Module 5. IMPORTANCE OF DOWNSTREAM PROCESSING AND PRIMARY SEPARATION TECHNIQUES:

Syllabus

1. Role and importance of downstream processing in biotechnological processes.
2. Problems and requirements of byproduct purification.
3. Economics of downstream processing in Biotechnology.
4. Cost cutting strategies,
5. Characteristics of biological mixtures,
6. Cell disruption methods for intracellular products,
7. Removal of insolubles, biomass (and particulate debris) separation techniques; flocculation and sedimentation,

- Centrifugation (ultra and differential) ,
- filtration methods and
- 8. Principle and Applications of Electrophoresis - their types.

Chapter 1 &2 Role, Important, problems and requirement of downstream processing

Question: Why downstream processing is important in bioprocessing

Question 2: What are the problem we phase in downstream processing and how to overcome the difficulties.

Unit process	Methodology	Role	Importance	Problem	requirement
Removal of insolubles	**Sedimentation**	Clarification	-cost effectiveness	- denaturation -space, scaleup	-time and temperature mgt
	Filtration - rotary drum,Vacuum filter, Plate and frame, horizontal plate, vertical leaf and candle type filters	Clarification -large-scale separation -when 10-40% solids -require cake discharge	-Simple and low cost - dilute, large and rigid	-Cake formation -Membrane fouling	- Filter aids -alternative process - Cross flow
	Centrifugation tubular bowl centrifuge, disc-bowel and basket type centrifuge	-Clarification -to wash accumulated solids	concentrated broth with lighter solid particles	-Density difference -cost	-pre treatment -technical skills
	Flocculation and floatation	-Clarification of -partial purification	-methods such as filtration and centrifugation are inefficient	- denaturation	-time and temperature mgt
Cell disruption	**Mechanical methods** -bead mill, -homogenizer -ultrasonication	releasing intracellular products	When product is not sensitive to grinding, pressure and pressure release	Denaturation	-time and temperature mgt
	Non-mechanical osmotic shock, detergent solubilisation and lipid dissolution methods	plays major role in cell disruption	When product is sensitive to grinding, pressure and pressure release	Chemical residues	Process optimisation
Extraction	**Liquid-liquid extraction**	achieves both concentration and purification in	-especially for the recovery of lipophilic compounds	Denaturation	-time and temperature mgt

		large scale	-Large scale		
	Supercritical fluid extraction	highly labile byproducts such as food aroma components and flavors	. This method handles varieties of samples such as solids, semisolids and liquids of different chemical nature.	cost	Process optimisation
	Aqueous two-phase extraction	recovery and purification	10-fold concentration	Denaturation	-time and temperature mgt
Concentration	methods such as evaporation, membrane filtration, ion exchange methods	recovery and purification	method achieves further concentration	Denaturation	-time and temperature mgt
Product purification	precipitation, chromatography, ultra filtration and electrophoresis	purifying biological solutes in a non-crystalline state	important in the preliminary stages of downstream processing of biological products. It is a well-established large scale industrial separation	Cost	Process optimisation
Product formulation	Artificial DRYING FREEZE DRYING Chemical formulation	Maintenance of the product activity and stability of the product during distribution and storage	protect protein from product decay and to enhance product solubility	cost	Process optimisation

- **Economics of downstream processing in Biotechnology.**

VTU: Write note on economics of downstream processing the biotechnology

Bioprocess economics:

- **Technological aspects** that are crucial for **establishing and operating** downstream processing **economically** is referred as **Bioprocess economics**
- Your emerging technology will never be able to attract investors unless it is **economically feasible one**.
- Therefore, the **economic aspects should be the basis for development and adoption of the biotechnological process or the strategies**, and hence it is very much necessary to have the blend of **scientific enthusiasm** with **economic awareness**.

Estimation of capital and operating cost

- Estimation of cost of **establishing a factory** and **subsequent running** it to obtain profit is very important.
- The cost of setting up of the factory is called **capital cost** including the fabrication of the entire plant and the preparation of land.
- The cost of running the production unit for production in terms of manpower, raw-material and utilities is called **operating cost**.
- Both the capital and operating cost are **depending on**
  - **the scale of operation** such as large scale, medium scale and small scale,
  - or the product profile like '**high value low volume product**' and '**low value high volume product'**, including the strategic importance of the project.
- **Proposed production process** is the basis for the cost estimation, but not on the rational approaches.
- There are different methods of cost estimation **but all depend on the lag period** before the start up as well as the **project implementation period**.
- It is important to note that **shorter the duration for the start up, more accurate the cost estimation will be**, and **shorter the project implementation period less expensive will be your capital cost**.

Classification of cost

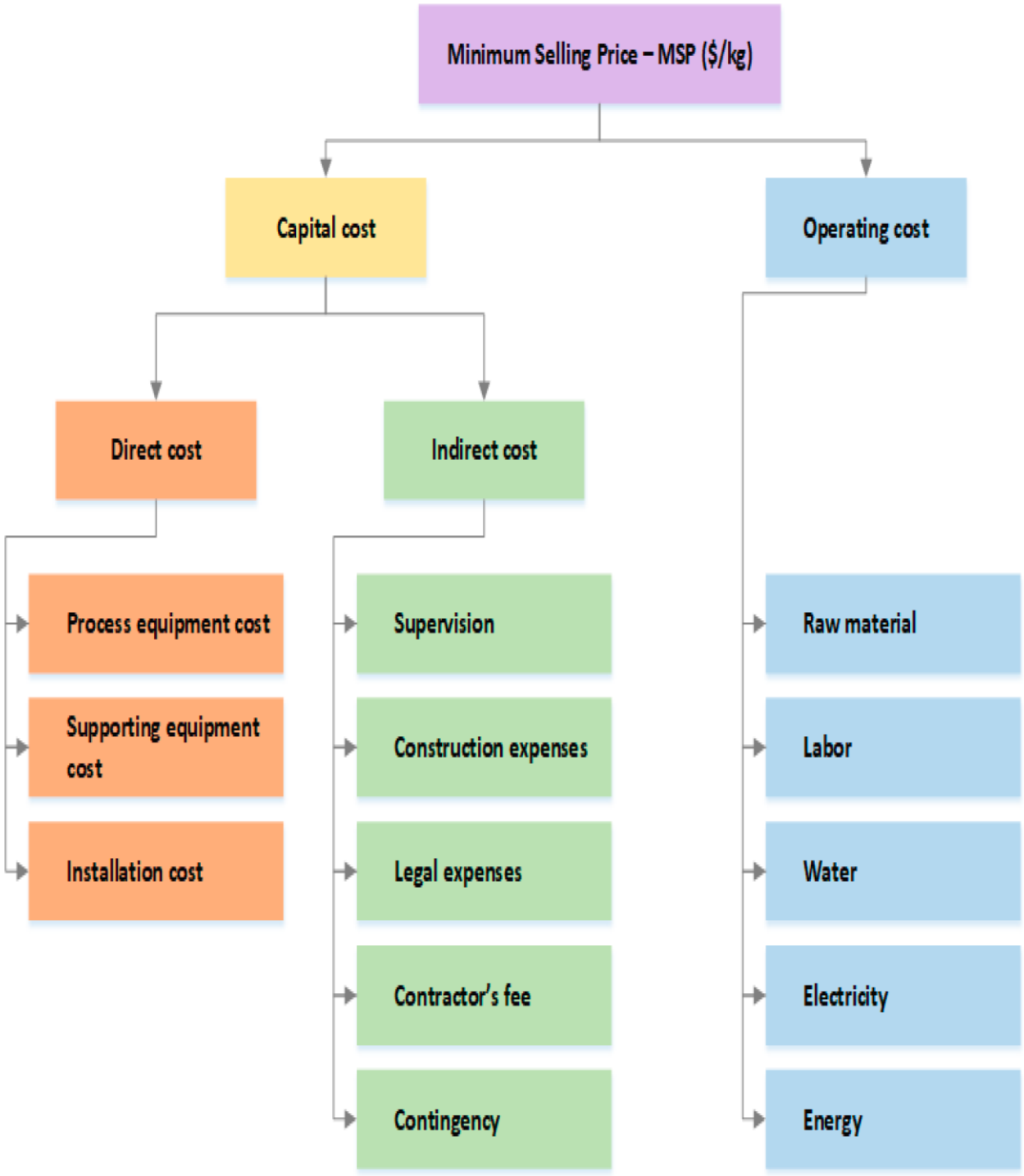
1. **Capital cost:**

- **Direct cost and indirect cost** are the two types of capital costs.
  - **Direct cost**: Cost incurred in establishing, building and furbishing the plant is known as **direct or fixed cost**,
  - **Indirect cost**: while working capital such as overheads, transport, engineering and taxes required for constructing the plant is called as **indirect or working capital**.
- Items normally incorporated into each category vary, but depends on the type of the cost estimation used.

2. **Operation cost**

- **Operating costs or the manufacture cost** is the measure of expenditure incurred on procurement, production, research and development, administration, quality assurance, storage, sales and marketing activities.
  - **Fixed cost:** Two types of operating costs are fixed cost such as taxes, depreciation and overheads,
  - **Variable costs** such as procurement, production, selling costs etc.
    - **Procurement cost** includes the cost of the raw materials, ingredients, chemicals, preservatives, packing materials, transport etc.
    - **Production cost** includes labor costs and benefits, electricity, water, training etc.
    - Research and development includes the cost incurred in developing product or process including survey activities.

- **Administration cost** includes phone, office supplies, and professional fees including cost incurred for coordinating with private and government agencies.
- **Quality assurance cost** includes the cost of running the laboratory and the in-process quality control to ensure product quality.
- **Storage or warehouse cost** includes the cost for running the cold store if any, handling, labor, transport etc.
- **Sales and marketing** cost includes cost of selling, promotional activities, advertisement etc.



Question: How to optimize bioprocessing by cost cutting

1. Cost cutting strategies in downstream processing industry

- The main objectives of all the business units are to make profits.
- Most of the entrepreneurs believe that best way to do that is by increasing sales.
- But that leads to corresponding increase in costs for the increased amount of work involved to increase the sales, but increased costs are just what need to be curtailed.
    - Therefore, effective cost cutting strategies is to **reduce fixed and variable cost to increasing profit margins**.
    - Many bioprocess industries are looking for ways to reduce operational overheads and support cost.
    - In response to changing business needs and competitive pressure companies deploy instruments and unit process to suit the need, which may leads to multiple systems performing same function and infrastructure duplication across the downstream processing units.
    - Such problems become more complicated when companies grow through mergers with the sole intension of acquiring brand names, technology or market share.
    - Companies that are gone through such transition are looking for ways to control and reduce the cost.
- But bioprocess industrial business strategies are always driven by return on investment (ROI).
- Hence the cost cutting strategies in downstream processing industries should always be targeted and significantly focused **at reducing both direct and indirect cost**.
- But before doing so, three important factors helps us to achieve our cost reduction goals are, building strong relationship with the trusted suppliers, developing a strong organizational structure and focusing relentlessly on execution of the cost cutting strategy. Cost cutting strategy should be formulated under the frame work of global trend so as to have a greater impact on the implementation.

Cost cutting strategy

Cost cutting strategies **consist of technology or cost analysis, cost cutting strategy formulation, and Cost cutting strategy implementation**.

<u>Technology or cost analysis</u>

- First step involved in cost cutting is to **analyze the technology or cost which is responsible for increasing the cost** so that we can formulate the strategy to control and reduce it.
- This process helps us to identify inefficiencies and expenses that can be solved through technologies and methodological changes.
- This process involves review of **organizational structure, business requirement, existing infrastructure, growth trends, growth factors, resources, expenses, and strategy and future plan**.
- Since these factors and their interdependency are complex, we have to analyze **these factors individually and together**.
- Each factor has to be analyzed with reference to the impact of other factors on each other and develop a road map for controlling and reducing the cost.
- **Organizational structure analysis** helps us to find out inefficient, surplus, and unskilled manpower, so that we can either improve the short coming or eliminate them from the organization.
- Find out all the requirements that is very **specifically required or not required** for running the business, so that the unnecessary resources, instrumentations, unit processes, technology, ingredients and materials can be eliminated.
- Analyze the **existing infrastructure** with reference to the present day relevancy, efficiency, utilities, productivity and maintenance.

- It is very much important to analyze the resources so that we can optimally utilize the resources without any waste.
- Analyze all the fixed and variable expenses so that we can identify the cost drivers.
- Fixed costs are those that are not related to the amount of sales or production.
- They usually include rent, insurance, and the costs incurred by the utilities in use, or for running the business, such as salaries, advertising etc.
- Fixed costs can change over a period of time, although the increase or decrease is not connected to production.
- Variable costs, however, are directly related to business activity. Raw materials and inventory are perfect examples of the variable costs of a business enterprise.
- Inventory has to be kept on hand in the retail industry, and with increased sales, there has to be an increase in the inventory too.
- Likewise, with raw materials, the more goods you produce the more raw materials you will need.
- So now, you must be wondering just how to lower those bills in order to control your business costs.
- Well, there isn't one cut and dry answer, and you will need to examine your whole business strategy and determine how to achieve cost-reduction without impacting your business adversely.
- Paradoxically, sometimes in order to save money you will need to spend money, such as upgrading the equipment in use.
- It is really a complicated issue as to how to lower those bills of fixed and variable cost.
- Most of the employers think of a single common cost that is firing of hired employees.
- This trend without analysis will make the situation more complicated as bluntly firing employees gives wrong signal and productivity may be affected due to deteriorating morale among the employees.
- Hence it is very important to analyze the present whole strategy and formulate the future plan to achieve cost reduction without impacting the business adversely.
- Cost cutting strategies can be adopted during the initiation of the new projects, during up gradation of the existing facilities or on the existing loss making facilities.
- Three important factors valuable at all the stages of cost cutting strategies are, **bioprocess design and economic analysis, Comparison of bioprocess alternatives, and bioprocess facility design, engineering and construction**.

1 Bioprocess design and economic analysis

- During the early stages of development, analysis of Cost-of-Goods (COG) identifies the principle “cost drivers” to the process and quantifies the sensitivity of COG to the impact of the values of these drivers.
- This helps us to formulate the cost-cutting strategies focused more on these aspects of the process that most greatly affect the COG, thereby driving the most cost reduction.
- In any process it is very important to understand the cost breakdown critically, so that we can identify the location of cost drivers so as to achieve the biggest saving potential.
- The major cost drivers in the bioprocess industries are raw material, utilities, labor and fixed costs.

2 Comparison of bioprocess alternatives

- Critically analyze the unit steps of downstream processing of bioproducts and compare with other process alternatives in term of its productivity, efficiency, product stability and cost.
- Simulate the process alternatives by mathematical models to determine if the potential cost advantages of process alternatives are worth the development effort, and to set the production target required for the process alternatives to be economically advantageous.

- While doing so we need to make sure that we are spending least time and resources to translate the cost cutting strategies to cost effective business unit.
- At this stage, we should focus on analyzing the process advantages of advanced alternative techniques such as bioreactor-based process to transgenic plant and animal systems, or from standards batch chromatography to countercurrent chromatography.
- In addition, we have to focus on other costs such as bioreactor equipment cost, cost of ingredients, filters, filter aids, resins, bioreactor molecules and other consumer items.

3 Bioprocess facility design, engineering and construction

- We need to have accurate and detailed COG simulation to determine the budget for bioprocess plant engineering and construction, to provide detailed specifications o the equipment manufacture, to generate efficient floor plan, utilities and equipment requirements.
- Results obtained from the process simulators can be recommended to for appropriate project execution and provide turnkey project execution resources for facility design, engineering, construction and validation so that the project is implemented in time to reduce the capital cost.
- This is very important as the operational burdens can be reduced, but unnecessary money spent on capital cost can not be reduce once the project is implemented.

Cost-cutting strategies formulation: bBasic objectives of cost cutting should be formulated in developing strategies that will translate into economically feasible business.

Chapter 5 Characteristics of biological mixtures

Question 1: Analyze characteristics of fermentation broth that are relevant for downstream processing.

The characteristics of biological mixture such as the

- **morphological features of cells,**
- **concentration of product of interest and impurities,**
- **physical and rheological characteristics of biological mixture** are very important because of its **influence on the isolation and recovery of the product of interest** during downstream processing.

- Since all these factors **influence the yield, purity and economics** of product recovery,
- **careful understanding of each of these factors** in the biological mixture prior to the recovery of the product of interest, and
- **careful selection of methods and instruments** are crucial to reduce the difficulties of product recovery.

- **Types and morphological features of the cells** are very important because without the **knowledge of the cells** either intracellular or extracellular products of interest cannot be efficiently being isolated from the insoluble.
- **Knowledge of the product of interest and the impurities** are also important because we will be able to isolate and purify the products from number of other impurities with **similar or dissimilar properties by carefully exploiting the discriminative physiochemical properties of these components in the biological mixtures**.
- **Physical and rheological characteristics of the biological mixtures** are very much important during, process design, reactor design and product recovery.
- Hence, the knowledge of the physicochemical properties of product of interest, impurities and solvent is very important during process design, instrument selection and recovery of the product of interest.

6.1 Morphological feature of the cells

- Product of interest may be recovered from **microbial, animal or plant cells grown** in the media or collected from the **natural source**.
- Cells and agglomerates of cells exhibit variations in the **size, shape and arrangements.**
- Wide varieties of microbial, animal or plant cells with size varying from **1 µm** as in the case of bacteria to **4000 µm** as in the case of cellular agglomerate are associated with the biological mixture.
- **Bacteria :** Bacteria are larger, single cells and can be shaped as rods, spheres, and spirals, with the size range from **0.03 to 0.1 µm** in diameter.

  Fungi: Fungi are larger than bacteria with the **size vary from 5-20 µm in diameter** and tend to grow into long filamentous cells.

  Mammalian cells: Most of the mammalian cells are of **similar size of around 20 µm in diameter**, but their shapes vary.

  Plant cells: Even though plat cells are similar to animals, they differ from one another in at least three major aspects.

- One difference is the **cell wall**, as it is a reinforced structure containing **cellulose** and **lignin** to make them rigid.
- Second difference, plants are **autotrophic** as it is energetically self-supporting.
- Third difference, plant cells have a **large vacuole**.

Concentration of product of interest and impurities

All products other than the product of interest are considered as impurities. One way of characterizing biological material is by size. Water molecule is the smallest of all and constitutes about **80% of the total volume of the biological fluids**.

Water molecules

- Water molecules may be in **free form** or may be **unavailable** for extraction if they are structural part.
- Water molecule consisting of an **oxygen atom** and **two-hydrogen atom**, and **two- hydrogen covalently bound at an angle of 104** ^{o}. Since the bond between hydrogen and oxygen is about 0.96 Å, volume occupied by the entire molecule is about **3 Å**, which is smallest of all in biological mixtures.

Sugars

- Even though many of the sugars are **chemically similar** to each other, they are structurally different.
- Glucose and fructose have the same empirical formula $C_6H_{12}O_6$, as shown below (Figure 6.2), but even **after having equal calories per unit weight, fructose tastes twice as sweet as equal quantity of glucose**.

6.2.3 Amino acids

- There are **20 amino acids occur in living organisms**, even though many **other synthetic amino acids gets incorporated into fermentation media**.
- Chemical features that are important for separation, are **α-amino acid terminal** and **carboxy terminal** as follows.
- α-amino acid terminal is **zwitterionic**, as they tend to have closely **separated positive and negative charges at one end of the molecule**, **while carboxylic terminal is asymmetric**, attached to **four groups, -H, -COO $^{-}$, -NH $^{3+}$**, and the **variable group –R**.

6.2.4 Lipids

- One more important component in the biological mixture is lipids.
- **Lipids are a diverse group of compounds** that have many key biological functions, such as **structural components of cell membranes**, **energy storage sources** and **intermediates in signaling pathways**.
- Lipids are **fat-soluble, naturally-occurring molecule, such as fats, oils, waxes, cholesterol, sterols, fat-soluble vitamins such as vitamins A, D, E and K, monoglycerides, diglycerides, and phospholipids**.
- Fats are a subgroup of lipids called **triglycerides**.
- Lipids also include molecules such **as fatty acids** and their derivatives such as **tri-, di-, and monoglycerides and phospholipids**, and other **sterol-containing metabolites such as cholesterol**.
- Lipids may be broadly defined as **hydrophobic or amphiphilic small** molecules that originate entirely or in part from two distinct types of biochemical subunits, ketoacyl and isoprene groups.
    - Using this approach, lipids may be **divided into eight categories** as **fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids and prenol lipids**.
- **Nevertheless, in unsaturated fats, some of these hydrogen atoms are "missing",** which always occurs in adjacent pairs of carbon atoms forming a double bond with each other.
    - Because unsaturated fats have "kink" or bend, the molecules do not stack together easily, and so they stay fluid at room temperature.

Nucleic acids

- The final groups of components of biological fluids are nucleic acids and the key feature of these molecules is their **'base', a purine or pyrimidine group attached to one of the molecules**.

- Nucleic acids are **linear, unbranched polymers of nucleotides** and these head groups can be polymerized to DNA, similar to the way the zwitterions head groups of the amino acids are polymerized in to proteins.
- A nucleotide is composed of three parts**: pentose, base and phosphate group** (Figure 6.13).
- In DNA or RNA, a pentose is associated with only one phosphate group, but a cellular free nucleotide, such as ATP may contain more than one phosphate group. If all phosphate groups are removed, a nucleotide becomes a nucleoside.
- There are five different bases, **Adenine, Cytosine, Guanine, Thymine, and Uracil**, each is denoted by a single letter as A, C, G, T and U, among them A, C, G and T exist in DNA, and A, C, G and U exist in RNA (Figure 6.14).

Polymers

- Of the different species of the components of the biological mixtures that we have discussed, **water is the smallest** and the lipids are probably the largest.
- Each of the **building blocks that we have studied above can become part of polymer of high molecular weight**.
- The sugars form **polysaccharides**.
- Polysaccharides are **powdery compounds**, usually insoluble in water and tasteless.
- **Starch and cellulose occur abundantly in plant life**.
- **An RNA molecule is a linear polymer** in which the monomers like nucleotides are linked together by means of phosphodiester bridges, or bonds, which link the 3' carbon in the ribose of one nucleotide to the 5' carbon in the ribose of the adjacent nucleotide.
- Lipids do not form chemically bound polymers, but forms macromolecular liquid crystals in conjugation with cholesterol.
- Polymers of amino acids, protein has an immense interest in bioseperation as many proteins such as enzymes, insulin, and hormones have pharmacological activity and have a commercial value.

Physical and Rheological Characteristic

- Even though the density of the **dry mass is around 1400 kg/m^3**, the density of the **fermentation broth is lower around 1100 kg/m^3** due to higher moisture content of about 70-80%, and density of the **fungal biomass after filtration is around 1030 kg/m^3** due the entrapped water content of about 15% due to capillary force.
- Since the recovery of the biomass by discriminating the density alone is difficult**, rheological properties of the fermentation, broth is important for most o**f the recovery steps such as centrifugation and filtration methods.
- With few exceptions as in the case of the production of polysaccharides, fermentation broth after the removal of insoluble are water like and with the **help of simple Newtonian and non-Newtonian models rheological characteristics of the biological fluids can be understood**.
- Viscosity of the biological fluids gets affected by the **concentration, size and shapes of the biomass, concentration of the byproducts** such as polysaccharides produced by the microbes and to some extent by nutrients and excreted impurities.
- **In the case of Newtonian fluid, the relationship between the shear stress and the strain rate is linear**, the constant of proportionality being the coefficient of viscosity.
- **While in the case of a** non-Newtonian fluid, the **relation between the shear stress and the strain rate is nonlinear, and can even be time-dependent**.

Therefore, before going for designing of downstream processing unit steps knowledge of physico-chemical properties of the solvents, solutes and microorganisms are very important

VTU: Illustrate cell disruption method used for intracellular release

Chapter 6: Cell disruption methods

- There are many methods to disrupt the cells of different types but any selected method must ensure that the labile product of interest are not denatured by the process or lost during the process or contaminated with unwarranted impurities.
- Cell disruption methods are classified into mechanical and non-mechanical methods based on the whether the mechanical means using specialist equipment are used to disrupt the cells.
- During the intracellular product release, knowledge of the location of product of interest is very important in selecting a suitable cell disruption method.
- If not carefully selected the target product may be contaminated with the other products, which are very difficult to separate by subsequent purification techniques.
- There are few methods, which selectively release the product of interest during disruption.
- Yield of the product release is directly proportional to the disruption process.
- For this reason during the release of intracellular enzymes, total protein released is taken to calculate the yield than the specific activity of the target enzyme alone.
- The factor that contributes to the difference in the disruption process is the location of the product within the cells, degree of disintegration, and the degree of the denaturation of the product during disruption.
- Best way to achieve high yield is to disrupt the cell until maximum yield.
- Hence, we need to stop the cell disruption before the completions of the product release to avoid loss.
- The rate for mechanical disruption can be calculated by first order process (Figure 8.9).

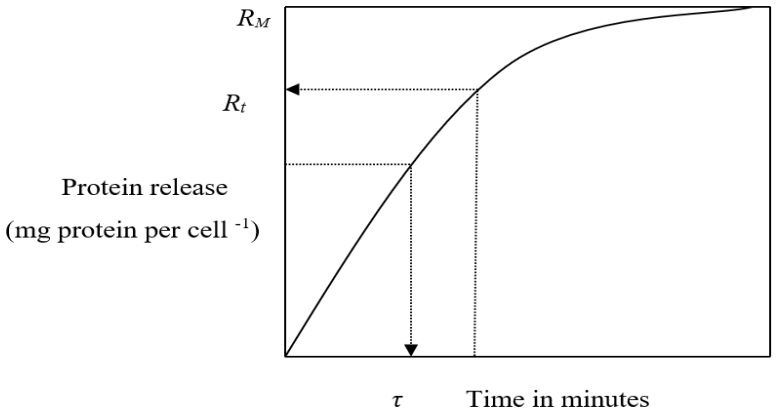


Figure 8.9 Shape of the protein release curve for first–order kinetics

- For the **time dependent cell disruption** process such as bead milling and ultrasonication, rate can be given as

$$R = R_M(1-e^{-t/\tau}) \quad \ldots (8.1)$$

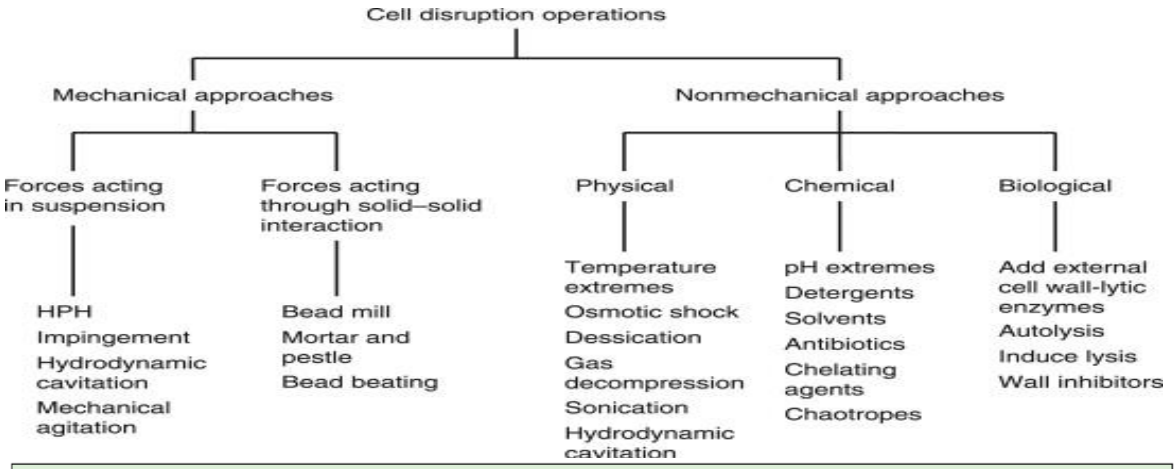
where R is concentration of the released protein, R_M is the maximum concentration of the protein released, τ is the first order kinetics and t is the disruption time.

The optimum time depending on the biological components such as cell type and the product, as well as the method of the extraction

- Similarly for the process based on the **number of passage of cells** through the device such as homogenization or French press, the rate equation is

$$R = R_M(1-e^{-n/\tau}) \quad \ldots (8.2)$$

where n is number of passes through the process. Non mechanical methods such as chemical and enzymatic cell disruption are also a time dependent process.



Cell disruption methods

(A) Mechanical	(B) Chemical	(C) Thermal/thermo-chemical	(E) Biological
1. Solid shear • Bead milling/bead beating • Grinding (with/without cryogens) • Homogenisers (high speed) • Mechanical cell press **2. Liquid shear** • High pressure homogeniser (microfluidiser)	• Solvent extractions • Acidic/alkaline • Ionic liquids • Chelating agents • Detergents • Osmosis • Oxidation • Nanoparticles • Supercritical fluid extraction	• Autoclave • Steam explosion • Hydrothermal liquefaction • Freeze drying	• Antibiotics • Enzymes (lytic, autolysis) • Phage
		(D) Electromagnetic	**(F) Current**
		• Microwaves (with/without solvents) • Ultrasound	• Pulsed electric field

Mechanical method

- Homogenization in a Warring blender (blade types), grinding with abrasives and ultrasonication are the small-scale methods, and are suitable for laboratory work.
- Whereas, homogenization using orifice type homogenizer or crushing using bead mills are widely used in larger scale, are most popular unit steps in chemical process and food industry.
- In the mechanical cell disruption, the rate of protein released is generally proved proportional to the amount of releasable protein from the cells.

$$\frac{dC}{dt} = -kC \quad \ldots$$

(8.3)

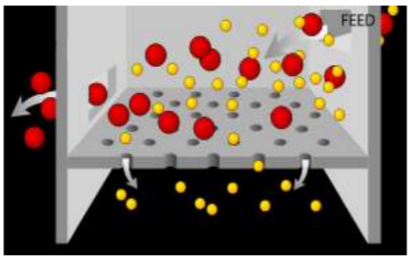
where C represents the protein content remaining associated with the cells, t is the time and k is a release constant dependent on the system. First order rate constant design depends on the design and speed of the agitator, bead loading, bead size, cell concentration, and temperature.

Chapter 7: Removal of Insolubles

- Products of downstream processing are mostly encountered as **suspended solids that are insoluble in water** and **dissolved solutes that are soluble in water**.
- **Concentration of biomass, cell debris and other insolubles**, as that of the products in the biological fluids are critical in formulating a strategy for the recovery of product of interest.
- Concentration of the particulates may vary from as low as **0.1%** as in the case of plant or animal cells to **as high as 60%v/v** as in the case of fermentation broth.
- Depending on the process used the **size of the particles range from 1µm for microorganisms to about 1 mm diameter** for nutrients.
- **Removal of insolubles** regardless of the intracellular or intracellular origin is performed at the **initial stage of the downstream processing prior to cell disruption**.
- So the primary step in bioseperation would always involves **the removal of suspended particles** consisting of **whole cells, cell debris, organelles, slims, DNA and RNA**.
- These insolubles are removed from the process liquid by **solid liquid separation process** such as **filtration, centrifugation, sedimentation, flocculation, electro-precipitation, and gravity settling**.

Removal of Insolubles

- Separation of cells, cell debris or other particulate matter
- Typical operations to achieve this:



1) Filtration
2) Centrifugation
3) Sedimentation
4) Flocculation a process where a solute comes out of solution in the form of floc or flakes.

Sedimentation

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

Centrifugation

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

Filtration

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

Chapter 9: Centrifugation

- Centrifugation is used to separate materials of different density when a **force greater than gravity is desired**.
- Centrifugation method is utilized to separate the **cellular debris from the released protein**.
- Example-a key role in many industrial processes, including the production of **insulin, is to separate liquid phases and solids from each other**.
- It **depends on particles size**, **density difference** between the cells and the broth and **broth viscosity**.
- It is based on the **behavior of particles in an applied centrifugal field**.
- More **dense components of the mixture move away from the axis of the centrifuge** while **less dense components of the mixture move towards the axis**

PRINCIPLE

- Centrifugation is used to separate **materials of different density** when a force greater than gravity is desired.
- The particles will tend to **sediment under the influence of gravity**.
- If the particles suspended in a liquid are so small or have a density so close to that of the liquid, then the **force of gravity fails to sediment** the particles into a separate layer.
- So the basis of centrifugation techniques is to **exert a larger force than the gravitational force** to enhance the effective sedimentation force for the separating such particles from the liquid.
- Particles which differ in density, shape or size can be separated since they **sediment at different rates in the centrifugal field**, each particle sedimenting at a rate which is proportional to the applied centrifugal field.

➢ The rate at which the sedimentation occurs in centrifugation is expressed in terms of sedimentation coefficient and is given by the formula:

$$S = V/\omega^2 r$$

Where, V = sedimentation of the molecules

ω = Rotation of the rotor in radians/sec. (angular velocity)

r = Distance in cm, from the centre of the rotor

Settling velocity is represented as follows.

$$v_g = \left[\frac{qg}{2\pi\, lr^2\omega^2}\right]$$

Types of centrifuges

- All the centrifuges available depend on the **common principle that an object rotating around the central axis at a constant radial distance from the point is acted on by a force**.
- At this constant speed **objects constantly change direction and accelerate**.

- When the solutes rotates in the cylindrical container, **content of the fluids and the solids exert an equal and opposite force**, called **centrifugal force**, outward to the wall of the container.
- As the cylinder rotates **biological fluid is admitted at the center** through the centrally located inlet, as the cylinder rotates around its central axis.
- On reaching the **end of the inlet biological fluid gets thrown immediately outward to the wall of the container**.
- The liquid and solids are acted upon by the **vertical gravitational force and horizontal centrifugal force**.
-

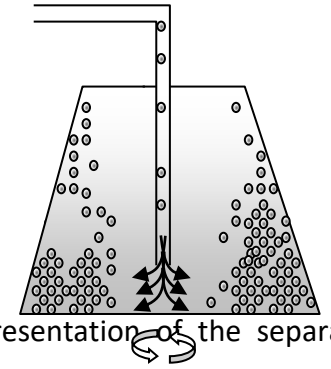
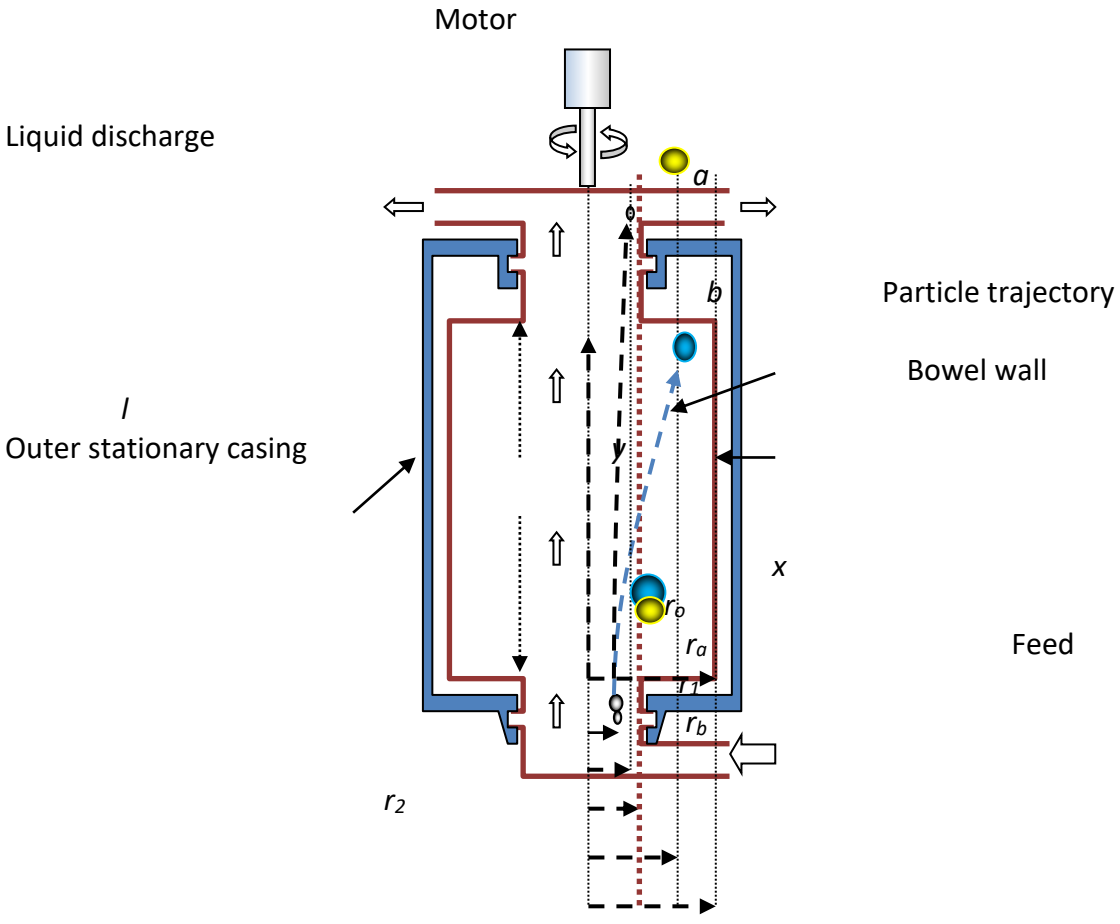


Figure 10.5 Diagrammatic representation of the separation of particles by centrifugation in a rotating bowel.

- There are three basic types of large scale centrifuges are presently used for the separation of insolubles from the biological mixtures.
- They are **tubular-bowel centrifuge, disc type centrifuge** and **basket type centrifuge**.

a. Tubular-bowel centrifuge



- Tubular-bowel centrifuge consists of **elongated narrow cylindrical bowel**, with the bowel dimension varies from **8 to 15 cm in diameter** and about **150 cm in height**.
- Bowel is suspended from top and rotates at the speed of **10,000 rpm** in an **outer stationary casing**.
- Biological fluid to be cleared of insolubles is **fed through the bottom** and **clarified liquid is removed from the to**p.
- Insolubles get deposited on the **bowel's inner wall surface as a thick paste**/ separated through insoluble outlet.
- Feed can be fed through the centrifuge **until no solutes escapes through the outlet**.
- The biological fluids enter the inlet situated at the bottom and we can assume that all the liquid moves upward at the uniform velocity, carrying insolubles along with it.
- Insolubles are assumed to be moving radically at its terminal settling velocity.
- A insoluble of a given size, ***a*** **at a given rotation speed is removed along with the liquid if the sufficient resident time is not given for it to reach the wall of the bowel** and **on the other hand if sufficient resident time is available to reach the wall of the bowel, particular size *b* gets retained in the bowel. Length of the bowel is *l* meter.**

2. Disc bowel centrifuge

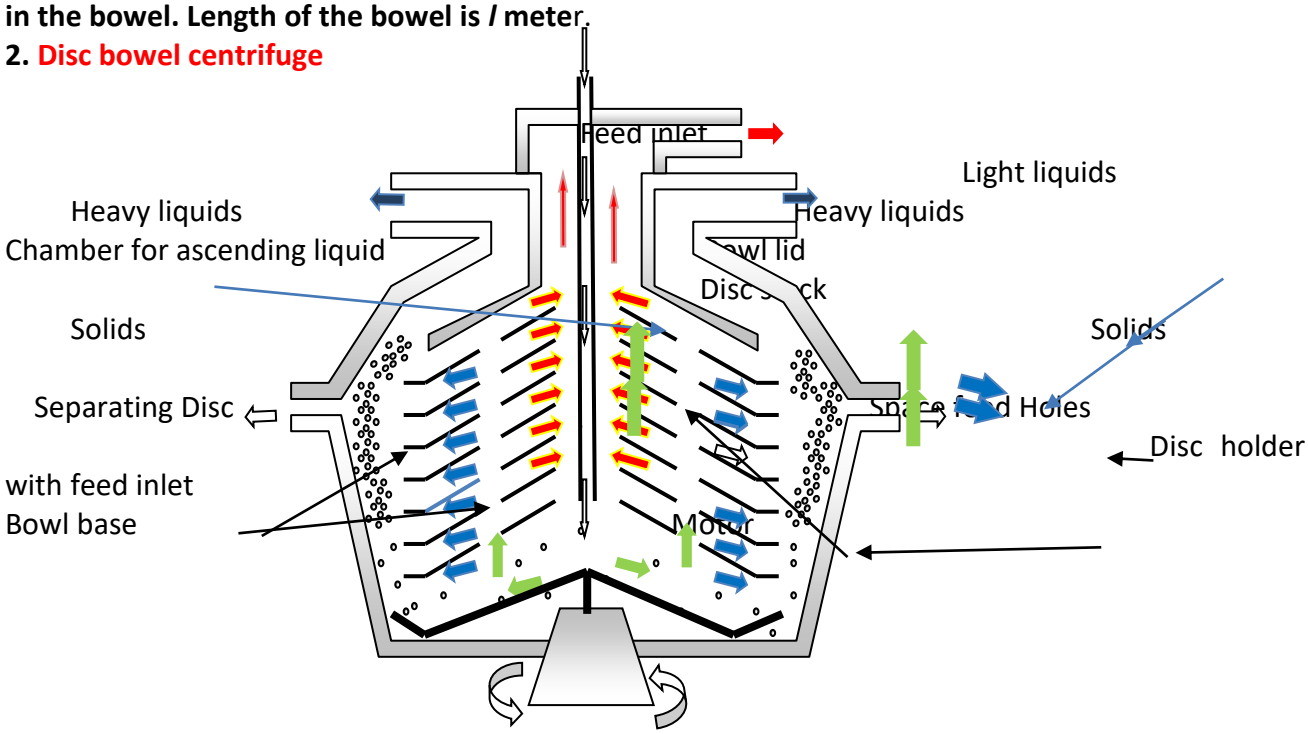


Figure 10.7 Diagrammatic representation disc type centrifuges.

- Disc bowel centrifuge is commonly used in the bioseperation, since it offers **continuous separation**.
- The **stacked conical disc allows a large sedimentation area** to be contained in a relatively compact volume.
- The feed enters **the actual compartment at the bottom of the bowel** and travels upward through **vertical spaced feed hole**, filling the spaces between the discs.
- The holes divide the vertical assembly into an **inner section and outer section**.
- **Inner section contains light liquids** which flow over the upper side of the discs and towards the inner outlet, and **outer section contains heavy liquids** which flow beneath the underside of a disk to the periphery of the bowl.
- Solids are thrown towards the outer wall and collected through the solid outlets.
- Disc bowel centrifuge is a **shallow wide cylindrical** bottom driven bowl rotating at moderate speed of 6000 rpm in a **stationary casing of 30 to 100 cm of diameter.**

- Bowl contains closely spaced metal discs located one above the other with fixed clearance of about **0.5 to 2 mm by keeping spacer bar** (Figure 10.7).
- Feed enters at the top, opens up at the bottom, and clarified liquid flows out an annual slit near the feed. Solids are removed continuously out of an orifice on the side of the centrifuge.

10.2.3 Basket centrifuge

The basic type of the centrifuge commonly used is the **basket centrifuge rotating around vertical axis** as represented in the figure 10.9.

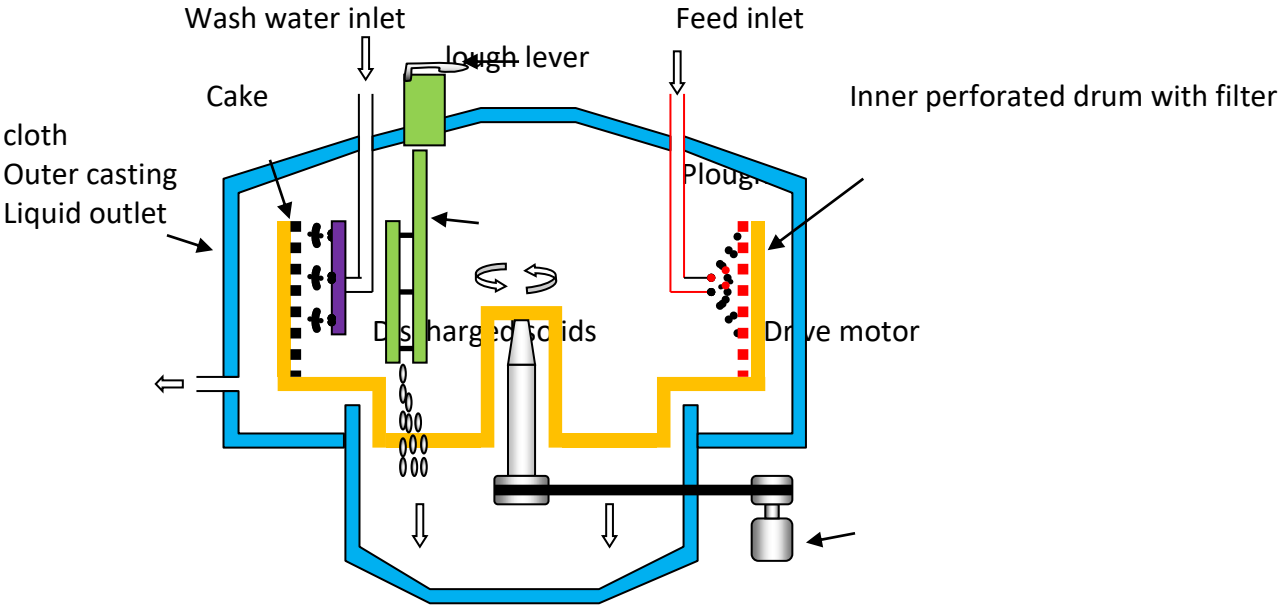


Figure 10.9 Diagrammatic representation of the vertical basket centrifuge

- Biological fluids are fed into **the rotating basket with filtering cloth on the slotted jacket.**
- **Liquid** passes through the cloth and solids are left on the basket walls.
- When the basket is full of material, the feed is finished and, the rotating drum speed is removed to **scrap the solid cake from the inner surface of the perforated drum by scrape plates**, **screw conveyer** or plough dragged near to the cake by moving lever to operate plough.
- If necessary, washing of the cake can be performed using an additional stage prior to the cake discharge.
- The theoretical prediction of rate of filtration in centrifugal filtration is not been so successful.
- Filtration during centrifugation is more complicated than ordinary process, because complications arise due to the increase of area from flow and driving force as we move away from the central axis of rotation, and change in the cake resistance.

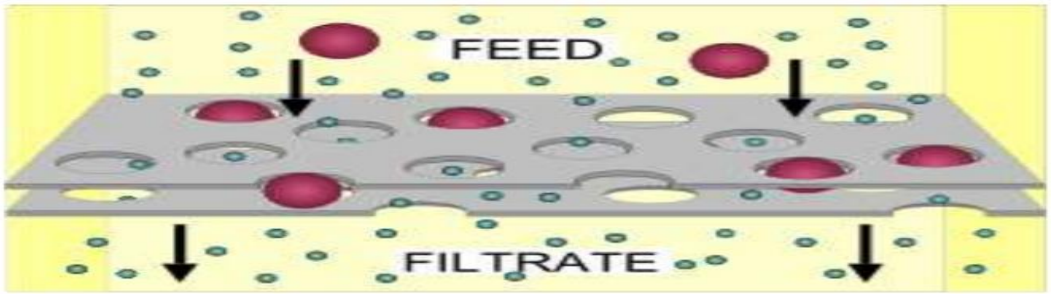
Chapter 9 **Filtration**

Introduction

- **Filtration** is commonly the mechanical or physical operation which is used for the separation of products like solids from fluids by interposing a medium through which only the fluid can pass.
- The fluid that passes through is called a filtrate.
- Filtration is the best established and most versatile method for removing insoluble from dilute streams like fermentation broths.

FILTERATION

The separation of solids from a suspension in a liquid by means of a porous medium or screen which retains the solids and allows the liquid to pass is termed filtration.



Principle of Filtration

Since the filter medium is permeable only to the fluid, it retains the solid particles and permits only the fluid to pass through which is collected as the filtrate. The volume of filtrate collected per unit time (dV/dt) is termed as the rate of filtration.

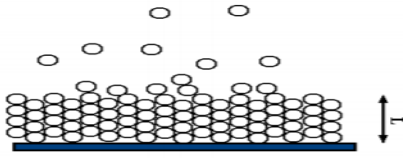
As the filtration proceeds, solid particle accumulate on the filter medium forming a packed bed of solids, called filter cake.

As the thickness of the cake increases

→ resistance to flow of filtrate increases

→ rate of filtration gradually decreases.

If rate is maintained to be constant then pressure difference driving force (-ΔP) will increase.



herefore, a batch filter is operated either at constant pressure or at constant rate.

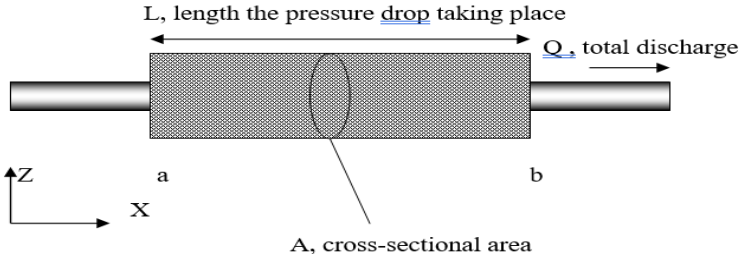
9.3 Basic theory of filtration

Darcy's law relates the **flow rate** through a porous bed of solids to the **pressure drop** causing that flow.

$$Q = \frac{-kA(P_a - P_b)}{\mu L} \quad \text{....}$$

(9.1)

The total discharge, Q in m³/s, is equal to the product of the **permeability, *k*** in m² of the medium, the **cross-sectional area, *A*** to flow, and the **pressure drop, ($P_b - P_a$)**, all divided by the **dynamic viscosity, *μ*** in kg/m·s or Pa·s, and the **length, *L*** the pressure drop is taking place over (Figure 9.7).



The negative sign shows that the fluids flow from high pressure to low pressure. So if the change in pressure is negative, in the *x*-direction, then the flow will be positive, in the *x*-direction.

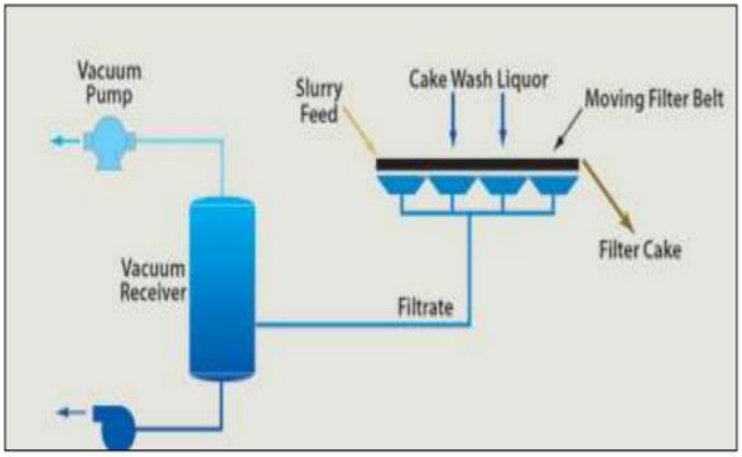
Basic differential equation for filtration at constant pressure drop

$$\frac{1}{A}\frac{dV}{dt} = \frac{\Delta p}{\mu(R_M + R_C)} \quad \text{.... (9.8)}$$

***R_M*, the medium resistance** is a constant and independent of the cake. But ***R_c* the cake resistance** varies with the ***V*, amount filtered**. But the exact nature of the type of variation of cake resistance depends on whether the cake is **compressible or incompressible**.

Types of filtration equipment

The industrial filtration equipment differs from laboratory filtration equipment only in the amount of material handled and in the necessity for low-cost operation.



- Filters can also be classified by operating cycle. Filters can be operated as **batch**, where the cake is removed after a run, or **continuous**, where the cake is removed continuously.

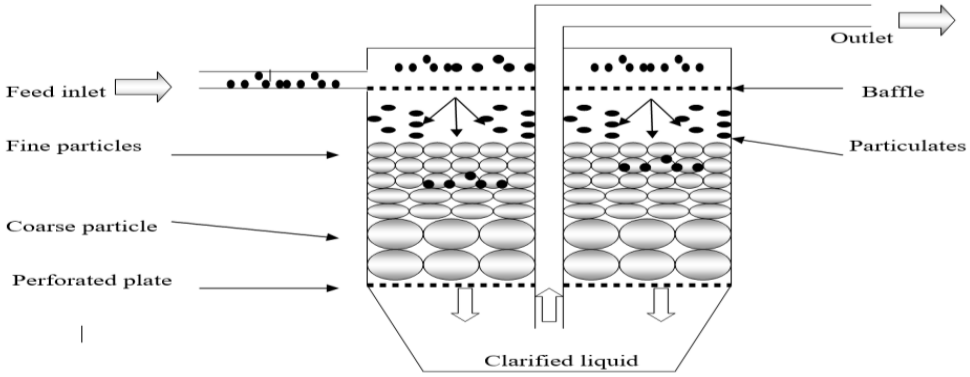
- In another classification, filters can be of the gravity type, where the liquid simply flows by means of a hydrostatic head, or pressure or vacuum can be used to increase the flow rates.

Some of the most important types of filters:

1. Bed filter
2. Plate-and-frame-filter
3. Leaf Filters
4. Continuous rotary filters
    a. Continuous rotary vacuum-drum filter
    b. Continuous rotary disk filter
    c. Continuous rotary horizontal filter

1. BED FILTER

- Such filters are useful mainly in cases where relatively small amounts of solids are to be removed from large amounts of water in clarifying the liquid.

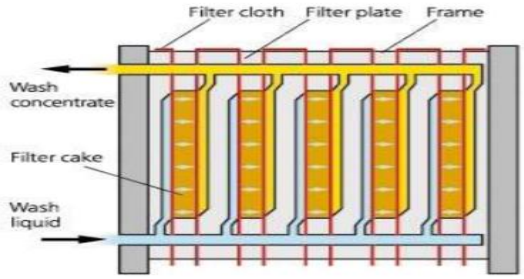


Bead or depth filters

- The simple type of filter that is the bed filters are relatively used when **small amount of biological fluids** has to be clarified.
- Often the **bottom layer is composed of coarse pieces of gravel** resting on **a perforated or slotted plate**, and above the gravel is **fine sand which acts as the actual filter medium**.
- Feed is introduced at the **top onto a baffle which spreads the water** out and **clarified liquid is drawn out at the bottom**.
- Filtration continues until the particulates clog the sand to stop the filtration. Filters are reused after back washing to drive the particulated away. These filters can only be used on the precipitates that do not adhere strongly to the sand and can easily be removed by back washing.

2. PLATE-AND-FRAME-FILTER

The feed slurry is pumped which flows through the duct. The filtrate flows through the filter cloth and the solids build up as a cake on the frame side of the cloth. The filtrate flows between the filter cloth and the face of the plate through the channels to the outlet.



- Plate and frame is one of the important filtration methods. It consists of **plates and frames that are assembled alternately with filter cloth on each side of the plate**.

- **Plates have grooved with channels** through which filtrate can drain down along each plate.
- **Frames are with empty space in between the four sides** helps in **keeping sufficient cavity for the fed slurry** and the retentate in between two membranes.
- The biological fluids to be cleared of insolubles are **pumped into the press** and **feed flow through the duct into each of the frames so as to fill the frames**.
- From here the **feed pass through the filter cloth** and **the retained solids build as a cake on the frame side of the cloth**.
- **Filtrate flows through the space in between filter cloth and the pace of the frames**, and then through the channel to the outlet

3. Leaf Filters

This filter is useful for many purposes but is not economical for handling large quantities of sludge or for washing with a small amount of fresh water. The wash water often channels in the cake and large volumes of wash water may be needed.

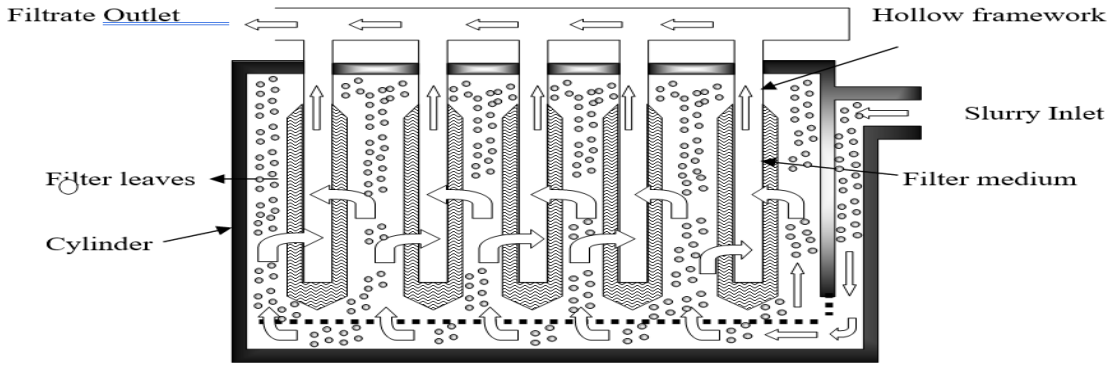


Figure 9.3 Diagrammatic representation of pressure leaf filter.

- This type of equipments consisting of **number of filter leaves**.
- Each leaf consisting of a **metal frame work of grooved plates covered with fine wire meshes or filter cloth**, **precoated with cellulose fiber**.
- **Numbers of such leaves are hung in parallel in a closed tank**.
- Biological fluids to be cleared of the insolubles or filtered of biomass are **forced under the pressure through the filter medium**, where the particulated deposited on the outer surface of the leaves.
- Filtrate flows through the **hollow frame work and out through a header** outlet.
- **Washing liquid flows** through the same path and hence washing of this type of filters are more efficient than the plate and frame filters.
- **The shells are opened to remove the biomass** by blowing the air in the reverse direction or to remove the unwanted insolubles by washing the cake with pressure along with the effluents.
- One more disadvantages associated with the pressure leaf filters are its batch mode of operation, but they can be automated for the filtering, washing and cleaning cycle.

4. Continuous rotary filters

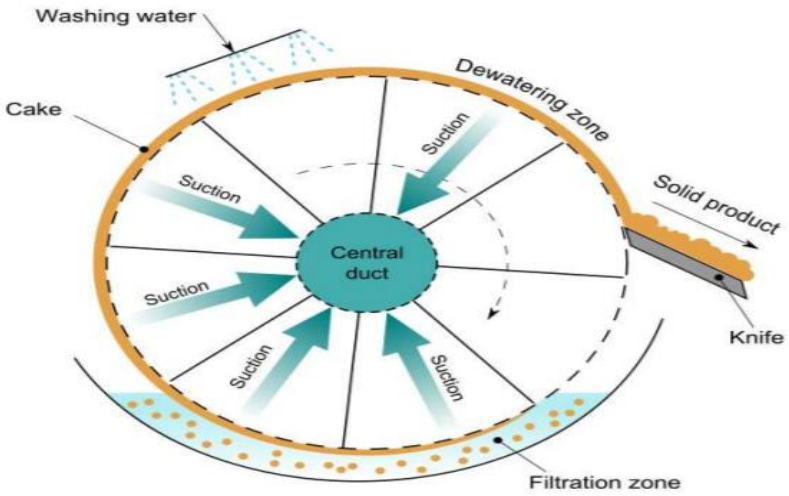
✓ A number of such filters are as follows:

a. Continuous rotary vacuum-drum filter

b. Continuous rotary disk filter

c. Continuous rotary horizontal filter

A. CONTINUOUS ROTARY VACUUM-DRUM FILTER



B. CONTINUOUS ROTARY DISK FILTER

The filter consist of concentric vertical disks mounted on a horizontal rotating shaft. The filter operates on the same principle as the vacuum rotary-drum filter. Each disk in hollow and covered with a filter cloth and is partly submerged in the slurry.

C. CONTINUOUS ROTARY HORIZONTAL FILTER

This type is a vacuum filter with the rotating annular filtering surface divided into sectors. As the horizontal filter rotates, it successively receives slurry, is washed, is dried, and the cake is scraped off.

Chapter 10: VTU: Explain two different types of electrophoresis

- Movement of particles by an electric force.
- Gel is the matrix perfused by buffer salts
  - Matrix will
    - reduce effects of diffusion
    - stabilize the system against turbulence (thermal agitation)
    - Introduce sieving action that allows separation based on molecular size
  - Buffer salts will
    - prevent sample interactions
    - create more conductivity

Electrophoresis

- Mobility = applied voltage x net charge on the molecule / function of molecule (shape, size and other physical matter)
- The electrophoretic mobility, μ (m ^{2}/Vs), is the observed electrophoretic velocity, v (m/s), divided by electric field strength, E (V/m):

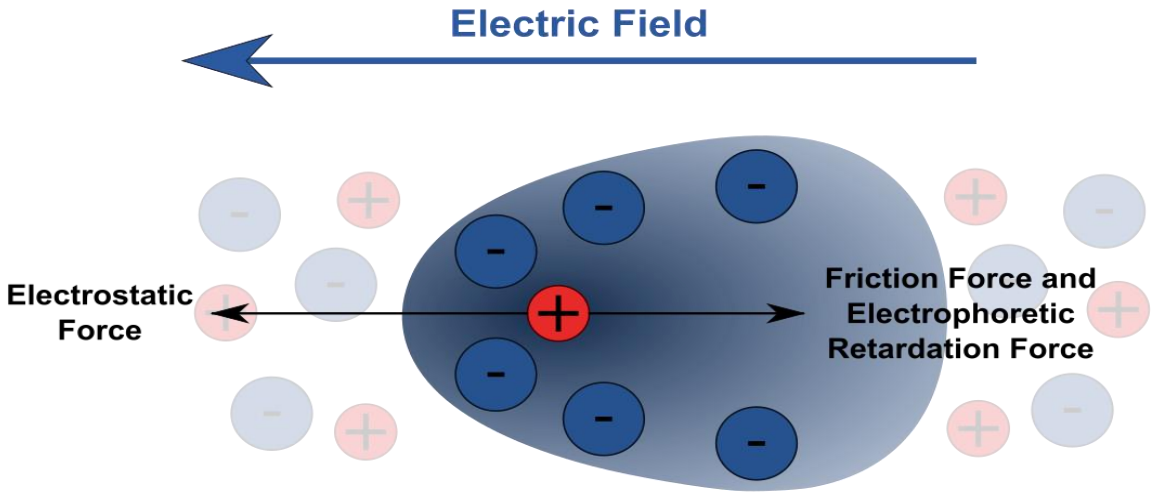
$\mu = v/E$

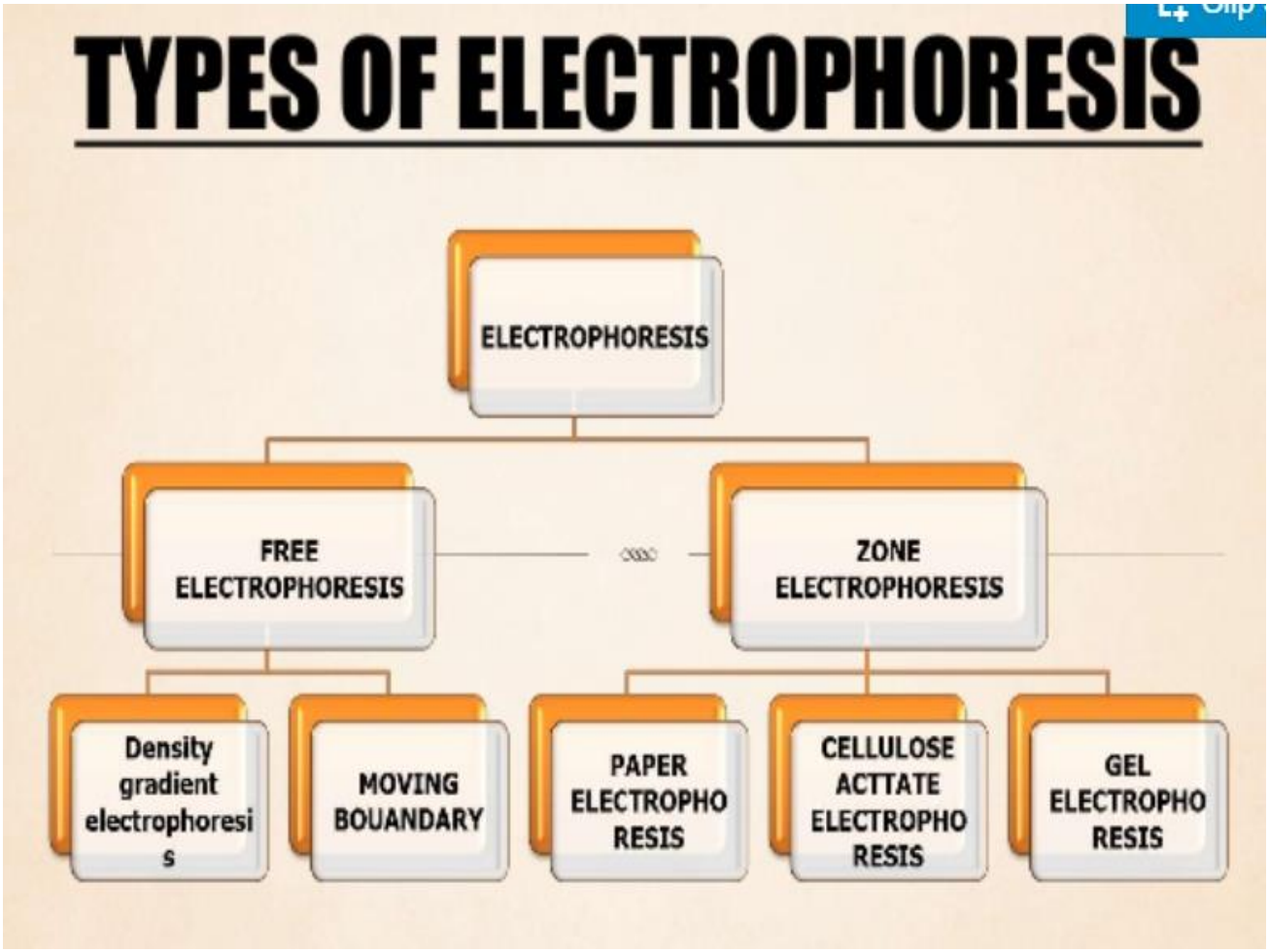
- Force: Total electric field is the effective charge and field strength
- Opposing force is friction and pore size
- When charge/mass is constant then large molecules move slower than small molecules.

EQUATION:

$$V = \frac{E * q}{f}$$

V = velocity of migration of the molecule.
E = electric field in volts per cm
q = net electric charge on the molecule
f = frictional coefficient




TYPES OF ELECTROPHORESIS
ELECTROPHORESIS
FREE ELECTROPHORESIS
ZONE ELECTROPHORESIS
Density gradient electrophoresis
MOVING BOUANDARY
PAPER ELECTROPHO RESIS
CELLULOSE ACTTATE ELECTROPHO RESIS
GEL ELECTROPHO RESIS

TYPES OF ELECTROPHORESIS

- Zone Electrophoresis
- Slab Gel Electrophoresis
- Disc Electrophoresis
- Isoelectric Focusing Electrophoresis
- 2-Dimensional Electrophoresis
- Capillary Electrophoresis
- Microchip Electrophoresis

PRINCIPLE

- Any charged ion or molecule migrates when placed in an electric field. The rate of migration depend upon its net charge, size, shape and the applied electric current.

Zone Electrophoresis:

- Produce zones of proteins that are heterogeneous and physically separated from one another
- Classified according to type and structure of the support material e.g AGE, CAE, PAGE etc

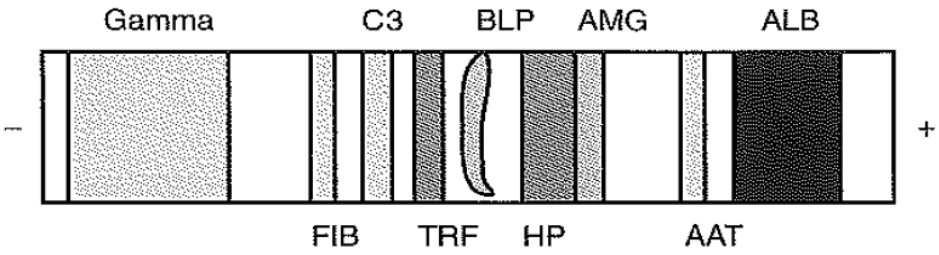
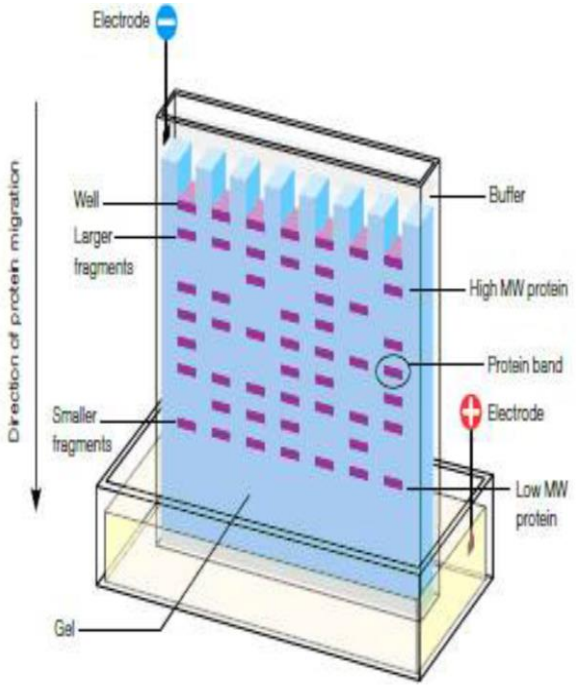


Figure 6-3 A simplified schematic drawing of a protein pattern from the serum of a subject with haptoglobin type 2-1 (separation by PAGE). Some zones contain more than the one protein shown, as demonstrated by immunological techniques. *AAT*, Alpha$_1$-antitrypsin; *ALB*, albumin; *AMG*, alpha$_2$-macroglobulin; *BLP*, beta-lipoprotein; *C3*, complement 3; *FIB*, fibrinogen; *gamma*, gamma-globulin; *HP*, haptoglobin; *TRF*, transferrin.

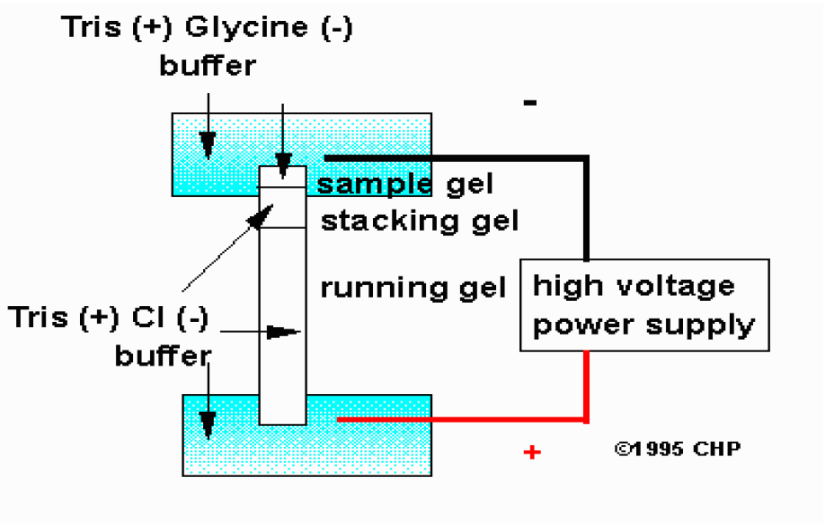
Slab Gel Electrophoresis:

- Use of a rectangular gel regardless of the thickness
- Main advantage – ability to simultaneously separate several samples in one run
- Primary method used in clinical chemistry lab
- Gels (usually agarose) may be cast on sheet of plastic backing or completely encased within a plastic walled cell allowing horizontal or vertical electrophoresis and submersion for cooling, if needed.
- May be cast with additives like:
  - Ampholytes which create a pH gradient, or
  - Sodium dodecyl sulfate (SDS) that denatures protiens



Disc Electrophoresis:

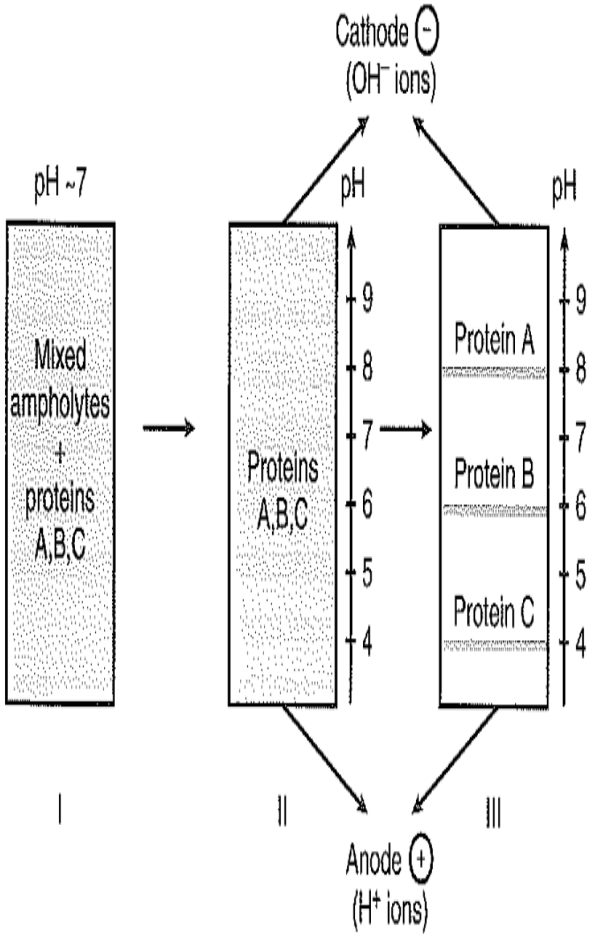
- 3-gel system:
  - small-pore separating gel (running gel),
  - a larger-pore spacer gel (stacking gel),
  - and a thin layer of large-pore monomer solution (sample gel) – containing about 3 µL of serum
- The different composition cause ***disc***ontinuities in the electrophoresis matrix
- During electrophoresis, all proteins migrate easily through the large-pore gels and stack up on the separation gel in a very thin zone
- This improves resolution and concentrates protein components at the border (or starting zone)
- Separation occurs at the bottom separation gel by the molecular sieve phenomenon



Schematic representation of Disc Electrophoresis

Isoelectric Focusing Electrophoresis:

- Separates amphoteric compounds with increased resolution in a medium possessing a stable pH gradient
- The protein migrate to a zone in the medium where the pH of the gel matches its pI
- At this point, the charge of the protein becomes zero and its migration ceases – it become "focused".
- Regions associated with a given pH are very narrow – enough to separate proteins that differ in their pI values by only 0.02pH units
- A high voltage power source is needed because carrier ampholytes are used in relatively high concentrations
- Thus, the electrophoretic matrix must be cooled
- IEF is used in neonatal screening programs to test for variant Hb



Capillary Electrophoresis:

- Separation in narrow-bore fused silica capillaries (inner diameter 25 – 75µm) filled with buffer – gel media can also be used
- Sample is loaded after filling capillary with buffer, and electric field applied
- Borate is a classic CE buffer that generates relatively low current and heat, even with a high ionic strength
- Electro-osmotic flow (EOF) controls the amount of time solutes remain in the capillary (also Electroendosmostic flow or endosmosis)
  - EOF = bulk flow of liquid towards the cathode upon application of an electric field and it is superimposed on electrophoretic migration
- Cations migrate fastest due to EOF and electrophoretic attraction towards the cathode

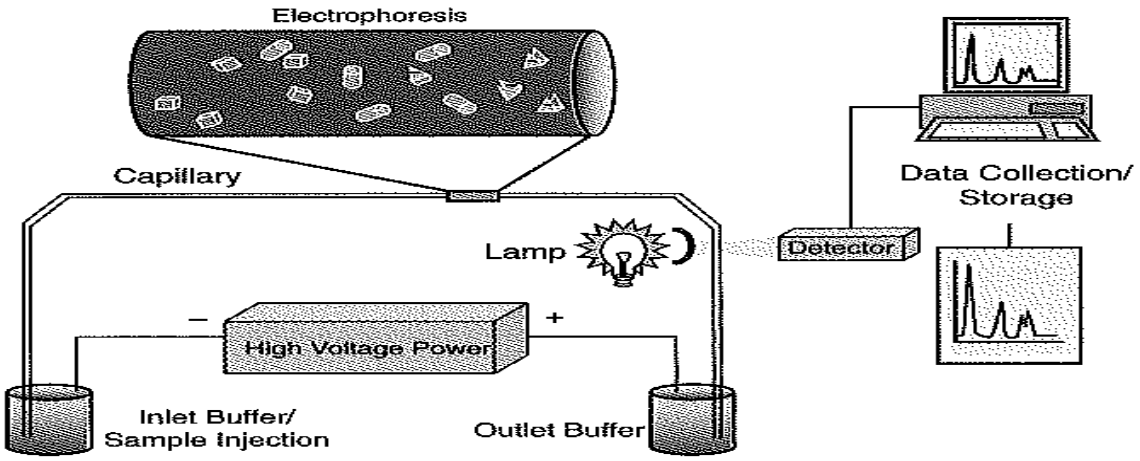


Figure 6-5 Schematic for CE instrumentation.

2-Dimensional Electrophoresis:

- Uses charge-dependent IEF (first dimension) and molecular weight-dependent electrophoresis (in the second)
- 1st dimension – carried out in a large-pore medium like agarose or large-pore PAG; to which ampholytes are added to yield a pH gradient
- 2nd dimension is often polyacrylamide in a linear or gradient format
- It achieves the highest resolving power for the separation of DNA fragments
  - 1st dimension – normal AGE
  - 2nd dimension – ethidium bromide is added to the gel to open up the fragments and cause changes in their mobility
- Method of choice when complex samples need to be arrayed for characterization, as in proteomics.