MARUDHAR KESARI JAIN COLLEGE FOR WOMEN (AUTONOMOUS)

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PG and Department of Biotechnology

IInd M.Sc. Biotechnology – Semester - III

E-Notes (Study Material)

Core Course 9-: BIOPROCESS TECHNOLOGY
Unit: III Introduction to bioproducts and bio separation. Primary recovery process: Cell disruption methods. Cell lysis and Flocculation: Osmotic and mechanical methods of lysis. Flocculation by electrolysis; polymorphic flocculation. Precipitation methods. Filtration: Principles, Conventional, Crossflow filtration. Sedimentation: Principles, Sedimentation coefficients. Extraction Principles, Liquid liquid extraction, aqueous two-phase extraction, supercritical fluid extraction. (20 Hours)
Learning Objectives: Outline the basis of Bioprocess Engineering
Course Outcome: Students will gain a comprehensive understanding of the basic principles underlying fermentation processes, including the types of fermentations and their applications in various industries.

Introduction of I. Bioproducts

Classification and characteristics of bioproducts

Characteristic of fermentation broth on downstreamprocessing.

Broad classification of bioproducts.

Primary and secondary metabolite.

II. Bioseparations

Introduction to bioseparations.

Stages of downstream processing.

Basic principles of engineering analysis.

Criteria for process development

Bioproducts:

- Chemical substances made by living things ranging from small molecules to higher molecules (macromolecules).
- Derived byextractionfrom original host or bysynthesisinbioreactor containing cells or

enzymes.

1. **Definition and Scope:**

- **Bioproducts**: Products derived from biological processes or materials, such as plants, animals, or microorganisms.
- **Renewable Resources**: Unlike fossil fuels or non-renewable resources, bioproducts are based on materials that can be replenished naturally over time.

2. Categories of Bioproducts:

- **Biochemicals**: These are chemical products derived from biological sources or processes, such as biofuels (ethanol, biodiesel), biodegradable plastics, and various industrial enzymes.
- **Biomaterials**: These include materials used in medical devices, implants, or as replacements for synthetic materials. Examples are bioplastics and tissue engineering scaffolds.
- **Biopharmaceuticals**: Medicines derived from biological sources, including vaccines, monoclonal antibodies, and gene therapies. These are produced using biotechnology methods and often involve genetic engineering.
- **Bioenergy**: Energy derived from biological sources, such as bioethanol from crops, biogas from organic waste, and biomass energy. These alternatives are key to reducing reliance on fossil fuels.
- **Agricultural Products**: Products like bio-pesticides and bio-fertilizers that help enhance agricultural productivity while being environmentally friendly.

3. Production Methods:

- **Biotechnology**: Involves the use of living organisms or their systems to develop products. This includes genetic engineering, fermentation, and cell culture technologies.
- **Bioconversion**: The process of converting organic materials into useful products using biological agents. For instance, using microorganisms to produce biofuels or pharmaceuticals.

4. Benefits:

- **Environmental Impact**: Bioproducts often have a lower environmental footprint compared to conventional products. They can reduce waste, lower greenhouse gas emissions, and minimize pollution.
- **Sustainability**: They offer a sustainable alternative to products made from non-

renewable resources, supporting a circular economy.

- **Economic Opportunities**: The bioproducts industry can create new job opportunities, drive innovation, and stimulate economic growth in sectors like agriculture, manufacturing, and healthcare.

Introduction to Bioseparation

1. Overview of Bioseparation: Bioseparation is a critical field in biotechnology and chemical engineering that focuses on isolating and purifying biological products from complex mixtures. It involves separating biological molecules such as proteins, nucleic acids, cells, or other biomolecules from each other or from non-biological contaminants.

2. Importance of Bioseparation:

- **Biopharmaceuticals: Purification of proteins and peptides for drug development and production.**
- **Environmental Applications: Removing contaminants from waste streams.**
- **Food and Beverage Industry: Ensuring purity and quality of food products.**
- **Research: Isolating specific biomolecules for scientific studies.**

3. Key Components of Bioseparation Processes:

- **Feed Stream: The initial mixture containing the target biomolecule along with contaminants.**
- **Target Molecule: The biomolecule or biological product of interest.**
- **Separation Techniques: Methods used to isolate the target molecule from the feed stream.**

4. Common Bioseparation Techniques:

- **Filtration: Using porous membranes or filters to separate particles based on size.**
  - ***Microfiltration:*** **Removes larger particles like cells.**
  - ***Ultrafiltration:*** **Separates smaller molecules such as proteins from solvents.**
  - ***Nanofiltration:*** **Filters out even smaller ions and molecules.**
- **Centrifugation: Utilizing centrifugal force to separate components based on density.**
  - ***Differential Centrifugation:*** **Separates cell components.**
  - ***Density Gradient Centrifugation:*** **Separates molecules based on density gradients.**
- **Chromatography: Separating biomolecules based on their interactions with a stationary phase and a mobile phase.**

- ***Affinity Chromatography:*** **Uses specific interactions between the target molecule and a ligand.**
- ***Ion Exchange Chromatography:*** **Separates based on charge differences.**
- *Size Exclusion Chromatography:* Separates based on size and shape.

- **Electrophoresis: Using an electric field to separate biomolecules based on their size and charge.**
  - ***Agarose Gel Electrophoresis:*** **Common for nucleic acids.**
  - ***SDS-PAGE:*** **Used for protein separation.**
- **Precipitation: Involves adding chemicals to cause specific biomolecules to aggregate and precipitate out of solution.**
- **Extraction: Using solvents to separate biomolecules based on their solubility in different phases.**

5. Challenges in Bioseparation:

- **Specificity: Achieving high selectivity for the target molecule while minimizing the loss of other valuable components.**
- **Scalability: Transitioning from laboratory-scale to industrial-scale processes without compromising efficiency or quality.**
- **Cost: Managing costs associated with reagents, equipment, and labor.**

6. Trends and Innovations:

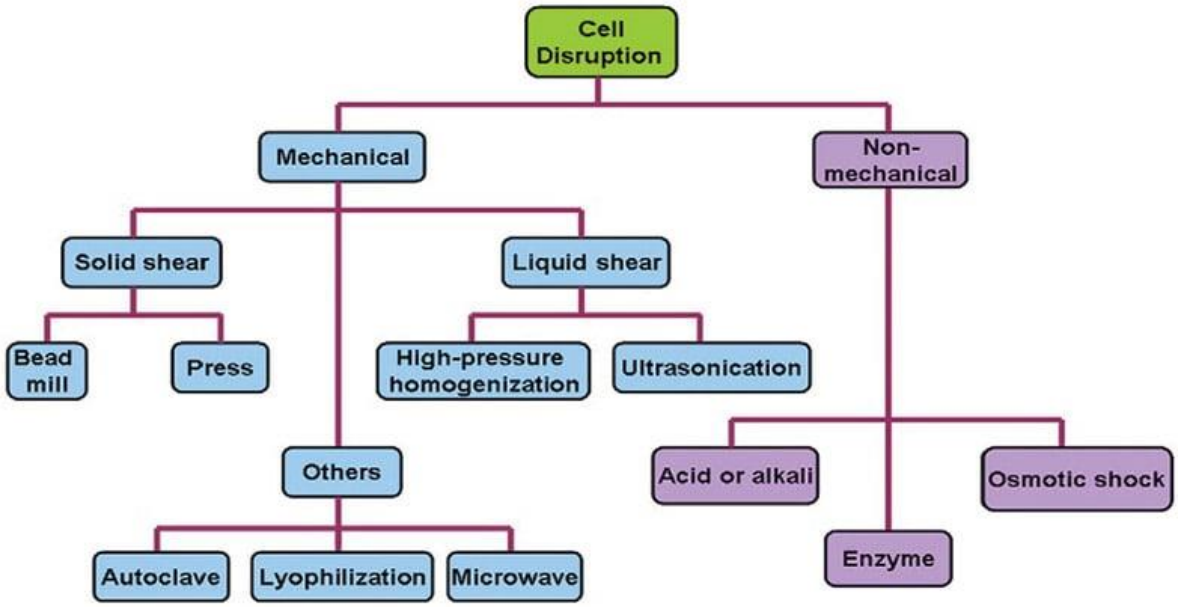
- **Enhanced Affinity Materials: Development of new ligands and materials for improved selectivity.**
- **Automation: Incorporation of robotic systems for high-throughput processing.**
- **Green Chemistry: Designing processes that reduce waste and environmental impact.**

Cell Disruption- Definition, Methods, Types, Significance

Introduction

- 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.
- The method used may vary depending on the type of cell and its cell wall composition.
- Irrespective of the method used, the main aim is that the disruption must be effective and the method should not be too harsh so that the product recovered remains in its active form.

- Cell disruption methods can be categorised into mechanical methods and non-mechanical methods.
- Mechanical methods are divided into solid shear methods and liquid shear methods.
- Non-mechanical methods can be divided into physical methods, chemical methods and enzymatic methods.



Mechanical Methods of Cell Disruption

Mechanical methods are those methods that required some sort of force to separate out intracellular protein without adding chemical or enzyme

1. Mortar & pastel/grinding
2. Blender
3. Bead beating
4. Ultra sonication
5. Homogenization

Mortar & Pestle

- It involves the grinding of the cells such that they are disrupted.
- This does not have to be in suspension and is often done with plant samples frozen in liquid nitrogen.
- When the material has been disrupted, metabolites can be extracted by adding solvents.

Blenders

- The use of blenders which employ high speed can be used to disrupt cell walls.
- It is the same process used by centrifugation, which separates or concentrates materials suspended in a liquid medium.

Bead beating

- Glass or ceramic beads are used to crack open cells
- The kind of mechanical shear is gentle enough to keep organelles intact.
- It can be used with all kinds of cells, just add beads to an equal amount of cell suspension and vortex.

Ultrasonication

- Ultrasonic homogenizers work by inducing vibration in a titanium probe that is immersed in the cell solution.
- A process called cavitation occurs, in which tiny bubbles are formed and explode, producing a local shockwave and disrupting cell walls by pressure change.
- This method is very popular for disruption of plant and fungal cells.

Homogenization

- Liquid-based homogenization is the most widely used cell disruption technique for small volumes and cultured cells.
- Cells are lysed by forcing the cell or tissue suspension through a narrow space
- Homogenizers use shearing forces on the cell similar to the bead method.
- Homogenization can be performed by squeezing cells through a tube that is slightly smaller than beads beating.

Non-Mechanical Methods of Cell Disruption

Nonmechanical methods are further divided into three classes which are following :

A. Physical methods

1. Freeze-Thaw

- It is suitable when working with soft plant material and algae.
- Disruption is achieved via a series of freezing and thawing cycles.
- Freezing forms ice crystals, which expand upon thawing, and this ultimately causes the cell wall to rupture.

2. Microwave/ Thermolysis

- Microwave (along with autoclave and other high temperature methods) are used to disrupt the bonds within cell walls, and also to denature proteins.
- However, uncontrolled amount of heat can easily denature or damage target proteins and subtances.

3. Osmotic Shock

- Through the process of osmosis, water can be moved into the cell causing its volume to increase to the point that it bursts.

- The method however, can only work with animal cells and protozoa, since they do not have cell walls.

4. Electric Discharges

- It is also possible to achieve cell disruption via electrical discharges in mammalian and other cells that are bounded by plasma membranes only.

B. Chemical methods

- They are often used with plant cells (and sometimes in combination with shearing).
- Organic solvents such as toluene, ether, benzene, methanol, surfactants, and phenyl ethyl alcohol DMSO can be used to permeate cell walls.
- EDTA can be used specifically to disrupt the cell walls of gram negative bacteria, whose cell walls contain lipopolysaccharides that are stabilized by cations like Mg^{2+} and Ca^{2+}.
- EDTA will chelate the cations leaving holes in the cell walls.

C. Enzymatic methods

- Another strategy to achieve cell lysis is to use digestive enzymes which will decompose the microbial cell wall.
- Different cell types and strains have different kind of cell walls and membranes, and thus the used enzyme depends on microbe. For example, lysozyme is commonly used enzyme to digest cell wall of gram positive bacteria. Lysozyme hydrolyzes β-1-4-glucosidic bonds in the peptidoglycan.
- The cell wall of yeast and fungi differs significantly from the cell wall bacteria. One commonly used enzyme mixture for degradation of cell wall of yeast and fungi is Zymolyase.
- It has for example β-1,3 glucanase and β-1,3-glucan laminaripentao-hydrolase activities (Zymolyase | Yeast lytic enzyme).
- In addition, the enzymes that are commonly used for degradation of cell wall of yeast and fungi include different cellulases, pectinases, xylanases and chitinases.
- Enzymes such as beta(1-6) and beta(1-3) glycanases, proteases and mannase can also be used to disrupt the cell wall.

Significance of Cell Disruption

- Cell disruption is an essential part of biotechnology and the downstream processes related to the manufacturing of biological products.
- It is necessary for the extraction and retrieval of the desired products, as cell disruption significantly enhances the recovery of biological products.

References

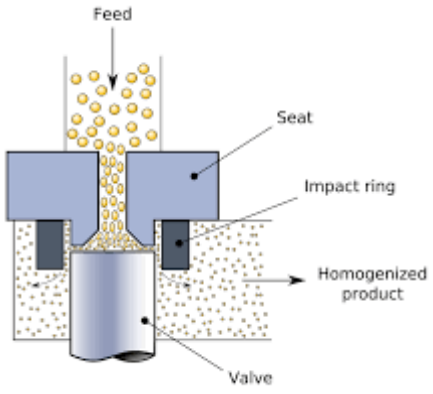
1. Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
2. file:///C:/Users/user/Downloads/Cell%20disruption%20methods.pdf
3. https://www.slideshare.net/sabanaeem1/cell-disruption
4. Chisti Y., Moo-Young M. (1986); Disruption of microbial cells for intracellular products; Enzyme Microb. Technol., vol. 8, April; doi: 0141 –0229/86/040194–11.

What is Cell Lysis?

Cell lysis is the process of breaking down the cell membrane to release the cell's internal contents. It's a crucial step in various biological and biochemical experiments, including protein extraction, nucleic acid isolation, and studying cellular components.

Types of Cell Lysis

1. **Physical Methods:**
    - **Mechanical Disruption:**
        - **Homogenization:** Grinding or shearing cells using a homogenizer or blender.
        - **Sonication:** Using high-frequency sound waves to break cell membranes.
        - **Freeze-Thaw Cycles:** Repeated freezing and thawing of cells to disrupt membranes



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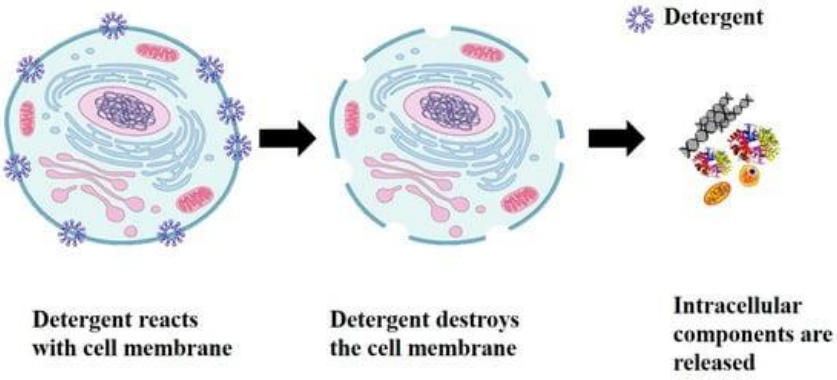
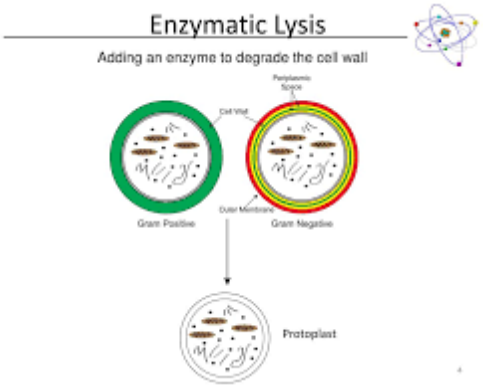
    - **Pressure:** High-pressure methods like French press can disrupt cell walls.
2. **Chemical Methods:**
    - **Detergents:** Using surfactants like Triton X-100 or SDS to solubilize cell membranes.



- **Hypotonic Solutions:** Using solutions with low salt concentrations can cause cells to swell and burst.

3. **Enzymatic Methods:**
   - **Lysozyme:** An enzyme that breaks down bacterial cell walls.
   - **Proteases:** Enzymes that degrade proteins, often used in combination with other methods.



Applications of Cell Lysis

1. **Protein Extraction:** Isolating proteins for analysis like Western blotting or enzyme assays.
2. **Nucleic Acid Isolation:** Extracting DNA or RNA for PCR, sequencing, or other molecular biology techniques.
3. **Cellular Component Study:** Analyzing organelles or other cell components.

Considerations for Cell Lysis

1. **Cell Type:** Different cells require different lysis methods. For example, bacterial cells may need enzyme treatment while mammalian cells might require detergents.
2. **Lysis Buffer Composition:** The choice of buffer can affect the outcome. For instance, buffers might contain salts, pH stabilizers, or protease inhibitors.
3. **Temperature:** Lysis conditions can be affected by temperature; some processes are better performed on ice to prevent degradation.

Lysis Buffer Components

- **Detergents:** Break down lipids in cell membranes (e.g., SDS, Triton X-100).
- **Salts:** Maintain osmotic balance (e.g., NaCl).
- **pH Buffers:** Maintain pH stability (e.g., Tris-HCl).
- **Protease Inhibitors:** Prevent protein degradation during lysis.

Troubleshooting Common Issues

- **Incomplete Lysis:** Try different methods or adjust buffer composition.
- **Protein Degradation:** Use protease inhibitors and keep samples on ice.
- **Viscosity Issues:** High viscosity can indicate DNA release; use DNase to degrade DNA if needed.

Applications of Flocculation in Bioprocess Technology

1. **Cell Harvesting:**
    - **Microbial Fermentation:** During microbial fermentation processes, cells and cell debris are suspended in the fermentation broth. Flocculation helps aggregate these cells, making it easier to separate them from the broth using centrifugation or filtration.
    - **Cell Culture:** In mammalian cell culture, flocculation can be used to aggregate and separate cells from the culture medium, aiding in the recovery and processing of cells for further analysis or product recovery.
2. **Protein Purification:**
    - **Clarification:** Flocculation can be employed to remove cell debris and other particulates from crude protein extracts. This step is crucial for clarifying the solution before more refined purification techniques, such as chromatography, are applied.
    - **Precipitation:** Flocculants can also be used to precipitate proteins or other biomolecules from solution, aiding in their concentration and purification.
3. **Biological Waste Treatment:**
    - **Effluent Treatment:** In bioprocessing facilities, waste streams containing residual cells, metabolic by-products, and other impurities are treated using flocculation. This helps in the removal of suspended solids and clarification of the effluent before further treatment or disposal.
4. **Downstream Processing:**
    - **Precipitation of Products:** Flocculation can be used to precipitate target products from a fermentation broth or cell lysate. This helps in concentrating

and purifying the product before further processing steps.

Types of Flocculants Used

1. **Inorganic Flocculants:**
    - **Aluminum Sulfate (Alum):** Commonly used in water treatment and can be applied in some bioprocesses for coagulation and flocculation.
    - **Ferric Chloride:** Another inorganic flocculant used in various applications for particle aggregation.
2. **Organic Flocculants:**
    - **Polymers:** Synthetic polymers, such as polyacrylamides, are often used in bioprocess technology for their effectiveness in flocculating cells and proteins. Natural polymers, such as guar gum or alginates, are also used in specific applications due to their biocompatibility and biodegradability.

Flocculation Process in Bioprocessing

1. **Preparation of Flocculant Solution:**
    - Flocculants are dissolved in water or buffer solutions to create an appropriate concentration for the process.
2. **Mixing:**
    - The flocculant solution is added to the bioprocess liquid (e.g., fermentation broth). Gentle mixing ensures uniform distribution and interaction of the flocculant with the particles or cells.
3. **Flocculation:**
    - Particles or cells aggregate into larger clumps or flocs. This can be facilitated by maintaining specific conditions such as optimal pH, temperature, and flocculant concentration.
4. **Separation:**
    - The formed flocs are separated from the liquid using techniques such as centrifugation, filtration, or sedimentation.

Considerations for Effective Flocculation

1. **Concentration and Type of Flocculant:**
    - The choice and concentration of flocculant must be optimized based on the specific requirements of the bioprocess and the characteristics of the suspended particles or cells.
2. **pH and Ionic Strength:**
    - The pH and ionic strength of the solution can affect flocculation efficiency. It

is important to adjust these parameters to optimize the process.

3. **Mixing Conditions:**
    - Proper mixing is crucial to ensure effective interaction between the flocculant and suspended particles, while avoiding excessive agitation that might break up the flocs.
4. **Temperature:**
    - The temperature of the solution can impact the flocculation process. Maintaining an appropriate temperature can enhance the effectiveness of flocculation.

Challenges and Troubleshooting

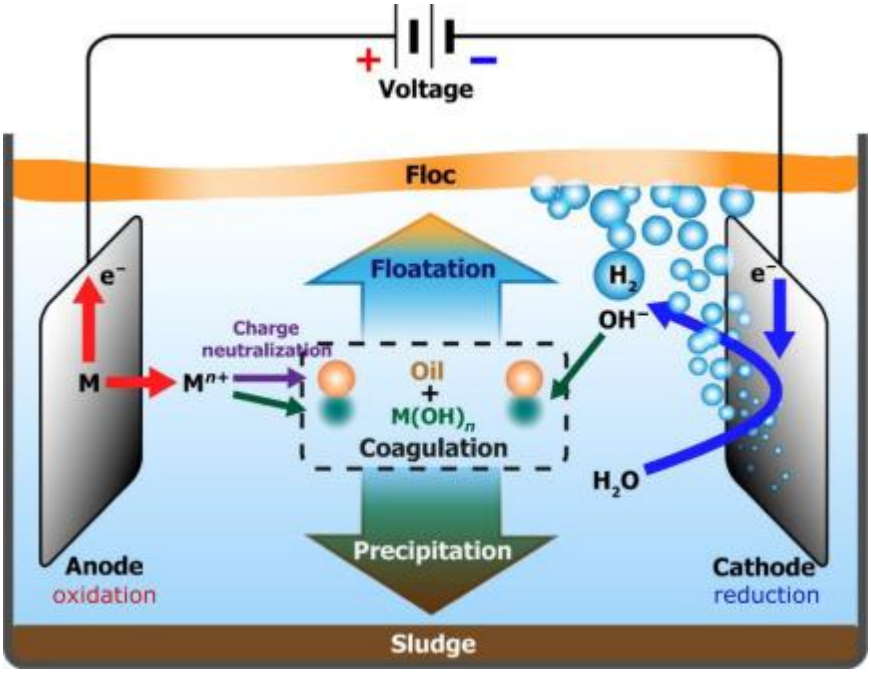
- **Incomplete Flocculation:** Adjust flocculant dosage or mixing conditions to improve flocculation efficiency.
- **Excessive Sludge Production:** Optimize the concentration of flocculants and manage the volume of generated sludge to avoid handling issues.
- **Floc Breakage:** Avoid excessive agitation and ensure optimal conditions to maintain floc stability.

Flocculation by Electrolysis

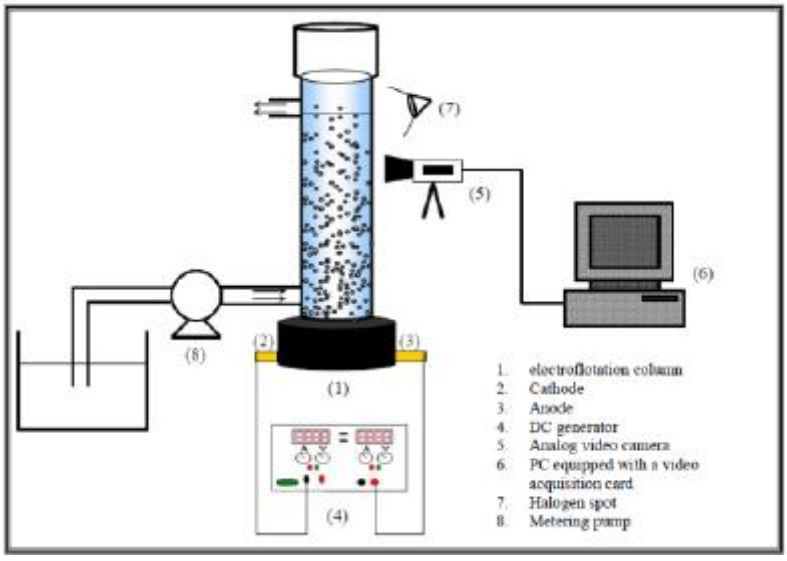
Flocculation by electrolysis involves using electrical currents to induce the formation of flocs, which can then be separated from a liquid. This technique is often used in water treatment and various industrial processes.

Mechanism:

1. **Electrolytic Coagulation:**
    - **Electrochemical Reaction:** Electrolysis involves passing an electric current through the liquid. This current causes electrochemical reactions at the electrodes, which can produce coagulant agents in situ (e.g., aluminum or iron salts).
    - **Formation of Flocs:** The coagulant agents formed through electrolysis neutralize the charges on suspended particles, leading to aggregation and formation of flocs.



2. **Electroflotation:**
    - **Bubble Formation:** Electrolysis generates gas bubbles (e.g., hydrogen or oxygen) at the electrodes. These bubbles adhere to the particles, causing them to float and form flocs that can be skimmed off the surface.



Applications:

- **Water Treatment:** Used for treating drinking water and wastewater to remove suspended solids, organic matter, and other contaminants.
- **Mining:** Applied for mineral processing and tailings management.
- **Food and Beverage Industry:** Used for clarifying liquids such as juice or wine.

Advantages:

- **In Situ Generation of Coagulants:** Coagulants are generated directly in the treatment

system, reducing the need for chemical storage and handling.

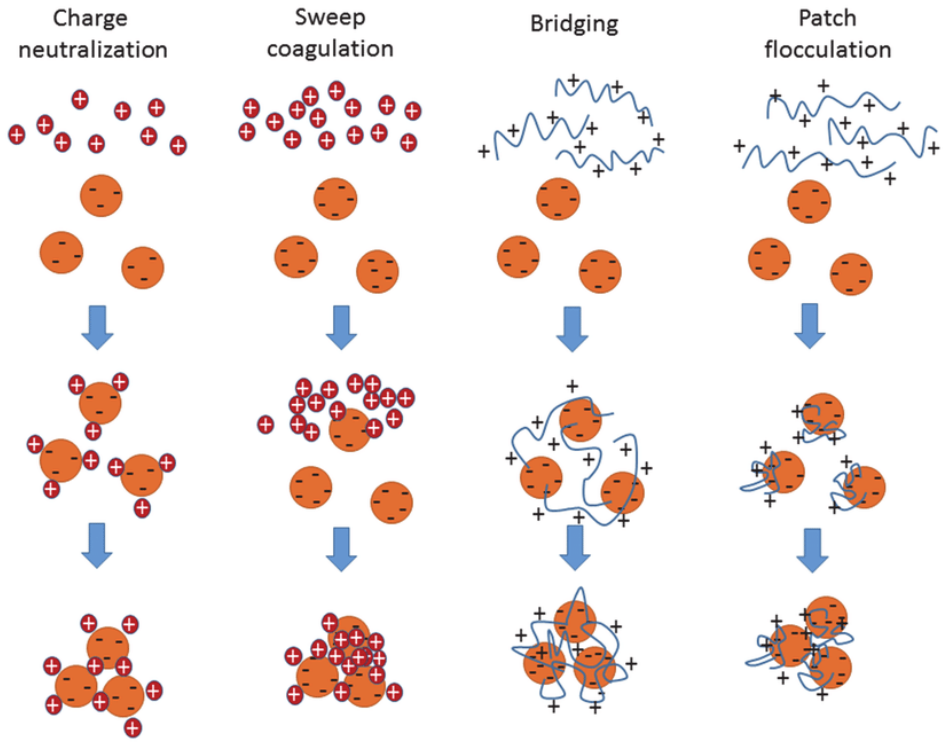
- **Reduced Chemical Costs:** Electrolysis can be cost-effective compared to purchasing and adding pre-made coagulants.

Considerations:

- **Energy Consumption:** Electrolysis can be energy-intensive, which may impact operational costs.
- **Electrode Maintenance:** Electrodes can degrade over time and may require regular maintenance or replacement.

Polymorphic Flocculation

Polymorphic flocculation refers to the flocculation of particles in different polymorphic forms or structures. This concept is relevant when dealing with materials that can exist in multiple crystal forms or structural configurations.



Mechanism:

1. **Polymorphic Materials:**
    - **Multiple Forms:** Some substances can exist in different polymorphic forms (e.g., different crystal structures of a compound). Each polymorph may have distinct physical and chemical properties.
    - **Flocculation Behavior:** The flocculation behavior can vary depending on the polymorphic form of the particles. Different forms may interact differently with flocculants.

2. **Flocculation Process:**
    - **Polymorph-Specific Interactions:** The efficiency of flocculation can be influenced by the polymorphic form of the particles. Some forms may flocculate more readily due to their surface properties or charge distribution.
    - **Optimizing Conditions:** Different polymorphs might require specific conditions or flocculants for effective aggregation.

Applications:

- **Pharmaceutical Industry:** Polymorphic flocculation is important in drug formulation, where different polymorphs of active pharmaceutical ingredients (APIs) may affect solubility and efficacy.
- **Material Science:** Used in the processing and purification of materials that exhibit polymorphism, such as pigments or catalysts.
- **Mineral Processing:** Relevant in the extraction and processing of minerals that can exist in multiple crystalline forms.

Considerations:

- **Polymorph Characterization:** Accurate characterization of the polymorphic forms is essential for optimizing flocculation conditions.
- **Tailoring Flocculants:** Different polymorphs may require different types or concentrations of flocculants to achieve optimal results.

Key Points for Both Techniques

1. **Optimization:** Both electrolysis-based and polymorphic flocculation processes need careful optimization of conditions (e.g., flocculant type and concentration, pH, temperature) to achieve the desired results.
2. **Monitoring:** Regular monitoring of the flocculation process is crucial to ensure consistent performance and to address any issues that arise.
3. **Application-Specific Adjustments:** Tailoring the process to the specific requirements of the application is essential for effective and efficient flocculation.

Additional reference

1. "Understanding Polymorphic Flocculation in Industrial Applications" K. H. Miller and C. J. Turner Chemical Engineering Research Center 2019
2. "Pharmaceutical Crystals: Properties, Analysis, and Applications"A. J. Williams and S. L. Brice Wiley 2018

Practice questions

1. Discuss the principle and process of flocculation by electrolysis.

2. Explain how this method is applied in water treatment and its advantages and limitations.
3. Provide examples of specific applications where electrolysis-based flocculation is particularly beneficial.

Precipitation methods

The precipitation step is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants. The underlying mechanism of precipitation is to alter the solvation potential of the solvent, more specifically, by lowering the solubility of the solute by addition of a reagent.

1. Isoelectric precipitation

Acids and bases to change the pH of a solution until the isoelectric point of the compound is reached and pH equals pI, when there is then no overall charge on the molecule and its solubility is decreased.

2. Salting out precipitation

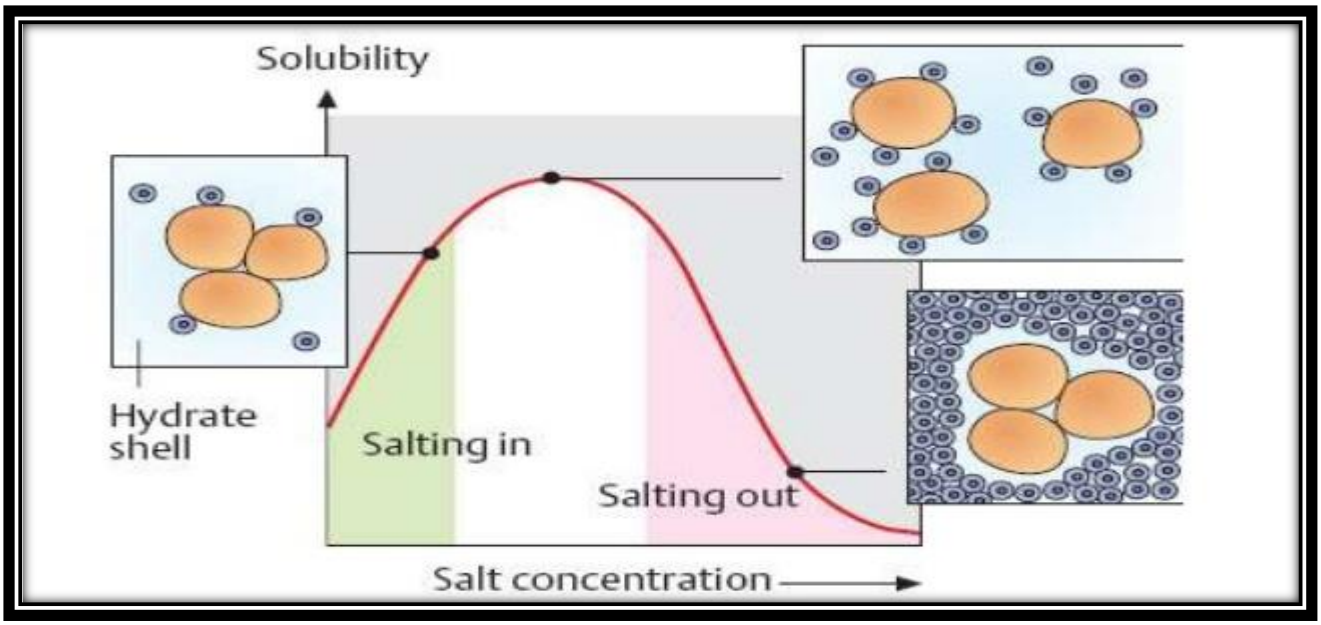
Salts such as ammonium and sodium sulfate are used for the recovery and fractionation of proteins. The salt removes water from the surface of the protein revealing hydrophobic patches, which come together causing the protein to precipitate. The most hydrophobic proteins will precipitate first, thus allowing fractionation to take place. This technique is also termed "salting out."

1- At low concentrations of salt → the solubility increases. This could be explained by the following: •

Salt molecules stabilize protein molecules by decreasing the electrostatic energy between the protein molecules which increase the solubility of proteins.

2- High concentration of salts → the solubility decreases, and protein precipitates. This could be explained by the following:

• because the excess ions (not bound to the protein) compete with proteins for the solvent. The decrease in solvation allows the proteins to aggregate and precipitate.



Ammonium sulfate $(NH_4)_2SO_4$ is commonly used because it is highly soluble and very effective. NaCl or KCl may be also be used to "salt out" proteins. Precipitate from solution as ionic strength is increased.

Ionic Strength = ½ S $M_iZ_i^2$

- M_i = Molarity of ion
- Z_i = Charge of ion
- 1M NaCl = ½ S (1 X 1^2) + (1 X 1^2)= 1
- 1M $CaCl_2$ = ½ S (1 X 2^2) + (2 X 1^2) = 3
- 1M $(NH_4)_2SO_4$ = ½ S (2 X 1^2) + (1 X 2^2) = 3

3. Organic solvents precipitation

Addition of miscible solvents such as ethanol or methanol to a solution may cause proteins in the solution to precipitate. The solvation layer around the protein will decrease as the organic solvent progressively displaces water from the protein surface and binds it in hydration layers around the organic solvent molecules.The principal causes of aggregation are likely to be electrostatic and dipolar van der Waals forces.

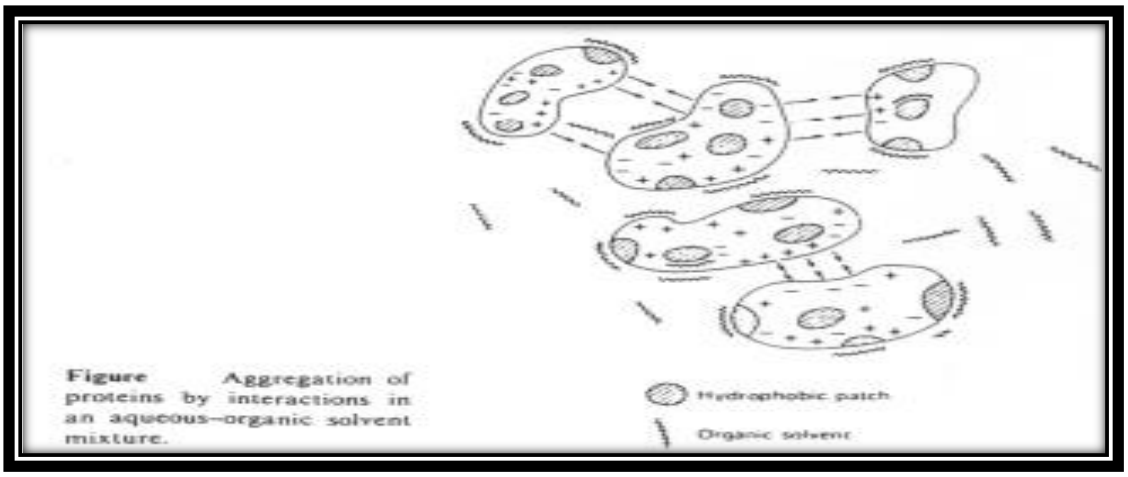


Figure Aggregation of proteins by interactions in an aqueous–organic solvent mixture.

4. Nonionic polymers precipitation

Nonionic polymers such as polyethylene glycol (PEG) and dextrans can be used in the precipitation of proteins and are similar in behavior to organic solvents.

5. Polyelectrolyte's precipitation

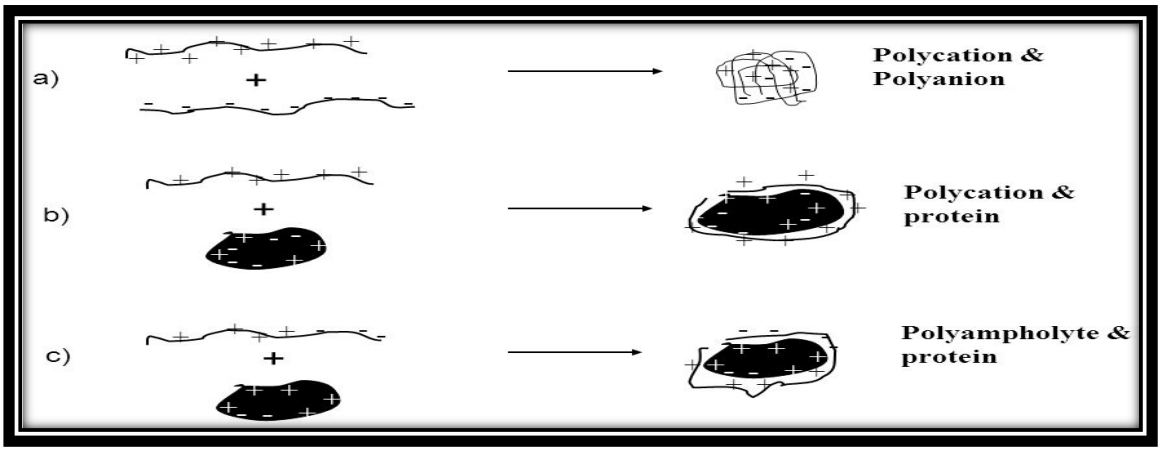
It can be used in the precipitation of a range of compounds. Alginate, carboxymethyl cellulose, polyacrylic acid, tannic acid and polyphosphates can form extended networks between protein molecules in solution.

• Polyanions and polycations interact with proteins below or above the isoelectric points.

• These interactions may result in soluble complexes or formation of amorphous precipitates.

• Protein precipitation by polyelectrolytes may lead to closely packed aggregates that are conveniently separated by settling or can generate open textured aggregates that can be separated by filtration.

• The precipitated proteins are recovered from the insoluble protein-polyelectrolyte complex aggregates by redissolution achieved by pH or ionic strength adjustment.



6- Protein binding dyes (triazine dyes) bind to and precipitate certain classes of protein.

7- Affinity precipitants are an area of much current interest in that they are able to bind to, and precipitate, compounds selectively

8- Thermal precipitation

Heat treatment as a selective precipitation and purification step for various thermostable products and in the deactivation of cell proteases. In this method, cell extracts are heated to a temperature at which many proteins denature and precipitate, where the protein of interest is more stable and stays soluble.

9- Heavy metals salts precipitation

Heavy metal salts usually contain Hg^{+2}, Pb^{+2}, Ag^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

Filtration is a fundamental separation process used in various industries, including water treatment, pharmaceuticals, and food processing, to remove particulates, microorganisms, and other contaminants from liquids and gases. Here's an overview of the principles of filtration, conventional filtration methods, and crossflow filtration:

Principles of Filtration

Filtration is based on the principle of separating solids from fluids (liquids or gases) by passing the mixture through a filter medium. The filter medium retains the solid particles while allowing the fluid to pass through.

Key Principles:

1. **Size Exclusion:** Particles larger than the pores of the filter medium are trapped, while smaller particles pass through.
2. **Pressure Difference:** Filtration often requires a pressure difference (applied pressure or vacuum) to drive the fluid through the filter medium.
3. **Filter Medium:** The filter medium can be made of various materials such as paper, cloth, membrane, or porous materials, and its characteristics (e.g., pore size) determine the efficiency of the filtration process.

Conventional Filtration

Conventional filtration methods are widely used in both laboratory and industrial settings. They typically involve passing a liquid or gas through a filter medium to separate out particles.

Types of Conventional Filtration:

1. **Gravity Filtration:**
    - **Process:** The liquid is allowed to flow through the filter medium by gravity. This is the simplest form of filtration and is often used for low-viscosity liquids.
    - **Applications:** Used in laboratories for separating solids from liquids in a funnel with filter paper.
2. **Vacuum Filtration:**
    - **Process:** A vacuum is applied to the filtrate side to accelerate the filtration process. The vacuum creates a pressure differential that helps pull the liquid through the filter medium more quickly.
    - **Applications:** Commonly used in laboratories and industrial processes where faster filtration is needed.
3. **Pressure Filtration:**
    - **Process:** Pressure is applied to the feed side of the filter to drive the liquid through the filter medium. This is used for thicker or more viscous liquids.
    - **Applications:** Used in various industries, including chemical and pharmaceutical processing.
4. **Deep Bed Filtration:**
    - **Process:** The filter medium consists of a thick bed of granular or fibrous material. Particles are trapped within the depth of the filter medium rather than on the surface.
    - **Applications:** Often used for filtering larger volumes of liquids or gases, such as in water treatment plants.

Crossflow Filtration

Crossflow filtration (also known as tangential flow filtration) is a specialized filtration technique where the feed solution flows parallel to the filter membrane rather than perpendicular to it.

Principle:

- **Flow Pattern:** In crossflow filtration, the feed solution flows tangentially along the surface of the filter membrane. This design minimizes the buildup of filtered material (fouling) on the membrane surface.
- **Shear Force:** The tangential flow creates a shear force that helps to keep the filter surface clean by sweeping away the accumulated particles.

Types of Crossflow Filtration:

1. **Microfiltration (MF):**
    - **Pore Size:** Typically used for particles in the range of 0.1 to 10 micrometers.
    - **Applications:** Used for water purification, beverage clarification, and protein concentration.
2. **Ultrafiltration (UF):**
    - **Pore Size:** Filters particles and macromolecules in the range of 1 to 100 nanometers.
    - **Applications:** Used for separating proteins, polysaccharides, and other large molecules from solutions.
3. **Nanofiltration (NF):**
    - **Pore Size:** Filters particles and molecules in the range of 1 nanometer.
    - **Applications:** Used for water softening, removing organic compounds, and certain salts.
4. **Reverse Osmosis (RO):**
    - **Pore Size:** Filters ions and small molecules in the range of angstroms.

- **Applications:** Used for desalination of seawater and production of ultra-pure water.

Advantages of Crossflow Filtration:

- **Reduced Fouling:** The tangential flow helps to minimize fouling and clogging of the filter membrane, which can lead to longer operating times and reduced cleaning frequency.
- **Higher Flux Rates:** Typically allows for higher flow rates and better efficiency in processing large volumes.
- **Scalability:** Suitable for large-scale industrial applications due to its efficiency and effectiveness in handling complex mixtures.

Disadvantages of Crossflow Filtration:

- **Complexity and Cost:** Generally more complex and expensive compared to conventional filtration methods.
- **Energy Consumption:** May require higher energy input to maintain the crossflow and pressure differential.

Basic Principles of Sedimentation and Sedimentation Coefficient | Centrifugation

Basic Principles of Sedimentation:

The rate of sedimentation is dependent upon the applied centrifugal field (G) being directed readily outwards; this is determined by the square of the angular velocity of the rotor (ω in radians s^{-1}) and the radians (r, in centimeters) of the particle from the axis of the rotation, according to the equation

$$G = \omega^2 r.$$

Since one revolution of the rotor is equal to 2 radians, its angular velocity, in radians s^{-1}, can be readily expressed in terms of revolutions per minute (rev min^{-1}), the common way of expressing rotor speed being

$$\omega = 2\pi \text{rev min}^{-1}/60$$

The centrifugal field (G) in terms of rev min^{-1} is then

$$G = 4\pi 2 \ (\text{rev min}^{-1})^2 r/3600$$

and is generally expressed as a multiple of the earth's gravitational field ($g = 981$ cm s^{-1}), i.e., the ratio of the weight of the particle in the centrifugal field to the weight of the same particle when acted on by gravity alone, and is then referred to as the relative centrifugal field (RCF) or more commonly as the 'number times g'.

Hence:

$$RCF = 4\pi^2 (\text{rev min}^{-1})^2 \, r/3600 \times 981,$$

which may be shortened to give

$$RCF = (1.118 \times 10^{-5}) (\text{rev min}^{-1})^2 \, r.$$

Because rotors are different from various manufactures, we use RCF to represent the centrifugation force.

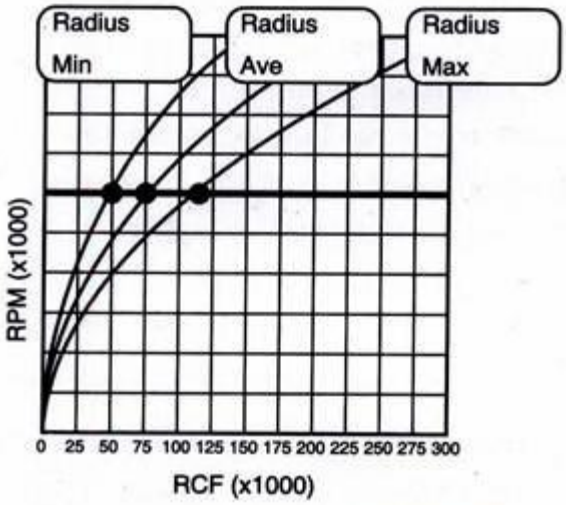


Fig. 5.1: Relation between RCF and RPM

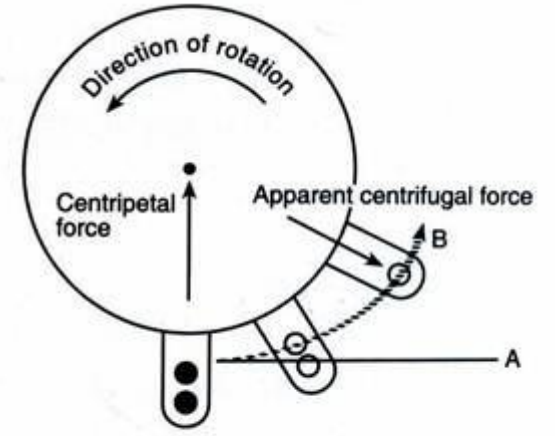


Fig. 5.2: Different forces during centrifugation

When conditions for the centrifugal separation of particles are reported, therefore, rotor speed, radial dimensions and time of operation of the rotor must all be quoted. Since biochemical experiments are usually conducted with particles dissolved or suspended in solution, the rate of sedimentation of a particle is dependent not only upon the applied centrifugal field but also upon the mass of the particle, which may be expressed as the product of its volume and density, the density and viscosity of the medium in which it is sedimenting and the extent to which its shape deviates from spherical.

When particle sediments it must displace some of the solution in which it is suspended, resulting in an apparent up-thrust on the particle equal to the weight of the liquid displaced. If a particle is assumed to be spherical and of known volume and density, the latter being corrected for the buoyancy due to the density of the medium, then the net outward force (F) it experiences when centrifuged at an angular velocity of ω radians s^{-1} is given by

$$F = 4/3\pi r^3_p (\rho_p - \rho_m)\omega^2 r,$$

where $4/3\pi r^3_p$ is the volume of a sphere of radius r_p, p_p is the density of the particle, p_m is the density of the suspending medium, and r is the distance of the particle from the centre of rotation. Particles, however, generate friction as they migrate through the solution. If a particle is rigid and spherical and moving at a known velocity, then the frictional force (F_0) opposing motion is given by

$$F_0 = vf,$$

where v is the velocity or sedimentation rate of the particle, and f is the frictional coefficient of the particle in the solvent. The frictional coefficient of a particle is the function of its size, shape and hydration, and of the viscosity of the medium, and according to the Stokes equation, for an un-hydrated spherical particle, is given by

$$F = 6\pi\eta r_p,$$

where η is the viscosity coefficient of the medium.

For asymmetric and/or hydrated particles, the actual radius of the particle in is replaced by the effective of Stokes radius, r_{eff}. An un-hydrated, spherical particle of known volume and density, and present in a medium of constant density, therefore accelerates in a centrifugal field, its velocity increasing until the net force of sedimentation equals the frictional force resisting its motion through the medium, i.e.,

$$F = F_0 \text{ or } 4/3\pi r^3_p (\rho_p - P_m)\omega^2 r = 6\pi\eta r_p v.$$

In practice, the balancing of these forces occurs quickly and the particle reaches a constant velocity because the frictional resistance increases with the velocity of the particle. Under these conditions, the net force acting on the particle is zero. Hence, the particle no longer accelerates but achieves a maximum velocity, with the result that it now sediments at a constant rate. Its rate of sedimentation (v) is then given by

$$v = dr/dt = 2r^2_p (\rho_p - p_m)\omega^2 r = 9\eta.$$

It is evident from this equation that the sedimentation rate of a given particle is proportional to its size, to the difference in density between the particle and the medium and to the applied centrifugal field. It is zero when the densities of the particle and medium are equal; it decreases when the viscosity of the medium increases, and increases as the force field increases. However, since the equation involves the square of the particle radius, it is apparent that the size of the particle has the greatest influence upon its sedimentation rate.

Sedimentation Coefficient (S):

When the frictional force balances the driving force, $\frac{dv}{dt} = 0$

$$\omega^2 r(m_p - m_s) - f \cdot v = 0$$

$$S = \frac{v_t}{\omega^2 x} = \frac{m(1 - \bar{v}_2 \rho)}{f},$$

where S = terminal velocity / unit acceleration

Sedimentation coefficients have units of sec. 10^{-13} sec is called 1 Svedberg (or 1 S).
m = particle mass

f = frictional coefficient of the particle in the solvent

p = density of solution

v = particle velocity.

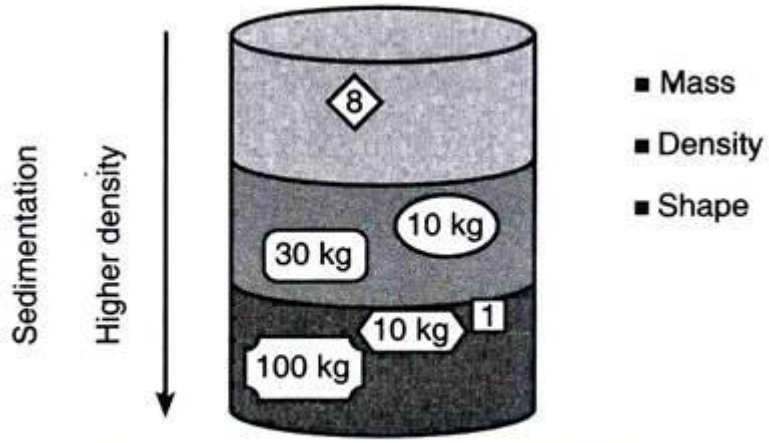


Fig. 5.3: Separation by Sedimentation

Characters of Sediment Coefficient (S):

1. S is increased for particle of larger mass (because sedimenting force in α man (1 – vr)).

2. S is increased for particle of larger density (equal volume).

3. S is increased for more compact structures (shape) of equal particle mass (frictional coefficient is less).

4. S is increased with rotational speed.

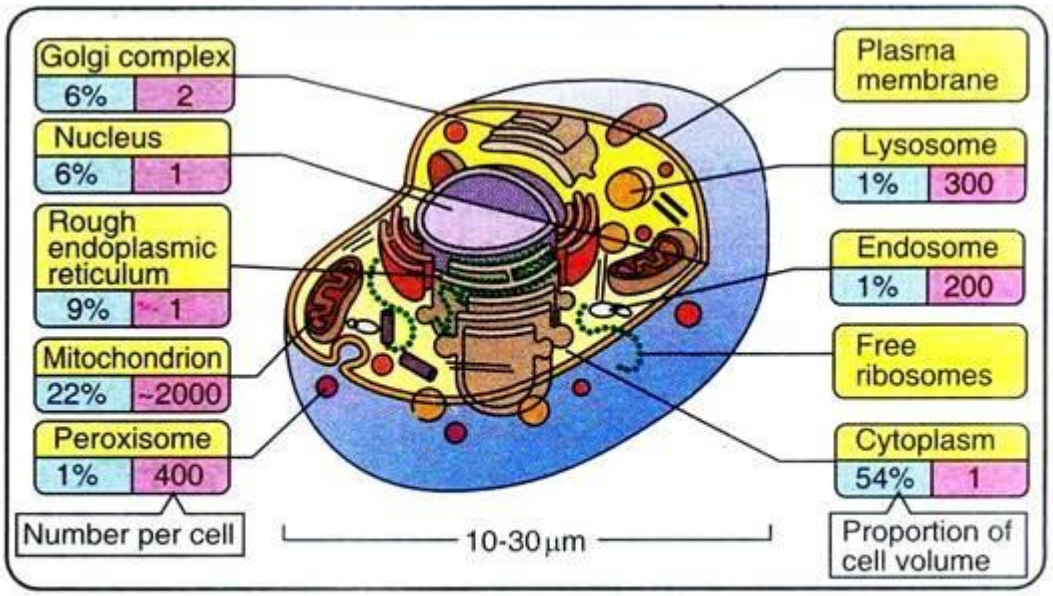


Fig. 5.4: Distribution of various organelles in cells

Extraction refers to a division process containing the division of a component from a medium. A few examples of the same are liquid extraction and solid-phase extraction. The form of extraction in which contaminants are removed from the medium consisting of a certain compound is called washing. Extraction is used in everyday activities, which are as simple as making tea, where the tea leaves, when boiled in water, remove the caffeine and other components of the tea leaves into the water. The components that can get dissolved in the natural solvents are extracted from the textile distilled throughout extraction. The leftovers of the extraction are studied for their quality and quantity.

Instances:

Instances of removed substances are lubricants, stains, residues of surfactants, fibre spin finishes, residual grease in wool, dyes, pesticides, acids, thickeners, heavy metal salts, etc.

Types of Extraction:

Liquid-liquid Extraction:

This type generally uses a funnel called a separatory funnel to separate a solute through one phase in another by mixing two unmixable phases. It is usually about separating the organic or the natural compounds from an aqueous phase to an organic one.

Solid-liquid Extraction:

This extraction is into the use of Soxhlet extractors. A solid compound containing the impurities is put in a thimble responsible for removing the impurities, for they are insoluble in the solvent chosen for extracting. Still, the wanted component has some solubility (at least).

Extraction of Metals:

Extraction of metals is done mainly from the ores with the help of various processes. The main process that aid in extracting metals and their refinement is called metallurgy. Mining is the procedure that involves the extraction of metals from the ore transferred to the surface of the Earth.

The type of the ore helps in deciding the steps to be taken in the extraction of metals, but some basic steps are to be followed:

- Enhancement of ore
- Enhanced ore used for the extraction of metal
- purifying of the contaminated metal

Methods of Extraction:

Various steps are followed in each of the extraction methods to have a complete separation of impurities and the desired component. The rising polarity of solvents indicates the character of

the removed particles. Diverse methods of extraction play a significant role in the extraction process. For small tests, Soxhlet extractors are used, and computer-aided finish analysers are used for bigger samples.

The most used methods of extraction are maceration, reflux extraction, and percolation, which frequently use organic or natural solvents and are in the requirement of large quantities of solvents and take much time in extraction.

The natural methods of extractions have a variety of modern and more organic methods, such as pressurized liquid extraction and supercritical fluid extraction. These modern methods of extraction are being used for the extraction of organic items, and the benefits of these extractions are greater than the traditional like, with less time usage, more selectivity, and less use of natural solvents.

Maceration:

This method of extraction is very easy and uncomplicated. But it has a few weaknesses like it takes more time in extraction and the inefficiency of the extraction. This method of extraction is generally applied in thermolabile substances extraction. When other solvents like luteolin, orient side were employed with this method of extraction, the inefficiency was the highest.

Percolation:

This method of extraction is more proficient than maceration because this method of extraction is continuous, and it keeps on changing the old and inundated solvent with a new and refreshed solvent.

Decoction:

This method of extraction has extracts that have a huge amount of dirt which is water-soluble. However, this extraction method could be used but cannot be applied for extracting volatile or thermolabile substances.

Reflux Extraction:

This method of extraction is far enhanced in the efficiency arena than the maceration and percolation. It needs a very low amount of solvent and employs less time in extraction. Though it is a grade above the maceration and percolation in the efficiency department, it still does not extract thermolabile organic items. This is superior to the extraction method called decoction also as it yielded the most out of puerarin and baicalin.

Soxhlet Extraction:

This method of extraction is like a beneficial combination of the methods, i.e., percolation and reflux extraction. It uses the notion of reflux to extract parsley along with refreshed solvent. This method of extraction needs a reduced amount of time and solvents than percolation and

maceration. This continuous extraction procedure might cause thermal degeneration if used in high temperatures and takes excessive time to extract the substance.

Pressurised Liquid Extraction (PLE):

This method of extraction uses extreme pressure for extraction. This extreme pressure results in the greater incursion of the solvents in the mixture as the pressure helps maintain high solubility.

Supercritical Fluid Extraction (SFE):

This extraction method employs supercritical fluids to play the part of solvents in the extraction process. These solvents have the capacity of dissolving a great number of organic items.

Reference Book:

1. **Bioprocess Engineering: Basic Concepts" by Michael L. Shuler and Fikret Kargi**
2. **"Introduction to Biochemical Engineering" by B. S. Bajaj**
3. **Bioseparation: Principles and Applications" by Shakti B. B. and W. G. H. M**
4. **Downstream Industrial Biotechnology: Recovery and Purification of Bioproducts" by Wolfgang G. Glasser**