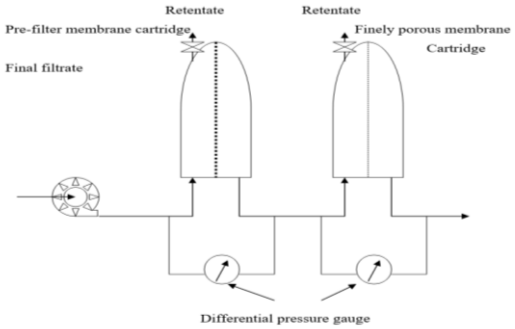


Figure 18.30 Schematic representation of in-line two cartridge filter system.

VTU: Elucidate on **Solute polarization** and **cake formation** in membrane ultra filtration

Question 3. Give an account of **causes, consequences and control techniques** during ultrafiltration of biological fluids 6 marks

- Molecular size is the basis for the separation of components of a solution using membrane as a molecular sieve.
- n membrane separation processes, liquid mixture comes in contact with the feed side of the membrane, membrane permeates only particular species along it, and permeate enriched with these species comes from the downstream side of the membrane.
- Since the membrane permeates different species of the feed at different rates, concentration gradient takes place in the fluids on both sides of the membrane.
- In the dead end filtration, rejected solutes accumulate as cake and in cross flow filtration with turbulent flow only thin layer of boundary layer is developed. T
- his boundary layer is poorly mixed causing concentration polarization and membrane fouling resulting in serious consequences of reduced or standstill flux. Concentration polarization is minimized by increasing the rate of mixing, and membrane fouling is minimized by adopting filtration strategy.
- Performance characteristics of the membranes with cross-flow type mechanisms are depending on two parameters such as **filtrate flux** and **solute rejection coefficient**.

  The following equation represents the Filtration flux *J* of the membrane

  $$J_i = \frac{Qf}{A} \quad \text{... (18.51)}$$

  where Qf is the filtrate flow rate and the A is the membrane area.

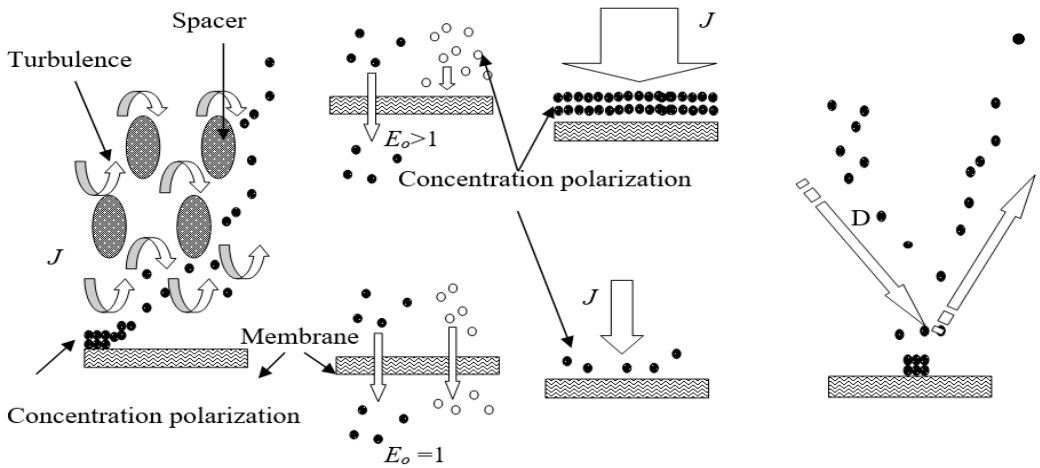
  The measured solute rejection coefficient R_m is represented as follows

- $$R_m = 1 - \frac{C_p}{C_b} \quad \text{... (18.52)}$$
- where C_p and C_b are the concentration of the solutes species in permeate and in the bulk of the solution. The value of the R_m = 1 indicates that the species concentration in permeate is zero, and the value of R_m = 0 indicates that the species are allowed to pass through the solution.
- The concentration polarization and the membrane fouling influence both **flux and the rejection characteristics of the membranes**.
- **Concentration polarization** is the accumulation of the completely or partially rejected solutes just above the surface of the membranes.

- **Membrane fouling** is the deposition of the solute particles on the membrane surface or within the pore resulting in the deterioration of the membrane performance such as reduced flow rate with the filtration time and increased rejection coefficient at constant operation parameters.
- All these phenomena are because of **membrane characteristics, membrane module, filtration methodology, and solute and solution properties**.

1. Concentration polarization

- . Most import aspects for the high flux through the membrane is the **degree of mixing in the feed solution**.
- Concentration polarization is common in **ultrafiltration membrane during protein purification** and in **microfiltration during cell suspension separation**.
- Concentration polarization has serious consequences especially in ultrafiltration.
- Concentration polarization causes the membrane fouling due to decomposition of colloidal and macromolecular material on the membrane surface leading to the consequences of **reduced flux and damage of the membrane**.
- **Increase in the degree of mixing reduces the concentration polarization** by quickly driving the solutes to the bulk of the feed.
- Four most critical factors that effects the magnitude of concentration is the **boundary layer thickness δ, the membrane enrichment E_o, Volume flux through the membrane J,** and **the diffusion coefficient of the solute in the boundary layer D**.
- Most straight forward way of reducing concentration polarization is by increasing the turbulence mixing, reducing selectivity, reducing volume, and reducing diffusion.



a. Turbulence mixing b. Selectivity c. Total volume flow d. Diffusion coefficient

2. Membrane fouling

- Membrane fouling is the process in which suspended or dissolved solids deposits on the external membrane surface, on the membrane pores, or within the membrane pores, resulting in a decrease in performance of a membrane.
- Flux through the membrane decreases slowly with time in all the membrane based separation process due to fouling caused by a variety of factors such as **slime formation**, microbial growth, **deposition of macromolecule**s especially in ultrafiltration, **colloidal deposition** and **physical compaction** of the membrane particularly in reverse osmosis due to high pressure.
- Fouling is the generic name for a number of phenomenon that cause irreversible change in membrane properties and effect of which can only be reversed if at all by cleaning.
- Fouling is responsible for at least part of the initial fall of membrane performance and is responsible for long-term reduction in the flux across the membrane.
- In protein purification, the main mode of fouling is macromolecular adsorption, pore blockage and cake formation.
- Adsorption occurs due to combination of hydrophobic and electrostatic interaction.
- Pore blockage occurs when solutes such as protein mixtures, cells and cell debris lodge at the pore entrance or adhere to the pore wall, resulting in the reduction of the pore diameter or non-availability of pore for the permeate flow.
- Cake formation is consequences of concentration polarization and consequent formation of a concentrated layer close to the membrane surface.
- Membrane fouling is a major problem associated with wide varieties of the membrane based separation process, resulting in severe fall of flux and deterioration of the product quality.
- In biofouling, bacteria grow on the surface of the susceptible membrane resulting in fouling.
- Organic fouling by organic components such as oil or grease is most common in water purification system and industrial effluent treatment.
- Ultrafiltration membranes are flushed with hot water at higher possible circulation rate, treated with acids or alkali agents depending on the nature of the layer, treated with hot detergent solution, and flushed with water to remove detergents.

VTU: Explain reverse osmosis in detail

3. Application of Membrane separation techniques: Reverse Osmotic technique

- Reverse osmosis membranes have the smallest pore structure, with pore diameter ranging from approximately 5-15 A^0 (0.5 nm - 1.5 nm).
- The extremely small pore size with the molecular weight of about 200 allows only small fractions of organic molecules and uncharged solutes to pass through the semi-permeable membrane along with the water, but rejects more than 95-99% of inorganic salts and charged organics due to charge repulsion established at the membrane surface.
- Reverse osmosis membrane eliminates the dissolved solids, bacteria, viruses and other colloids contained in the water.
- Reverse osmosis is a pressure driven membrane diffusion process of desalting water using membranes that are permeable to water but essentially impermeable to salt.
- Presently reverse osmosis membranes at 800-1000psi with a target salt rejection of about 99 % desalinate seawater with 3.5 weight percentage salt.
- Of recently, reverse osmosis process at pressures in the 150–400 psi range to desalinate brackish water with salt concentrations of 0.2–0.5 weight percentage typically operated with a target salt rejection of about 99 %.
- Reverse osmosis membranes are categorized into three types. First category is the reverse osmosis membrane that works at 200-1000psi pressure to desalinate seawater and brackish water with 0.5 to 5% weight salt solution. Second category of reverse osmosis membranes works at low pressure nanofiltration at 100-200psi to remove salt solution of 200-5000ppm salt solution. Third category of reverse osmosis membranes used for the nanofiltration of solutes from organic salt solution.
- Reverse osmosis is commonly used for producing potable water from sea or brackish water, ultrapure water for food processing, pharmaceutical grade water, and water for chemical, pulp and paper industry.
- Reverse osmosis has a potential application in municipal and industrial waste treatment, process water for boilers, de-watering of feed streams, and processing high-temperature feed-streams.

Application of reverse osmosis system

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- About 50% of the reverse osmosis systems are installed in the desalination of the brackish or seawater, 40% of the systems are used in the production of ultrapure water, pharmaceuticals and power generation industries, and remaining systems are installed in the food processing and pollution control.

Concentration of enzymes

- Reverse osmosis is simply ultrafiltration using a membrane with pores small enough to allow the passage of solvent molecules only.
  Hhence Reverse osmosis process thus helps us to concentrate and recover enzyme solutions from low molecular weight solutes such as ammonium sulphate from enzymes after enzyme precipitation.

Concentration of sugar

- Reverse osmosis process is efficient in concentrating sugar solution from 3 to 15% of sugar to solutions with sugar concentration between 25 and 30%.

Dialysis

- RO effectively remove some of the middle molecular weights metabolites such as urea and creatinine in blood, but not metabolites with the molecular weight between 1000 and 10000.

Blood oxygenation

- Blood oxygenators are used during surgery when the patient's lungs cannot function normally. Blood is generally circulated on the outside of the fibers to maintain good mass transfer with minimal pressure drop through the device.

Organic solvent separation

- Reverse osmotic membranes are successfully used in the separation of small solvent molecules from larger hydrocarbons in mixtures resulting from the extraction of vacuum residual oil in refineries.

Food Industry

- Reverse osmosis is an efficient and economical operation for the concentrating liquid foods such as orange juice and tomato pulp, compared to the heat treatment process.

Brackish water desalination

- Reverse osmosis membranes reduce the salinity of the seawater from as high as 10, 00 mg/l to 500mg/l. with the rejection of 90%. Christmas type or tapered type of module provides very high filtration rate.

Seawater desalination

- Since seawater contains 3-4% of salt, membranes such as polyamide hollow fine fibers and interfacial composites with 99.3% rejection or more can only produce water with less than 500mg/l of salt

Ultra pure water purification

- Ultrapure water require for research and electronic industry is prepared using municipal water with the dissolved salts of 200ppm using ultra pure water purification system consisting of complex array of operations. Typical plant removes 98% of all the salts and dissolved particles with the recovery rate of 90% or more.

Water and waste water treatment

- In the industry, rain water collected from storm is purified by reverse osmosis system and used for boiler plants to prevent the scaling and corrosion of the equipments. Reverse osmosis is also used in the treatment of effluents and brackish ground water. Reverse osmosis system is effective in the recovery of pollutants in the industrial effluents before discharging it in to reservoirs.

VTU: Write short notes on precipitation method a) with salt b) using organic salt

4. Critically evaluate the protein precipitation techniques

Protein precipitation: methods

1. In the physiological conditions of biological fluids at **0.15 -0.22 M** most of the protein exists in soluble form.
2. **Solubility of the protein and electrolyte colloid** is due to the balance between **attractive and repulsive force** between colloidal particles not allowing them to form **aggregates**.
3. But solubility of the protein can be changed by
   - changing the charges,
   - changing the pH or
   - changing the dielectric constant.
4. Protein precipitation depends on

- either by modifying the solvent property
- or by modifying the solute property

5. **Modifying the solvent property**: changing pH, ionic strength, dielectric constant or water solubility
6. **Modifying the solute property:** using precipitating agent such as metals, polyelectrolyte or affinity reagents
7. **Precipitation:** Reduced the bulk of the volume by 10-15 folds (Concentration), purifies the product by 15-20 folds(Purification), and stabilizes the products more than years(stabilization).

Solubility

- Cohn's equation is an empirical equation that correlates the protein solubility and the salt molality in the salting-out region.
- Cohn equation, where log solubility varies inversely as an ionic strength, and straight line plot results in the slope *K*, and the intercept on the *y*-axis, *β,* which is the hypothetical solubility at zero ionic strength.
- Position of the straight line varies with the nature of the protein which in turn varies with the temperature and pH of the protein.

$$\ln S = \beta - kI$$

- where ***S* is the solubility** of the given protein, and ***I* is the ionic strength** of the given solution.
- ***β* is constant** for particular protein that depends on the **pH and temperature of the solution**. The constant ***k* is the salting out constant** that is characteristic of the protein being salted out, and it is

independent of the temperature and pH of the solution at or above the isoelectric point, but **dependent on the salt and the protein surface hydrophobicity**. Cohn's equation is valid only in the salting-out region and relates the effect of pH and effect of neutral salts

1. Isoelectric precipitation by change in pH

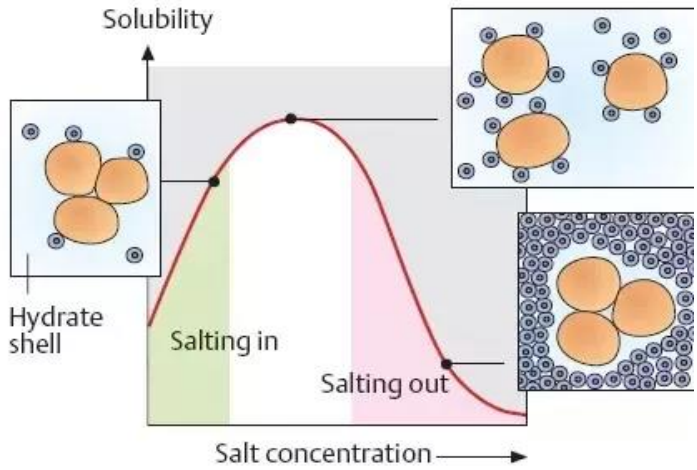
- Among other things, the solubility of protein **depends on the pH of the solution**.
- Protein can be either **positively or negatively charged** due to the **terminal amine (-NH_2) and carboxyl (-COOH) groups**, and **the side chain groups**.
- **Lowering pH of the solution increases the negative charges on proteins** and **increasing pH of the solution increases the positive charges on proteins**.
- Isoelectric point of the protein is the **intermediate pH at which a protein molecule has a net charge of zero**, because of the counterbalance positive and negative charges.
- Therefore, the **net charge on the protein, either positive or negative, can interact with water molecules**.
- This results in the **protein molecule dissociating itself from other protein molecules and becoming more soluble**.
- On the other hand, protein **is the least soluble when the pH of the solution is at its isoelectric point**
- The net charge on protein is **affected by pH of its surrounding solvent** and can become more positively or negatively charged due to the loss or gain of protons (H^+).
- The pI value can affect the solubility of a molecule at a given pH, and at this point such **molecules have minimum solubility in water or salt solutions leading to precipitation**

2 Change in ionic strength (Salting-in and salting-out)

- Surface of the globular protein facing the solvent contains **positive charged region, negative charged region, uncharged polar region, non-polar hydrophilic region hydrophobic region**.
- Proteins such as enzymes at physiological **salt conditions of around 0.15-0.2M and neutral pH are highly soluble** even at 40 % of protein solution.
- This solubility is due to the polar interaction with aqueous solvent, ionic interaction with the salts present and to some extent, repulsive electrostatic forces between like-charged molecules or small aggregates of molecules.
- Among other things, the solubility of protein **depends on the salt concentration in the solution**.

With the increase in the concentration in salt of such solution various charged groups on a protein molecule gets stabilized by the salt, thus attracting protein into the solution and enhancing the solubility of protein .

- The increase in the solubility at a given pH and temperature with increasing salt concentration is **salting-in process**, which takes place within the ionic strength ranging from zero to 0.5M.
- **Addition of salt to the solvent offers the shielding effect of the dissociated ions and restructures the water in the vicinity of the ions**.
- To exploit this property to isolate and purify protein molecules, **stabilizing salt ions are removed from the solution either by dilution method to decrease the salt concentration or by dialysis method to remove the stabilizing salt from the solution**, or also by adjusting the pH at constant ionic strength to precipitate the product of interest leaving behind other protein impurities in the solution.
- However, a point of maximum protein solubility reaches as the salt concentration of salt increases, and further increase in the salt concentration results in **decreased availability of water to solubilize protein due to the removal of water from the water shield surrounding the hydrophobic area**.
- Here, addition of salt results in the alteration of the free energy contributions for the formation of a solute sized cavity in the solvent and the **electrostatic free energy contributions**.
- The cavity formation or hydrophobic contribution reduces the solubility in the presence of salt, and it exceeds the electrostatic contribution as the salt concentration is increased.
- Finally, protein precipitates due to the non-availability of sufficient water molecules to interact with protein molecules.
- Salting-out is the phenomenon of protein precipitation in the presence of excess salt.



3 Organic solvent mediated precipitation

- Adding water miscible solvents such as ethanol or methanol into aqueous extracts of the protein causes the precipitation of proteins in the solution.
- Primary effect is due to the reduction of the water activity and reduction of the solvating power of the water for charged hydrophobic protein molecules.
- The solvation layer around the protein decreases as the organic solvent progressively displaces water from the protein surface and binds it in hydration layers around the organic solvent molecules.
- The proteins with reduced hydration layer can aggregate by attractive electrostatic and dipole forces.
- Important parameters to consider during organic solvent precipitation are the temperature, pH and protein concentration in solution.
- Water miscible organic solvents decrease the dielectric constant of water, which in turn facilitates two proteins to come very close to each other.
- Decreased solubility of protein leads to aggregation and precipitation due to electrostatic and dipolar interaction between oppositely charged region of protein molecules
- Reduction in the dielectric constant of the medium during solvent precipitation is as follows.

  $ln\ S = \frac{k}{D^2} + log\ S_o$, S_0 is an extrapolated value of S, D is the dielectric constant of the reagent-water mixture and k is a constant that relates to the dielectric constant of the original aqueous medium.

  Water is a polar molecule – it has a partial negative charge near the oxygen atom due the unshared pairs of electrons, and partial positive charges near the hydrogen atoms
- Because of these charges, polar molecules, like proteins, DNA or RNA, can interact electrostatically with the water molecules, allowing them to easily suspend in water.
- Polar molecules can therefore be described as hydrophilic and non-polar molecules, which can't easily interact with water molecules, are hydrophobic.
- Nucleic acids are hydrophilic due to the negatively charged phosphate (PO3-) groups along the sugar phosphate backbone.
- The electrostatic attraction between the Cations in solution and the an ions on protein are dictated by Coulomb's Law, which is affected by the dielectric constant of the solution. Water has a **high dielectric constant**, which makes it fairly difficult for the Cationa and anions to come together. Ethanol on the other hand has a much lower dielectric constant, making it much easier for Cation to

interact with the anions, shield it's charge and make the nucleic acid less hydrophilic, causing it to drop out of solution.

4 Non-ionic polymer mediated precipitation

- Water-soluble ppolymers, such as dextrans and polyethylene glycols, cause aggregation of proteins without protein denaturation even at ambient temperature.
- The polymers are frequently used in protein precipitation because they have low flammability and are less likely to denature biomaterials than isoelectric precipitation.
- These polymers in solution attract water molecules away from the solvation layer around the protein, resulting in the increased protein-protein interactions and precipitation.
- PEG up to 20% are not too viscous and is commercially available polymer with most common of them is 6000 Da.
- It is used to precipitate plasma protein fibrinogen and globulin.
- These polymers reduce the availability of affective amount of water required for the solvation of protein and exclude the protein from part of the solution.
- Solubility of the protein in proportional to the accessible volume and it is a linear equation. $ln\ S = B - kC$

VTU: Describe the aqueous two-phase extraction technique in details

Introduction

- Aqueous two phase extraction (ATP) is one of the key separation methods in downstream processing
- Its biocompatibility is one of the advantages in protein partitioning with good selectivity at minimal protein loss
- ATP can be applied as a selective technique for a particular protein separation by integrating with affinity ligands.
- ABS is an excellent method to employ for the extraction of proteins/ enzymes and other labile biomolecules from crude cell extracts or other mixtures
- Aqueous two-phase systems are generally composed of a water solution of two structurally distinct hydrophilic polymers or of one polymer and certain salts

Principle:-

- In general, there are two major types of ATPS available, viz., polymer/polymer (e.g Polyethylene glycol/Dextran) and polymer/salt (e.g Polyethylene glycol/phosphate) system.
- It is formed by mixing two different water-soluble polymers or one water-soluble polymer and salt in water.
- When the limiting concentrations are exceeded, two immiscible aqueous phases are formed.
- The limiting concentrations depend on the type of phase forming components and on the pH, ionic strength and temperature of the solution.
- **PEG–dextran system**

- The "upper phase" is formed by the more hydrophobic polyethylene glycol (PEG), which is of lower density than the "lower phase," consisting of the more hydrophilic and denser dextran solution.
- Although PEG is inherently denser than water, it occupies the upper layer. This is believed to be due to its solvent 'ordering' properties, which excludes excess water, creating a low density water environment.
- The degree of polymerisation of PEG also affects the phase separation and the partitioning of molecules during extraction.

Factors Affecting

- **Biomolecule concentration :** Increase in protein conc and mol wt decreases their separation in upper phase.
- **Polymer concentration** : Viscosity play important role here so for extreme partition high content of polymer is to be chosen.
- **Surface properties of biomolecule** : surface net charge plays important role in hydrophobicity or hydrophillicity of biomolecule.

for e.g. ; lysine and glutamic acid have relatively low hydrophobicity and they partition favourably in the salt rich phase in the PEG/ salt ATP systems.

- **PH :** In PEG/Salt system as pH changes from acidic to basic the protein becomes less positive or more negative charge and negative charge protein prefer upper phase.
- **Temperature :** At high temp it is easy to form two phase system with small conc of PEG and salt . PEG/Dextran system will form two phase system at low temp.
- **Affinity ligand attach to polymers :** PEG has many sites to which other groups can attach . When hydrophobic group is attach the hydrophobic protein and amino acid will be separated in the upper phase.
- **Salt :** salt ion partition differently between the phases causing an uneven distribution in the system that generates a difference in electric potential between the phases.

Advantages

- They provide mild conditions that do not harm or denature unstable/labile biomolecules
- The interfacial stress (at the interface between the two layers) is far lower (400-fold less) than water-organic solvent systems used for solvent extraction, causing less damage to the molecule to be extracted
- The polymer layer stabilizes the extracted protein molecules, favouring a higher concentration of the desired protein in one of the layers, resulting in an effective extraction

Application

THE PURIFICATION OF RECOMBINANT PHARMACEUTICAL PROTEINS:-

•Human insulin-like growth factor 1 (IGF-1) accu- mulates in medium and cellular periplasmic space when expressed in *E. coli* with an endogenous secretary signal sequence.

• Due to its heterogeneity in form and location, low yield of IGF-1 was obtained using a typical recovery strategy.

•To enhance recovery yield, a new method, called *in situ* solubilization, involves addition of chaotrope and reductant to alkaline fermentation broth and provides re-covery of about 90% of all IGF-1 in an isolated super-natant. Then, an aqueous two-phase extraction procedure was employed which partitions soluble non-native IGF-1 and biomass solids into separate liquid phases

Chapter 9: supercritical extraction

- The formation of a supercritical fluid is the result of a dynamic equilibrium.
- When a material is heated to its specific critical temperature in a closed system, at constant pressure, a dynamic equilibrium is generated.
- This equilibrium includes the same number of molecules coming out of liquid phase to gas phase by gaining energy and going in to liquid phase from gas phase by losing energy.
- At this particular point, the phase curve between liquid and gas phases disappears and supercritical material appears.
- In order to understand the definition of SF better, a simple phase diagram can be used. Figure 1 displays an ideal phase diagram.
- For a pure material, a phase diagram shows the fields where the material is in the form of solid, liquid, and gas in terms of different temperature and pressure values.
- Curves, where two phases (solid-gas, solid-liquid and liquid-gas) exist together, defines the boundaries of the phase regions.
- These curves, for example, include sublimation for solid-gas boundary, melting for solid-liquid boundary, and vaporization for liquid-gas boundary.
- Other than these binary existence curves, there is a point where all three phases are present together in equilibrium; the triple point (TP).

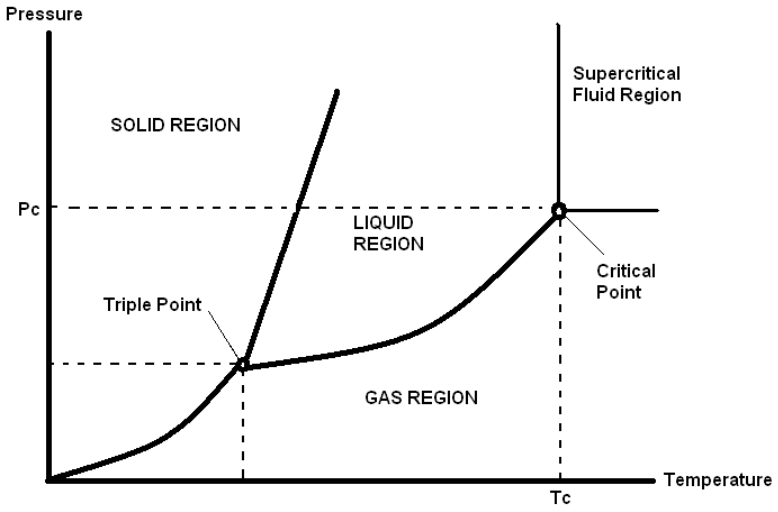


Figure 1 Schematic representation of an idealized phase diagram

- The unique physical properties of supercritical fluids, having values for density, diffusivity and viscosity values between liquids and gases, enables supercritical fluid extraction to be used for the extraction processes which cannot be done by liquids due to their high density and low diffusivity and by gases due to their inadequate density in order to extract and carry the components out.
- Complicated mixtures containing many components should be subject to an extraction process before they are separated via chromatography.
- An ideal extraction procedure should be fast, simple, and inexpensive. In addition, sample loss or decomposition should not be experienced at the end of the extraction.

- Following extraction, there should be a quantitative collection of each component. Ideally, the amount of unwanted materials coming from the extraction should be kept to a minimum and be easily disposable; the waste should not be harmful for environment. Unfortunately, traditional extraction methods often do not meet these requirements. In this regard, SFE has several advantages in comparison with traditional techniques.
- The extraction speed is dependent on the viscosity and diffusivity of the mobile phase. With a low viscosity and high diffusivity, the component which is to be extracted can pass through the mobile phase easily. The higher diffusivity and lower viscosity of supercritical fluids, as compared to regular extraction liquids, help the components to be extracted faster than other techniques. Thus, an extraction process can take just 10-60 minutes with SFE, while it would take hours or even days with classical methods.
- The dissolving efficiency of a supercritical fluid can be altered by temperature and pressure. In contrast, liquids are not affected by temperature and pressure changes as much. Therefore, SFE has the potential to be optimized to provide a better dissolving capacity.
- In classical methods, heating is required to get rid of the extraction liquid. However, this step causes the temperature-sensitive materials to decompose. For SFE, when the critical pressure is removed, a supercritical fluid transforms to gas phase. Because supercritical fluid solvents are chemically inert, harmless and inexpensive; they can be released to atmosphere without leaving any waste. Through this, extracted components can be obtained much more easily and sample loss is minimized.

Chapter 10: In-situ product recovery /integrated processing/hybrid DSP

- In situ/In stream recovery techniques Optimization of the process has also been investigated with the use of in situ processes to remove butanol.
- This technique consists in the removal of selective reaction products during fermentation. Different approaches exist to develop an integrated product recovery system.
- This system consists of a fermentation unit coupled to a product separation unit.
- Two different setups can be designed:
- - In situ recovery: the concentration step occurs inside the bioreactor, where the product is partially separated, Figure 3. The alcohol-depleted fermentation broth never leaves the bioreactor.

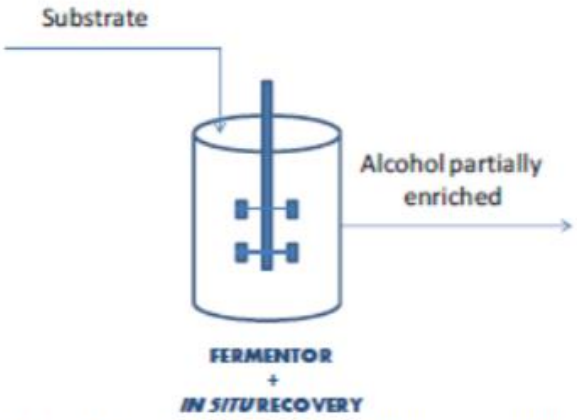


Figure 3. Fermentation In situ recovery process [18].

- In stream recovery: fermentation and primary separation are carried out concomitantly but in two distinct vessels. This implies the continuous pumping of the fermentation broth though another column containing the selective phase or material, which allows separating a fraction of the product,

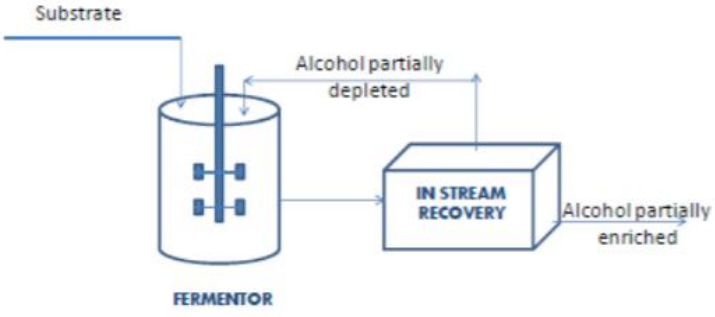


Figure 4. Fermentation In stream recovery process [18].

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- Figure 4. The alcohol – depleted stream is returned to the bioreactor.
- This can be an advantage in different ways, like enriching the end product concentration leading to lower downstream processing costs, improve the productivity by removal of the inhibition product, reduce the process flows since it achieves higher product concentrations and for last it also increases the product yield, reducing side reactions by the product removal.
- In both previous process configurations, there is a decrease in the toxic effect of the alcohol on bacterial cells due to a continuous removal of the product from the fermentation broth.
- In fact, if the butanol is recovered as fast as it is produced the fermentation inhibition can be avoided and therefore the microbial culture can achieve a higher product yield and productivity.

- Furthermore, by alleviating product toxicity, higher substrate concentrations in the fermentation broth can be considered (possibly in a fed-batch operation).
- The advantages and disadvantages of both configurations are in fact according to the type of the separation unit used, between adsorption, liquid-liquid extraction, stripping and more.
- The integrated product recovery techniques of the alcohols from aqueous broth can be based on the difference between physical or chemical properties of the different alcohols and water or on their interaction with an auxiliary agent or material.
- The end product enrichment depends on the selectivity of the ISPR (in situ process removal) technique but processes with lower separation factors can still obtain similar or higher product concentration in the concentrate, if operated at higher residual products concentrations.
- Therefore the concentrate concentration is strongly connected to the product concentration in the fermentation broth