IDHAYA COLLEGE FOR WOMEN, KUMBAKONAM

DEPARTMENT OF MICROBIOLOGY

Year : III, B.Sc.,

Semester : VI

Topic : Unit – III

Subject Name : Industrial Microbiology

Sub Code : 16SCCMB9

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2020-2021

Stages in Downstream Processing

The five stages are:

- ✓ **Solid-Liquid Separation**
- ✓ **Release of Intracellular Products**
- ✓ **Concentration (4) Purification by Chromatography**
- ✓ **Formulation.**

1. Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth (Note: If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation). Some authors use the term harvesting of microbial cells for the separation of cells from the culture medium. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

Flotation: When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

Flocculation: In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

Filtration: Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors—the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

Depth Filters: They are composed of a filamentous matrix such as glass wool, asbestos or filter paper. The particles are trapped within the matrix and the fluid passes out. Filamentous fungi can be removed by using depth filters.

Absolute Filters: These filters are with specific pore sizes that are smaller than the particles to be removed. Bacteria from culture medium can be removed by absolute filters.

Rotary Drum Vacuum Filters: These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10µm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth (Fig. 1). As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

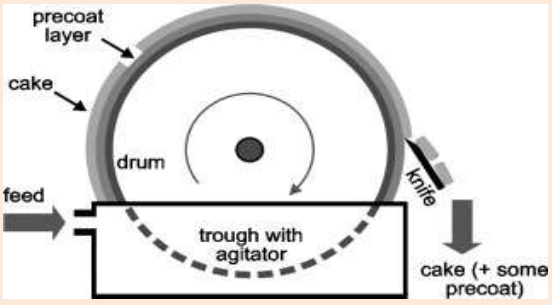


Fig.1, Diagrammatic Representation of a rotary drum vacuum filter

Membrane Filters: In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations—static filtration and cross-flow filtration (Fig. 2). In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.

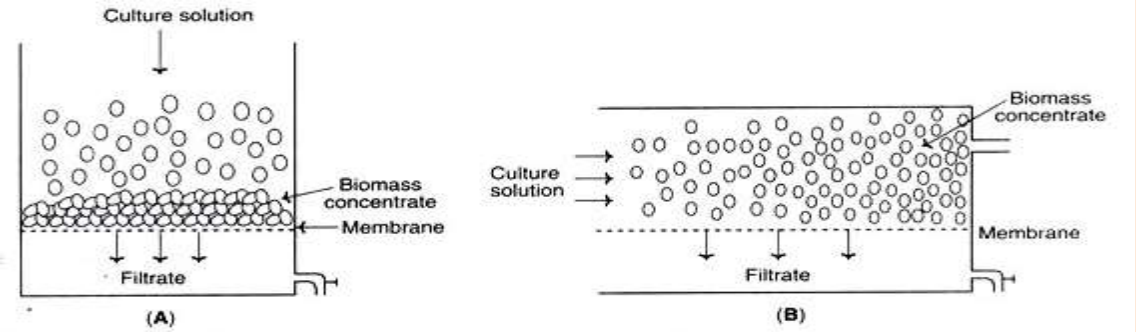


Fig.2. Filter systems for separation of biomass and culture filtrate

A. Static-flow filtration, B. Cross-flow filtration

Types of filtration processes: There are 3 major types of filtrations based on the particle sizes and other characters (Table.1). These are microfiltration, ultrafiltration and reverse osmosis.

Table.1. Major types of filtration processes with characteristic features

Type	Size of particles separated	Compound or particle separated
1. Microfiltration	0.1-10 um	Cells or cell fractions, virues
2. ultrafiltration	0.001-0.1 um	Compounds with molecular weights greater than 1000 (e.g. enzymes)
3. Reversed osmosis	0.0001-0.001 um	Compounds with molecular weights less than 1000 (e.g. lactose)

Centrifugation: The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus,

centrifugation is mostly used for separating solid particles from liquid phase (fluid/particle separation). Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale industrial centrifugation. However, in recent years, continuous flow industrial centrifuges have been developed. There is a continuous feeding of the slurry and collection of clarified fluid, while the solids deposited can be removed intermittently. The different types of centrifuges are depicted in (Fig. 3), and briefly described hereunder.

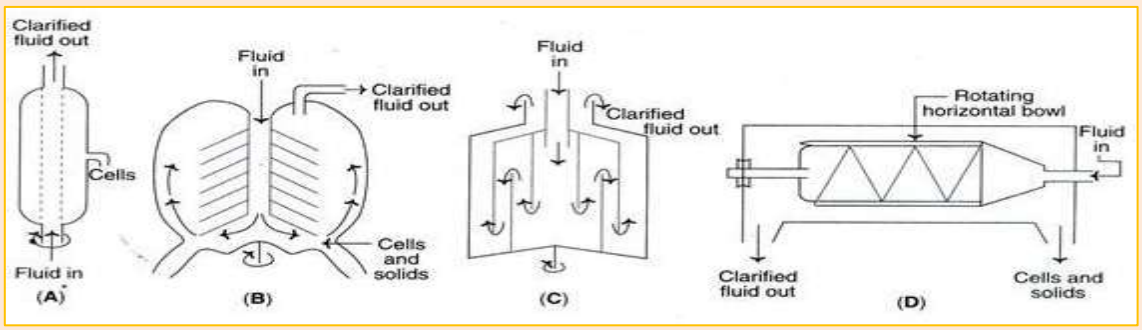


Fig. 3. Centrifuges commonly used in downstream processing

A. Tubular bowl centrifuge, B. Disc centrifuge, C. Multichamber centrifuge, D. Scroll centrifuge.

Tubular bowl centrifuge (Fig. 3.A): This is a simple and a small centrifuge, commonly used in pilot plants. Tubular bowl centrifuge can be operated at a high centrifugal speed, and can be run in both batch or continuous mode. The solids are removed manually.

Disc centrifuge (Fig. 3.B): It consists of several discs that separate the bowl into settling zones. The feed/slurry is fed through a central tube. The clarified fluid moves upwards while the solids settle at the lower surface.

Multi-chamber centrifuge (Fig. 3.C): This is basically a modification of tubular bowl type of centrifuge. It consists of several chambers connected in such a way that the feed flows in a zigzag fashion. There is a variation in the centrifugal force

in different chambers. The force is much higher in the periphery chambers, as a result smallest particles settle down in the outermost chamber.

Scroll centrifuge or decanter (Fig. 3.D): It is composed of a rotating horizontal bowl tapered at one end. The decanter is generally used to concentrate fluids with high solid concentration (biomass content 5-80%). The solids are deposited on the wall of the bowl which can be scrapped and removed from the narrow end.

2. Release of Intracellular Products

As already stated, there are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods. The outline of different techniques used for breakage of cells is given in Fig.4.

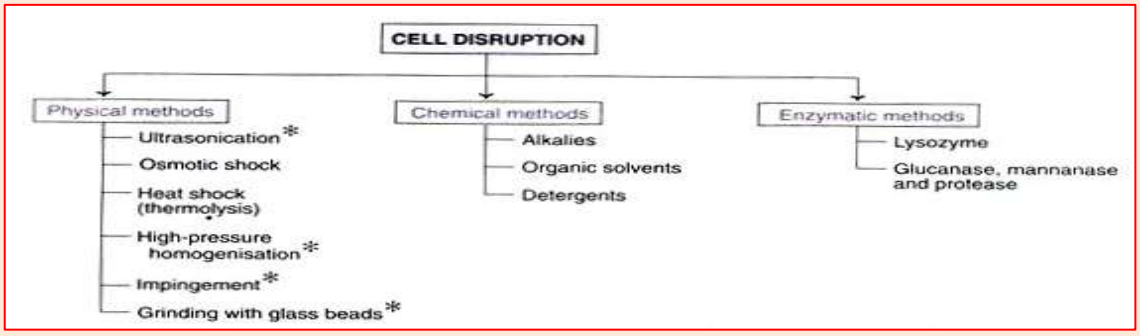


Fig.4. Major Methods for cell disruption to release the intracellular products

The selection of a particular method depends on the nature of the cells, since there is a wide variation in the property of cell disruption or breakage. For instance, Gram-negative bacteria and filamentous fungi can be more easily broken compared to Gram-positive bacteria arid yeasts.

Cell Disruption:

Physical methods of cell disruption: The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

Ultra sonication: Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock: This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis): Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization: This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

Impingement: In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking

E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads: The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.

A diagrammatic representation of a cell disrupter employing glass beeds is shown in (Fig. 5). It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

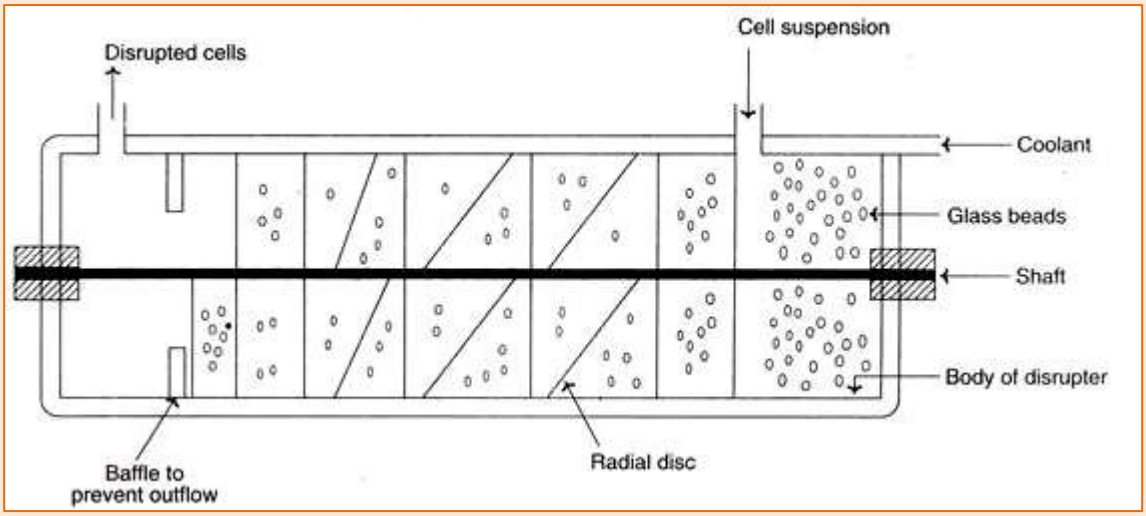


Fig .5. Diagrammatic Representation of a cell disrupter.

Mechanical and non-mechanical methods: Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non- mechanical in nature.

Chemical methods of cell disruption: Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

Alkalies: Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

Organic solvents: Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents: Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt

precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromatography for purification.

Enzymatic methods of cell disruption: Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram- positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

Combination of methods: In order to increase the efficiency of cell disintegration in a cost-effective manner, a combination of physical, chemical and enzymatic methods is employed.

3. CONCENTRATION

The filtrate that is free from suspended particles (cells, cell debris etc.) usually contains 80-98% of water. The desired product is a very minor constituent. The water has to be removed to achieve the product concentration. The commonly used techniques for concentrating biological products are evaporation, liquid-liquid extraction, membrane filtration, precipitation and adsorption. The actual procedure

adopted depends on the nature of the desired product (quality and quantity to be retained as far as possible) and the cost factor.

Evaporation: Water in the broth filtrate can be removed by a simple evaporation process. The evaporators, in general, have a heating device for supply of steam, and unit for the separation of concentrated product and vapour, a condenser for condensing vapour, accessories and control equipment. The capacity of the equipment is variable that may range from small laboratory scale to industrial scale. Some of the important types of evaporators in common use are briefly described.

Plate evaporators: The liquid to be concentrated flows over plates. As the steam is supplied, the liquid gets concentrated and becomes viscous.

Falling film evaporators: In this case, the liquid flows down long tubes which get distributed as a thin film over the heating surface. Falling film evaporators are suitable for removing water from viscous products of fermentation.

Forced film evaporators: The liquid films are mechanically driven and these devices are suitable for producing dry product concentrates.

Centrifugal forced film evaporators: These equipment evaporate the liquid very quickly (in seconds), hence suitable for concentrating even heat-labile substances. In these evaporators, a centrifugal force is used to pass on the liquid over heated plates or conical surfaces for instantaneous evaporation.

Liquid-Liquid Extraction: The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product. The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases. The process of liquid-liquid extraction may be broadly categorized as extraction of low molecular weight products and extraction of high molecular weight products.

Extraction of low molecular weight products: By using organic solvents, the lipophilic compounds can be conveniently extracted. However, it is quite difficult to extract hydrophilic compounds. Extraction of lipophilic products can be done by the following techniques.

Physical extraction: The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non-ionizing compounds.

Dissociation extraction: This technique is suitable for the extraction of invisible compounds. Certain antibiotics can be extracted by this procedure.

Reactive extraction: In this case, the desired product is made to react with a carrier molecule (e.g., phosphorus compound, aliphatic amine) and extracted into organic solvent. Reactive extraction procedure is quite useful for the extraction of certain compounds that are highly soluble in water (aqueous phase) e.g., organic acids.

Supercritical fluid (SCF) extraction: This technique differs from the above procedures, since the materials used for extraction are supercritical fluids (SCFs). SCFs are intermediates between gases and liquids and exist as fluids above their critical temperature and pressure. Supercritical CO_2, with a low critical temperature and pressure is commonly used in the extraction. Supercritical fluid extraction is rather expensive, hence not widely used (SCF has been used for the extraction of caffeine from coffee beans, and pigments and flavor ingredients from biological materials).

Extraction of high molecular weight compounds: Proteins are the most predominant high molecular weight products produced in fermentation industries. Organic solvents cannot be used for protein extraction, as they lose their biological activities. They are extracted by using an aqueous two-phase systems or reverse micelles formation.

Aqueous two-phase systems (ATPS): They can be prepared by mixing a polymer (e.g., polyethylene glycol) and a salt solution (ammonium sulfate) or two different polymers. Water is the main component in ATPS, but the two phases are not miscible. Cells and other solids remain in one phase while the proteins are transferred to other phase. The distribution of the desired product is based on its surface and ionic character and the nature of phases. The separation takes much longer time by ATPS.

Reverse miceller systems: Reverse micelles are stable aggregates of surfactant molecules and water in organic solvents. The proteins can be extracted from the aqueous medium by forming reverse micelles. In fact, the enzymes can be extracted by this procedure without loss of biological activity.

Membrane Filtration: Membrane filtration has become a common separation technique in industrial biotechnology. It can be conveniently used for the separation of biomolecules and particles, and for the concentration of fluids. The membrane filtration technique basically involves the use of a semipermeable membrane that selectively retains the particles/molecules that are bigger than the pore size while the smaller molecules pass through the membrane pores.

Membranes used in filtration are made up of polymeric materials such as polyethersulfone and polyvinyl di-fluoride. It is rather difficult to sterilize membrane filters. In recent years, micro-filters and ultrafiIters composed of ceramics and steel are available. Cleaning and sterilization of such filters are easy. The other types of membrane filtration techniques are described briefly.

Membrane adsorbers: They are micro- or macro porous membranes with ion exchange groups and/or affinity ligands. Membrane adsorbers can bind to proteins and retain them. Such proteins can be eluted by employing solutions in chromatography.

Pervaporation: This is a technique in which volatile products can be separated by a process of permeation through a membrane coupled with evaporation. Pervaporation is quite useful for the extraction, recovery and concentration of volatile products. However, this procedure has a limitation since it cannot be used for large scale separation of volatile products due to cost factor.

Perstraction: This is an advanced technique working on the principle of membrane filtration coupled with solvent extraction. The hydrophobic compounds can be recovered/ concentrated by this method.

Precipitation: Precipitation is the most commonly used technique in industry for the concentration of macromolecules such as proteins and polysaccharides. Further, precipitation technique can also be employed for the removal of certain unwanted byproducts e.g. nucleic acids, pigments.

Neutral salts, organic solvents, high molecular weight polymers (ionic or non-ionic), besides alteration in temperature and pH are used in precipitation. In addition to these non-specific protein precipitation reactions (i.e. the nature of the protein is unimportant), there are some protein specific precipitations e.g., affinity precipitation, ligand precipitation.

Neutral salts: The most commonly used salt is ammonium sulfate, since it is highly soluble, nontoxic to proteins and low-priced. Ammonium sulfate increases hydrophobic interactions between protein molecules that result in their precipitation. The precipitation of proteins is dependent on several factors such as protein concentration, pH and temperature.

Organic solvents: Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation. They reduce the dielectric constant of the medium and enhance electrostatic interaction between protein molecules that lead to precipitation. Since proteins are denatured by organic solvents, the precipitation process has to be carried out below 0°C.

Non-ionic polymers: Polyethylene glycol (PEG) is a high molecular weight non-ionic polymer that can precipitate proteins. It reduces the quantity of water available for protein solvation and precipitates protein. PEG does not denature proteins, besides being non-toxic.

Ionic polymers: The charged polymers such as polyacrylic acid and polyethyleneimine are used. They form complexes with oppositely charged protein molecules that causes charge neutralisation and precepitation.

Increase in temperature: The heat sensitive proteins can be precipitated by increasing the temperature.

Change in pH: Alterations in pH can also lead to protein precipitation.

Affinity precipitation: The affinity interaction (e.g., between antigen and antibody) is exploited for precipitation of proteins.

Precipitation by ligands: Ligands with specific binding sites for proteins have been successfully used for selective precipitation.

Adsorption: The biological products of fermentation can be concentrated by using solid adsorbent particles. In the early days, activated charcoal was used as the adsorbent material. In recent years, cellulose-based adsorbents are employed for protein concentration. And for concentration of low molecular weight compounds (vitamins, antibiotics, peptides) polystyrene, methacrylate and acrylate based matrices are used. The process of adsorption can be carried out by making a bed of adsorbent column and passing the culture broth through it. The desired product, held by the adsorbent, can be eluted.

4. PURIFICATION BY CHROMATOGRAPHY

The biological products of fermentation (proteins, pharmaceuticals, diagnostic compounds and research materials) are very effectively purified by

chromatography. It is basically an analytical technique dealing with the separation of closely related compounds from a mixture. Chromatography usually consists of a stationary phase and mobile phase. The stationary phase is the porous solid matrix packed in a column (equilibrated with a suitable solvent) on to which the mixture of compounds to be separated is loaded. The compounds are eluted by a mobile phase.

A single mobile phase may be used continuously or it may be changed appropriately to facilitate the release of desired compounds. The eluate from the column can be monitored continuously (e.g. protein elution can be monitored by ultraviolet adsorption at 280 nm), and collected in fractions of definite volumes.

The different types of chromatography techniques used for separation (mainly proteins) along with the principles are given in (Table. 2). A large number of matrices are commercially available for purification of proteins e.g., agarose, cellulose, polyacrylamide, porous silica, cross- linked dextran, polystyrene. Some of the important features of selected chromatographic techniques are briefly described.

Chromatography	*Principle*
Gel-filtration (size exclusion)	Size and shape
Ion-exchange	Net charge
Chromatofocussing	Net charge
Affinity	Biological affinity and molecular recognition
Hydrophobic interaction	Polarity (hydrophobicity of molecules)
Immobilized metal-ion affinity	Metal ion binding

Table.2. Chromatographic techniques along with the principal for separation of proteins

Gel-filtration chromatography: This is also referred to as size-exclusion chromatography. In this technique, the separation of molecules is based on the size, shape and molecular weight. The sponge-like gel beads with pores serve as molecular sieves for separation of smaller and bigger molecules. A solution mixture containing molecules of different sizes (e.g. different proteins) is applied to the column and eluted. The smaller molecules enter the gel beads through their pores and get trapped. On the other hand, the larger molecules cannot pass through the pores and therefore come out first with the mobile liquid. At the industrial scale, gel-filtration is particularly useful to remove salts and low molecular weight compounds from high molecular weight products.

Ion-exchange chromatography: It involves the separation of molecules based on their surface charges. Ion-exchangers are of two types (cation- exchangers which have negatively charged groups like carboxymethyl and sulfonate, and anion-exchangers with positively charged groups like diethylaminoethyl (DEAE). The most commonly used cation-exchangers are Dowex HCR and Amberlite IR, the anion-exchangers are Dowex SAR and Amberlite IRA.

In ion-exchange chromatography, the pH of the medium is very crucial, since the net charge varies with pH. In other words, the pH determines the effective charge on both the target molecule and the ion-exchanger. The ionic bound molecules can be eluted from the matrix by changing the pH of the eluant or by increasing the concentration of salt solution. Ion-exchange chromatography is useful for the purification of antibiotics, besides the purification of proteins.

Affinity chromatography: This is an elegant method for the purification of proteins from a complex mixture. Affinity chromatography is based on an interaction of a protein with an immobilized ligand. The ligand can be a specific antibody, substrate, substrate analogue or an inhibitor. The immobilized ligand on a solid matrix can be effectively used to fish out complementary structures. In Table 20.3, some examples of ligands used for the purification of proteins are given. The protein bound to the ligand can be eluted by reducing their interaction. This can be achieved by changing the pH of the buffer, altering the ionic strength or by using another free ligand molecule. The fresh ligand used has to be removed in the subsequent steps.

Ligand	*Type of protein*
Antibody	Antigen
Cofactor	Enzyme
Receptor	Hormone
Hapten	Antibody
Inhibitor	Enzyme
Lectins	Glycoproteins
Heparin	Coagulation factors
Metal ions	Metal ion binding proteins

Table.3.Examples of ligands used for separation of proteins by affinity chromatography

Hydrophobic interaction chromatography (HIC): This is based on the principle of weak hydrophobic interactions between the hydrophobic ligands (alkyl, aryl side chains on matrix) and hydrophobic amino acids of proteins. The differences in the composition of hydrophobic amino acids in proteins can be used for their separation. The elution of proteins can be done by lowering the salt concentration, decreasing the polarity of the medium or reducing the temperature.

5. FORMULATION

Formulation broadly refers to the maintenance of activity and stability of a biotechnological products during storage and distribution. The formulation of low molecular weight products (solvents, organic acids) can be achieved by concentrating them with removal of most of the water. For certain small molecules, (antibiotics, citric acid), formulation can be done by crystallization by adding salts. Proteins are highly susceptible for loss of biological activity; hence their formulation requires special care. Certain stabilizing additives are added to prolong the shelf life of protein. The stabilizers of protein formulation include sugars (sucrose, lactose), salts (sodium chloride, ammonium sulfate), polymers (polyethylene glycol) and polyhydric alcohols (glycerol). Proteins may be formulated in the form of solutions, suspensions or dry powders.

Drying: Drying is an essential component of product formulation. It basically involves the transfer of heat to a wet product for removal of moisture. Most of the biological products of fermentation are sensitive to heat, and therefore require gentle drying methods. Based on the method of heat transfer, drying devices may be categorized as contact, convection, radiation dryers. These three types of dryers are commercially available.

Spray drying: Spray drying is used for drying large volumes of liquids. In spray drying, small droplets of liquid containing the product are passed through a nozzle directing it over a stream of hot gas. The water evaporates and the solid particles are left behind.

Freeze-drying: Freeze-drying or lyophilization is the most preferred method for drying and formulation of a wide-range of products—pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. This is mainly because freeze-drying usually does not cause loss of biological activity of the desired product. Lyophilization is based on the principle of sublimation of a liquid from a frozen state. In the actual technique, the liquid containing the product is frozen and then dried in a freeze-dryer under vacuum. The vacuum can now be released and the product containing vials can be sealed e.g., penicillin can be freeze dried directly in ampules.

Integration of Different Processes: It is ideal to integrate the fermentation and downstream processing to finally get the desired product. However, this has not been practicable for various reasons. Integration of certain stages in downstream processing for purification of product has met with some success. For instance, protein concentration by extraction into two phase systems combined with clarification and purification can be done together.

Cell Disruption

- ✓ **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.

Types of cell disruption methods

There are two types of cell disruption method which are following

- Mechanical methods
- Non Mechanical methods

MECHANICAL METHODS

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

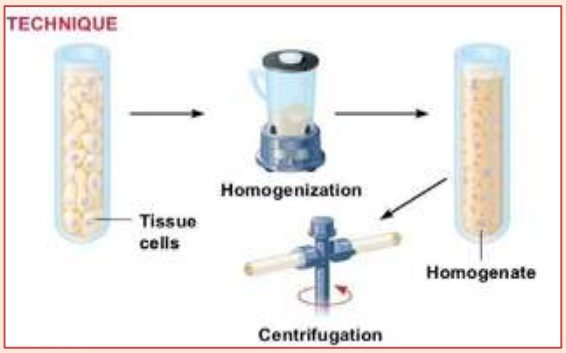
- Mortar & pastel/grinding
- Blender
- Bead beating
- Ultra sonication
- Homogenization

MORTAR & PESTLE

1. Just give the cells a good old grinding. This does not have to be in suspension and is often done with plant samples frozen in liquid nitrogen.
2. When the material has been disrupted, metabolites can be extracted by adding solvents.

BLENDERS

1. The use of blenders (high speed can be used to disrupt cell walls.
2. This 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 but this 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

ULTRA SONICATION

- 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 plant and fungal cells but comes at a disadvantage: It's very loud and has to be performed in an extra room

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

- Non mechanical methods are further divided into three class which are following

    Physical methods

    Freeze thaw

    Microwave/ Thermolysis

    Osmatic shock

    Electric discharges

- Chemical methods
- Enzymatic methods

FREEZE THAW

- This method used when working with soft plant material and algae.
- freezing is used to achieve cell disruption 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.

MICROWAVE

- Microwave (along with autoclave and other high temperature methods) are used to disrupt the bonds within cell walls, and also to denature proteins. This is a somewhat risky method, as the excess heat can quickly damage the rest of the cell.

OSMATIC SHOCK

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

- Note that this method can only work with animal cells and protozoa, since they do not have cell walls.

ELECTRICAL DISCHARGES

- ✓ It is also possible to achieve cell disruption via electrical discharges in mammalian cells.
- ✓ Cells that are bounded by plasma membranes and, unlike plant cells, have no cell wall.
- ✓ This method allows researchers to examine secretion by exocytosis, which is a process during which the membrane-bounded sphere (intracellular vesicle) shifts to and fuses with the plasma membrane

CHEMICAL METHOD

- ✓ 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.

ENZYMATIC METHODS

- ❖ Enzymes such as beta (1-6) and beta (1-3) glycanases, proteases and mannase can be used to disrupt the cell wall.
- ❖ This method is particularly useful for isolating the cell without the wall (protoplast).

IMMOBILIZATION OF CELL AND ENZYMES

Traditionally, free solution enzymes (e.g. in soluble or free form) react with the elements below to produce products. Such use of enzymes is intense, especially for industrial purposes, because the enzymes are fragile, and cannot be recycled.

Inactivation of enzymes (or cells) refers to the process of adding / adding enzymes (or cells) to inert support the stability and stability of its use. By using this method, enzymes are made more efficient and more expensive for their industrial use. Some workers view dysfunction as a goose with a golden egg in enzyme technology. Inactive enzymes keep their origin necessary for catalysis.

There are several advantages of immobilized enzymes:

- Stable and more efficient in function.
- Can be reused again and again.
- Products are enzyme-free.
- Ideal for multi-enzyme reaction systems.
- Control of enzyme function is easy.
- Suitable for industrial and medical use.
- Minimize effluent disposal problems.

Impaired enzymes are generally preferred over non-activated cells because of the specificity of the products that produce the products in a pure way. However, there are several advantages to using more inactive enzyme systems such as organelles and whole cells in addition to non-activated enzymes. Stainless cells have a natural environment through the presence of a cofactor (and its creative potential) and are especially suitable for many enzymatic reactions.

Methods of Immobilization:

The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding and cross-linking.

Adsorption:

Adsorption involves the physical binding of enzymes (or cells) on the surface of an inert support. The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE-cellulose, DEAE-sephadex). Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds (Fig. 1). Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

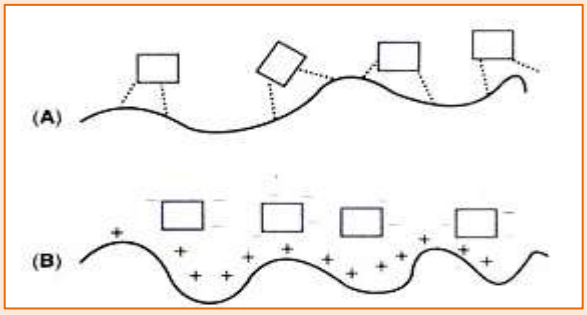


Fig. 1. Immobilization of enzymes by adsorption

A. by van der Waals forces, B. By adsorption bonding

Entrapment: Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix. The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through. In this technique, commonly referred to as lattice entrapment, the enzyme (or cell) is not subjected to strong binding forces and structural distortions.

Some deactivation may however, occur during immobilization process due to changes in pH or temperature or addition of solvents. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber. Enzymes can be entrapped by several ways.

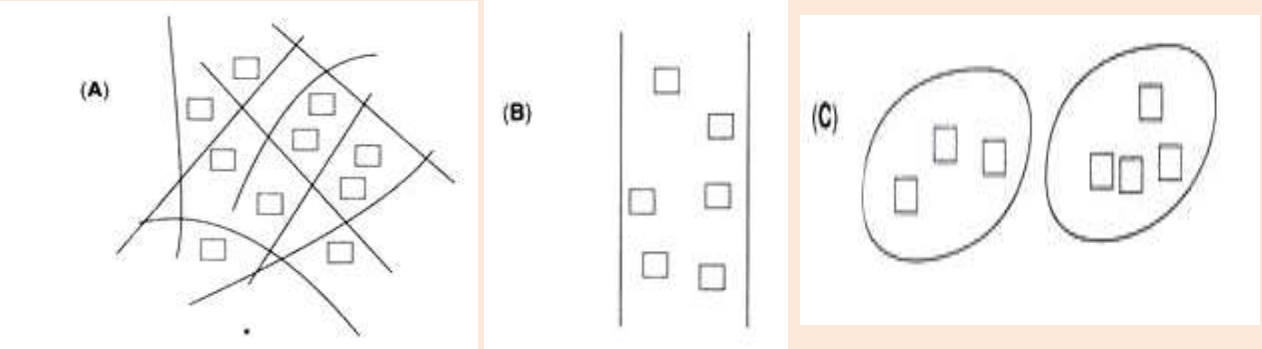


Fig. 2. Immobilization of enzymes by entrapment

A. Inclusion in gels, B. Inclusion in fibres, C. Inclusion in microcapsules

1. Enzyme inclusion in gels:

This is an entrapment of enzymes inside the gels (Fig. 2.A).

2. Enzyme inclusion in fibres:

The enzymes are trapped in a fibre format of the matrix (Fig. 2. B).

3. Enzyme inclusion in microcapsules:

In this case, the enzymes are trapped inside a microcapsule matrix (Fig. 2. C). The hydrophobic and hydrophilic forms of the matrix polymerise to form a microcapsule containing enzyme molecules inside. The major limitation for entrapment of enzymes is their leakage from the matrix. Most workers prefer to use the technique of entrapment for immobilization of whole cells. Entrapped cells are in use for industrial production of amino acids (L-isoleucine, L-aspartic acid), L-malic acid and hydroquinone.

Microencapsulation:

Microencapsulation is a type of entrapment. It refers to the process of spherical particle formation wherein a liquid or suspension is enclosed in a semipermeable membrane. The membrane may be polymeric, lipoidal, lipoprotein-based or non-ionic in nature. There are three distinct ways of microencapsulation.

- ✓ Building of special membrane reactors.
- ✓ Formation of emulsions.
- ✓ Stabilization of emulsions to form microcapsules.

Microencapsulation is recently being used for immobilization of enzymes and mammalian cells. For instance, pancreatic cells grown in cultures can be immobilized by microencapsulation. Hybridoma cells have also been immobilized successfully by this technique.

Covalent Binding:

Immobilization of the enzymes can be achieved by creation of covalent bonds between the chemical groups of enzymes and the chemical groups of the support (Fig.3). This technique is widely used. However, covalent binding is often associated with loss of some enzyme activity. The inert support usually requires pretreatment (to form pre-activated support) before it binds to enzyme. The following are the common methods of covalent binding.

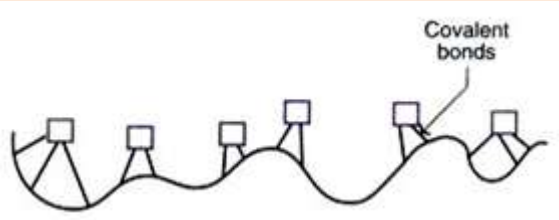
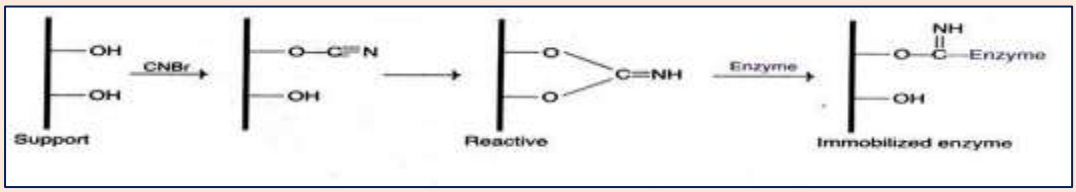
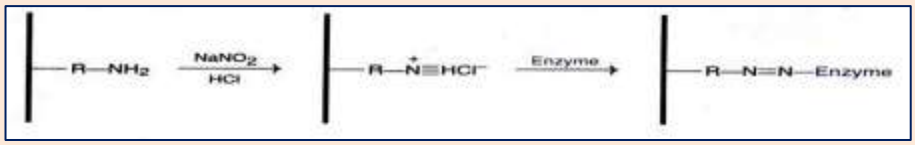


Fig.3. Covalent Binding

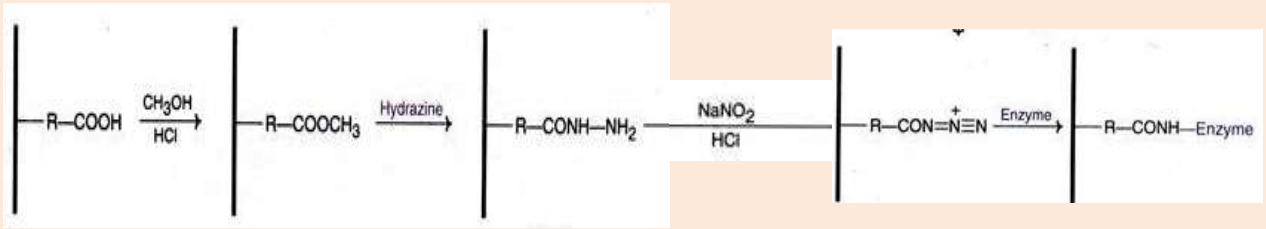
Cyanogen bromide activation: Inert support materials (cellulose, sepharose, sephadex) containing glycol groups are made by CNBr, which then binds the enzymes and activates them.



Diazotation: Other support materials (amino benzyl cellulose, amino derivatives of polystyrene, aminosilanized porous glass) are included when discussing treatment with NaNO2 and HCI. They, however, bind closely to the tyrosyl groups or histidyl enzymes.

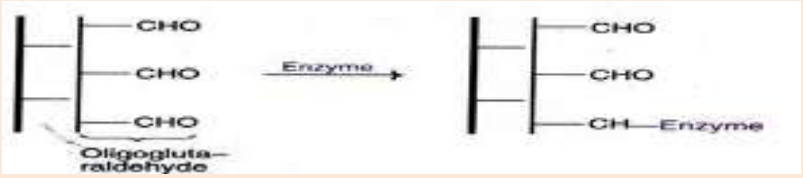


Peptide bond formation: Enzyme immobi¬lization can also be obtained by forming peptide bonds between amino (or carboxyl) support groups and carboxyl (or amino) groups of enzymes. The first support material is chemically treated to



form functional groups.

Activation by bigen or poly-functional reagents: Other reagents such as glutaraldehyde can be used to form bonds between amino groups of enzymes and amino groups of support (e.g. aminoethylcellulose, albumin , amino alkylated porous glass). This is illustrated.



Cross-Linking:

The absence of a solid support is a characteristic feature of immobilization of enzymes by cross- linking. The enzyme molecules are immobilized by creating cross-links between them, through the involvement of poly-functional reagents. These reagents in fact react with the enzyme molecules and create bridges which form the backbone to hold enzyme molecules (Fig. 4). There are several reagents in use for cross-linking. These include glutaraldehyde, diazobenzidine, hexamethylene diisocyanate and toluene di- isothiocyanate.

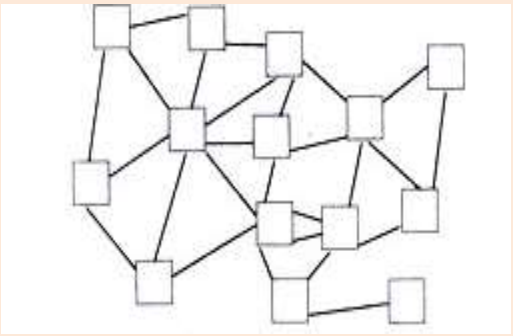


Fig. 4. immobilization of enzymes by cross- linking

Glutaraldehyde is the most widely used coordinating reagent. It interacts with lysyl residues of enzymes and forms the basis of Schiff. The cross-links formed between the enzyme and glutaraldehyde are irreversible and are able to withstand high pH and temperature. Glutaraldehyde linkages have been used successfully for the production of many industrial enzymes e.g. glucose isomerase, penicillin amidase. The linking plan is simple and ineffective. Unfortunately, it also risks the degradation of the enzyme by the poly-function reagent.

Choice of Immobilization Technique: The choice of a specific mechanism for the activation of enzymes is based on the trial and error process to select a positive

one. Among the factors that determine the process, enzyme activity for durability, durability, regeneration and cost characteristics are important.

Immobilization of L-amino acid acylase: L-Amino acid acylase was the first novel to be synthesized by a group of Japanese workers (Chibata and Tosa, 1969). More than 40 ways of becoming overwhelmed have been made by this group. Only three of them were found useful. They covalently bound iodoacetyl cellulose, ionic binding to DEAE-Sephadex and incorporated into polyacrylamide.

Stability of Soluble Enzymes: Some enzymes cannot be metabolized and must be used in soluble form e.g. Enzymes are used in fluid cleaners, certain diagnostic reagents and food additives. Such enzymes can be stabilized by using specific additives or by chemical modification. Stable enzymes have a long life, although they cannot be regenerated. Other important mechanisms of enzyme stability are briefly described.

Solvent Stabilization: Certain solvents at low levels stabilize enzymes, while high concentrations of enzymes obtain an e.k. acetone (5%) and ethanol (5%) can stop benzyl alcohol dehydro-genase.

Substrate Stabilization: The active site of the enzyme can be stabilized by adding substrates e.g. starch stabilizes amylase; sugar strengthens glucose uptake.

Stabilization by Polymers: Enzymes can be stabilized, especially against temperature, with the addition of polymers such as gelatin, albumin and polyethylene glycol.

Stabilization by Salts: The stability of ironloenzymes can be obtained by adding salts such as Ca, Fe, Mn, Cu and Zn eg. proteins can be stabilized by adding calcium.

Stabilization by Chemical Modifications: Enzymes can be stabilized by the conversion of relevant compounds without loss of biological activity. There are many types of chemical modifications.

- ✓ Addition of side chains of poly-amino e.g. polytyrosine, polyglycine.
- ✓ Acylation of enzymes by adding groups such as acetyl, propionyl and ancinyl.

Stabilization by Refolding: Theoretically, the stability of the enzymes is due to the hydrophobic interaction in the enzyme core. Therefore, it is suggested that enzymes can be stabilized by stabilizing hydrophobic interactions. For this purpose, the enzyme first occurs and is formed in one of the following ways (Fig. 5).

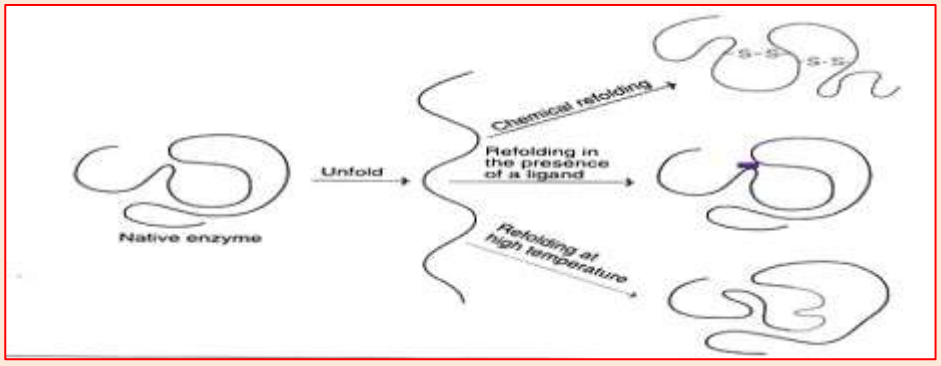


Fig.5. Stabilization of enzyme by Refolding

1. The enzyme can be chemically treated (e.g. urea and a disulfide) and then refolded.

2. The refolding can be done in the presence of low molecular weight ligands.

3. For certain enzymes, refolding at higher temperatures (around 50°C) stabilize them.

Stabilization by Site-Directed Mutagenesis: Site-directed mutagenesis has been successfully used to produce more stable and functionally more efficient enzymes e.g. subtilisin E.

Immobilization of Cells: Immobilized individual enzymes can be successfully used for single-step reactions. They are, however, not suitable for multi-enzyme reactions and for the reactions requiring cofactors. The whole cells or cellular organelles can be immobilized to serve as multi-enzyme systems. In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions, due to cost factor in isolating enzymes. For the enzymes which depend on the special arrangement of the membrane, cell immobilization is preferred.

Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as that used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are also employed.

Immobilized Viable Cells: The viability of the cells can be preserved by mild immobilization. Such immobilized cells are particularly useful for fermentations. Sometimes mammalian cell cultures are made to function as immobilized viable cells.

Immobilized Non-viable Cells: In many instances, immobilized non-viable cells are preferred over the enzymes or even the viable cells. This is mainly because of the costly isolation and purification processes. The best example is the

immobilization of cells containing glucose isomerase for the industrial production of high fructose syrup. Other important examples of microbial biocatalysts and their applications are given in (Table.1).

Immobilized microorganism (microbial biocatalyst)	*Application(s)*
Escherichia coli	For the synthesis of L-aspartic acid from fumaric acid and NH_3
Escherichia coli	For the production of L-tryptophan from indole and serine
Pseudomonas sp	Production of L-serine from glycine and methanol
Saccharomyces cerevisiae	Hydrolysis of sucrose
Saccharomyces sp	Large scale production of alcohol
Zymomonas mobilis	Synthesis of sorbitol and gluconic acid from glucose and fructose
Anthrobacter simplex	Synthesis of prednisolone from hydrocortisone
Pseudomonas chlororaphis	Production of acrylamide from acrylonitrile
Humicola sp	For the conversion of rifamycin B to rifamycin S
Bacteria and yeasts (several sp)	In biosensors

Table.1. The best example is the immobilization of cells in industrial application

Limitations of Immobilizing Eukaryotic Cells: Prokaryotic cells (particularly bacterial) are mainly used for immobilization. It is also possible to immobilize eukaryotic plant and animal cells. Due to the presence of cellular organelles, the metabolism of eukaryotic cells is slow. Thus, for the industrial production of biochemical, prokaryotic cells are preferred. However, for the production of complex proteins (e.g. immunoglobulin's) and for the proteins that undergo post-translational modifications, eukaryotic cells may be used.

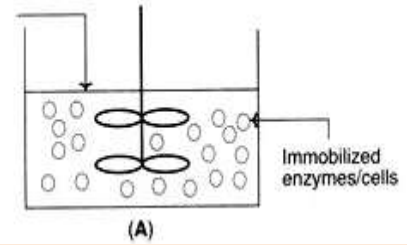
Effect of Immobilization on Enzyme Properties: Enzyme immobilization is frequently associated with alterations in enzyme properties, particularly the kinetic properties of enzymes.

Some of them are listed below: There is a substantial decrease in the enzyme specificity. This may be due to conformational changes that occur when the enzyme gets immobilized. The kinetic constants K_m and V_{max} of an immobilized enzyme differ from that of the native enzyme. This is because the conformational change of the enzyme will affect the affinity between enzyme and substrate.

Immobilized Enzyme Reactors: The immobilized enzymes cells are utilized in the industrial processes in the form of enzyme reactors. They are broadly of two

types — batch reactors and continuous reactors. The frequently used enzyme reactors are shownig.

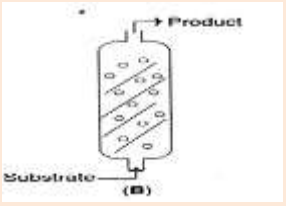
A. Batch Reactors: In batch reactors, the immobilized enzymes and substrates are placed, and the reaction is allowed to take place under constant stirring. As the reaction is completed, the product is separated from the enzyme (usually by denaturation).



Soluble enzymes are commonly used in batch reactors. It is rather difficult to separate the soluble enzymes from the products; hence there is a limitation of their reuse. However, special techniques have been developed for recovery of soluble enzymes, although this may result in loss of enzyme activity.

B. Stirred tank reactors:

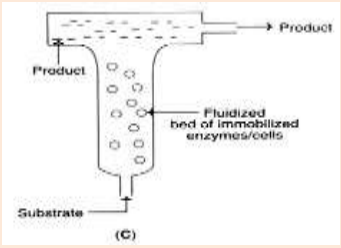
The simplest form of batch reactor is the stirred tank reactor. It is composed of a reactor fitted with a stirrer that allows good mixing, and appropriate temperature



and pH control. However, there may occur loss of some enzyme activity. A modification of stirred tank reactor is basket reactor. In this system, the enzyme is retained over the impeller blades. Both stirred tank reactor and basket reactor have a well-mixed flow pattern.

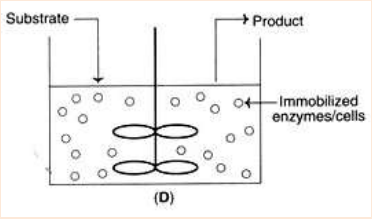
C. Plug flow type reactors:

These reactors are alternatives to flow pattern type of reactors. The flow rate of fluids controlled by a plug system. The plug flow type reactors may be in the form of packed bed or fluidized bed. These reactors are particularly useful when there occurs inadequate product formation in flow type reactors. Further, plug flow reactors are also useful for obtaining kinetic data on the reaction systems.

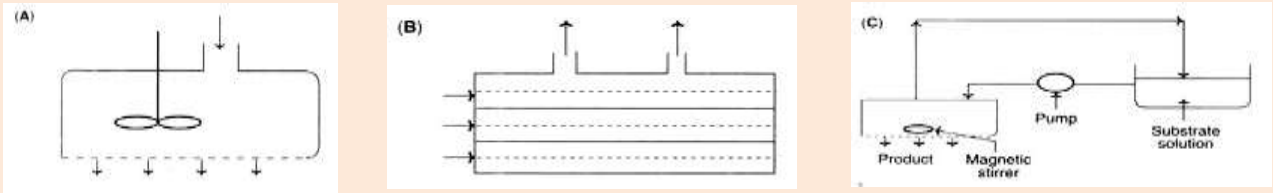


D. Continuous Reactors:

In continuous enzyme reactors, the substrate is added continuously while the product is removed simultaneously. Immobilized enzymes can also be used for continuous operation. Continuous reactors have certain advantages over batch reactors. These include control over the product formation, convenient operation of the system and easy automation of the entire process. There are mainly two types of continuous reactors-continuous stirred tank reactor (CSTR) and plug reactor (PR). A diagrammatic representation of CSTR is depicted in. CSTR is ideal for good product formation.



Membrane Reactors: Several membranes with a variety of chemical compositions can be used. The commonly used membrane materials include polysulfone, polyamide and cellulose acetate. The biocatalysts (enzymes or cells) are normally retained on the membranes of the reactor. The substrate is introduced into reactor while the product passes out. Good mixing in the reactor can be achieved by using stirrer (Fig. A). In a continuous membrane reactor, the biocatalysts are held over membrane layers on to which substrate molecules are passed (Fig. B).



In a recycle model membrane reactor, the contents (i.e. the solution containing enzymes, cofactors, and substrates along with freshly released product are recycled by using a pump (Fig. C). The product passes out which can be recovered.

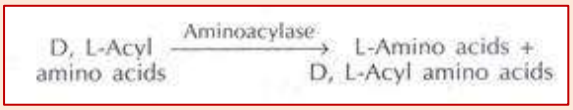
Applications of Immobilized Enzymes and Cells: Immobilized enzymes and cells are very widely used for industrial, analytical and therapeutic purpose, besides their involvement in food production and exploring the knowledge of biochemistry, microbiology and other allied specialties. A brief account of the industrial applications of immobilized cells is given in Table 1.

Manufacture of Commercial Products: A selected list of important immobilized enzymes and their industrial applications is given in Table 2. Some details on the manufacture of L-amino acids and high fructose syrup are given hereunder.

Immobilized enzyme	*Application(s)*
Aminoacylase	Production of L-amino acids from D, L-acyl amino acids
Glucose isomerase	Production of high fructose syrup from glucose (or starch)
Amylase	Production of glucose from starch
Invertase	Splitting of sucrose to glucose and fructose
β-Galactosidase	Splitting of lactose to glucose and galactose
Penicillin acylase	Commercial production of semi-synthetic penicillins
Aspartase	Production of aspartic acid from fumaric acid
Fumarase	Synthesis of malic acid from fumaric acid
Histidine ammonia lyase	Production of urocanic acid from histidine
Ribonuclease	Synthesis of nucleotides from RNA
Nitrilase	Production of acrylamide from acrylonitrile

Table.2. A selected list of important immobilized enzymes and their industrial applications

Production of L-Amino Acids: L-Amino acids (and not D-amino acids) are very important for use in food and feed supplements and medical purposes. The



chemical methods employed for their production result in a racemic mixture of D- and L-amino acids. They can be acylated to form D, L-acyl amino acids. The immobilized enzyme aminoacylase (frequently immobilized on DEAE sephadex) can selectively hydrolyse D, L-acyl amino acids to produce L-amino acids.

The free L-amino acids can separated from the un-hydrolysed D-acyl amino acids. The latter can be recemized to D, L-acyl amino acids and recycled through the enzyme reactor containing immobilized aminoacylase. Huge quantities of L-methionine, L-phenylalanine L-tryptophan and L-valine are produced worldwide by this approach.

Production of High Fructose Syrup:

- Fructose is the sweetest among monosaccharide's, and has twice the capacity of sucrose. Glucose is about 75% sweet as sucrose. Therefore, glucose (the most important monosaccharide) cannot replace sucrose to soften. So, there is a great need for fructose which is very sweet, but has the same calorific value as glucose or sucrose.
- High fructose syrup (HFS) contains an equal amount of sugar and fructose. HFS is almost like sucrose from a healthy diet standpoint. HFS can substitute for diabetes when preparing soft drinks, processed foods and baked.
- High fructose syrup can be produced from sugar by using a boiled glucose isomerase. Starch containing raw material (wheat, potatoes, corn) is subjected to hydrolysis to produce sugar. Glucose isomerase isomerizing glucose to fructose (Fig. 6). A product made by HFS that contains about 50% fructose. (Note: Some authors use the term high fructose corn syrup i.e. HFCS instead of HFS).

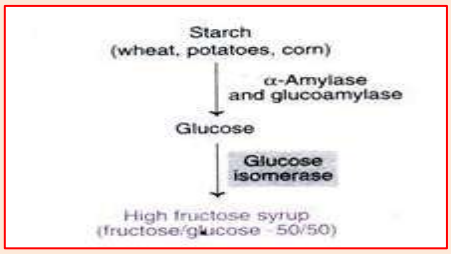


Fig.6. Production of High Fructose Syrup from starch

Glucose isomerase: This is an intracellular enzyme produced by a number of microorganisms. The species of Arthrobacter, Bacillus and Streptomyces are the preferred sources. Being an intracellular enzyme, the isolation of glucose isomerase without loss of biological activity requires special and costly techniques. Many a times, whole cells or partly broken cells are immobilized and used.

Immobilized Enzymes and Cells- Analytical Applications:

In Biochemical Analysis:

Immobilized enzymes (or cells) can be used for the development of precise and specific analytical techniques for the estimation of several biochemical compounds. The principle of analytical assay primarily involves the action of the immobilized enzyme on the substrate.

A decrease in the substrate concentration or an increase in the product level or an alteration in the cofactor concentration can be used for the assay. A selected list of examples of immobilized enzymes used in the assay of some substances is given in Table.3. Two types of detector systems are commonly employed.

Immobilized enzyme	*Substance assayed*
Glucose oxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Lactate dehydrogenase	Lactate
Alcohol oxidase	Alcohol
Hexokinase	ATP
Galactose oxidase	Galactose
Penicillinase	Penicillin
Ascorbic acid oxidase	Ascorbic acid
L-Amino acid oxidase	L-Amino acids
Cephalosporinase	Cephalosporin
Monoamine oxidase	Monoamine

Table 3. Selected examples of Immobilized Enzymes used in Analytical biochemistry

Thermistors are heat measuring devices which can record the heat generated in an enzyme catalysed reaction. Electrode devices are used for measuring potential differences in the reaction system. In the (Fig. 7.A, B and C) an enzyme thermistor and an enzyme electrode, along with a specific urease electrode are depicted.

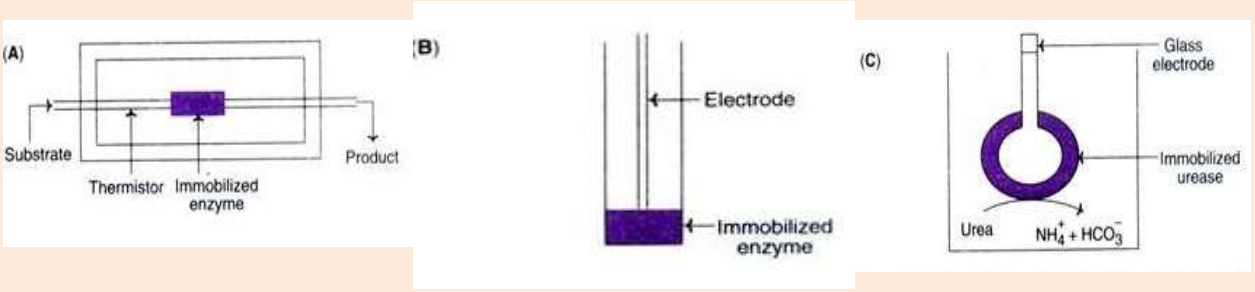


Fig.7. Enzymes used in Analytical biochemistry

A. Enzyme Thermistor, B. Enzyme Electrode, C. Urease Electrode

In Affinity Chromatography and Purification:

Immobilized enzymes can be used in affinity chromatography. Based on the property of affinity, it is possible to purify several compounds e.g. antigens, antibodies, cofactors.

QUALITY ASSURANCE

Defining and attempting to measure quality of care is not new. Infact, the quality of health care is an idea generally attributed to ernest Codman, a physician who first proposed the end result idea in 1869.Quality of care can be evaluated from the perceptive in individuals, populations or communities. The main aim of quality assurance us to achieved desired outcomes

DEFINITION OF QUALITY

Quality means usually think in terms of an excellent product or service that fulfills or exceeds our expectations.Quality is defined “as the degree to which a set of inherent characteristics fulfills requirement”.

DEFINITION OF QUALITY CARE

Quality care is the term used to describe care and services that allow recipients to attain and maintain their highest level of physical and psychological health.

DEFINITION OF QUALITY ASSURANCE

Quality assurance is achievable through on going evaluation of patient care which would assure the hospital that all that was done for the patient.sakharkar B.M (1999)

Quality Assurance is a program adopted by an institution that is designed to promote the best possible care.Deloughery(1995)

PURPUSE OF QUALITY ASSURANCE

- Help patients by improving quality of care.
- Assess competence of medical staff, serve as an impetus to keep up to date and prevent future mistakes.
- Bring to notice of hospital administration the deficiencies and in correcting the causative factors.
- Help to exercise a regulatory function.
- Restricting undesirable procedures.

PRINCIPLES OF QUALITY ASSURANCE

- ✓ Quality assurance is a never ending process of creative destruction, with rapid advances in science and technology and medical knowledge continuous updating is essential.
- ✓ The emphasis is on establishing professional excellence patient satisfaction at a reasonable cost.
- ✓ Quality is not proportionate to the use of sophisticated technology or to be expense incurred.
- ✓ Technical imperative should not insist on prolonging life at any lost with no consideration to quality of life.

EFFECT OF POOR QUALITY CARE

If medical care given is substandard or it is of poor quality, its effects may be immediately noticed e.g. Fall from cot, wound infection, sudden death etc, poor quality care can effect:-

- patients
- family
- society
- hospitals
- staff

EFFECTS ON PATIENTS:-

- Physical discomfort: e.g. Disturbed sleep due to noise of staff talking loudly, cleaning utensils etc. Wound infection poor quality of food causing abdominal pain IV fluid going out vein causing swelling or thrombophlebitis
- Mental stress
- Rise in complication
- Higher mortality rate
- Loss of working days
- fIncreased expenses

EFFECTS ON FAMILY

1. Inconveniences
2. Higher expenses
3. Frequent changes
4. Loss of trust

5. Black mailing, particularly for iatrogenic complications, refusing to pay the bills.

EFFECTS ON SOCIETY:-

- ✓ Increased prevalence of certain disease
- ✓ Increased risk of certain infections
- ✓ Diminished productivity, unhealthy person is medically more demanding and economically less productive.
- ✓ Avoiding use of scientific hospital management and taking treatment from other places.

EFFECTS ON HOSPITAL:-

- ➢ Increased length of stay leading – overcrowding in public hospitals, cross infection, reduction in turnover leading to longer waiting list for routine admissions, higher expenses, and shortage of linen.
- ➢ Higher rate of complications leading to additional investigations, additional medications, need for revision surgery.
- ➢ Accidents and mishaps
- ➢ Hospital image getting tarnished
- ➢ Adverse publicity by media which undermines other good services.

EFFECTS ON STAFF:-

- ✓ Reduced motivation
- ✓ Indiscipline
- ✓ Risk of infection to staff
- ✓ Difficulty to attract good staff
- ✓ Fast turn over

- ✓ Frustration.

TYPES OF QUALITY ASSURANCE

EXTERNAL QUALITY ASSURANCE:-

Quality assurance can be evaluated by independent assessors (or) people from outside the institution/hospital.

INTERNAL QUALITY ASSURANCE:-

Quality assurance can be evaluated by local assessors (or) senior person from the same institution/hospital.

QUALITY ASSURANCE COMMITTEE

Thecommitteeshouldconsistofthefollowing.

- ✓ Medical administrator
- ✓ Two seniorclinicians
- ✓ Pathologist
- ✓ Radiologist
- ✓ Nurse administrator
- ✓ Medical records officer – secretary
- ✓ Additional personnel such as super specialist and consultants can be

FUNCTIONS OF QAC

Coordination:-

- ✓ Collecting information
- ✓ Consider activities that should be related, e.g. Quality appraisal and continuing education
- ✓ Communication across patient care disciplines

- ✓ Co –ordinate actions of hospital authority groups.

Information:-

- ✓ Provide a centralized source of reports to the board.
- ✓ Suggest head for intervention to hospital authority groups.

Planning:-

Establish priorities

Consultation:-

Provide specific assistance, usually through the coordinator. Response:-

Internally, acknowledge issues of importance to individuals and departments when suggesting high priority areas for immediate attention.

Externally, provide the organization home for responding to quality requirement of external agencies of any e.g. medical companies.

Search for expertise:-

Operate openly, not behind closed doors, seek out the specific clinical and or management expertise necessary to reach sound conclusions.

Follow up:-

Committee members must recognize that their major functions are

- To coordinate not to control
- To inform, not to scold

- To plan and suggests priorities not to do detailed studies in committee and To recommend report, not to intervene directly.

IDINTYFICATION OF VALUSE

Emphasizes the need to clarify the social, institutional, professional and individual values, along with the advances in scientific knowledge which influence nursing practice. Examination of these beliefs offers insight into what clients, nurses and others think is important in nursing care.

IDENTIFY STANDADS AND CRITERIA

The standards and criteria derived from the values describe the level of nursing care considered acceptable. These standards may range from minimal to achievable, excellent or comprehensive. Standards represent the agreed upon level of excellence, whereas criteria are specific, measurable statements which reflect the intent of the standard and can be compared to actual nursing practice.

SECURE MEASURMENTS

- The next component involves the measurement of current nursing practice against the established standards and criteria. There are many methods which could be used to perform the comparison including concurrent and retrospective audit, direct observation of nurse or patient performance, questionnaire, patient or nurse interview and knowledge testing.
- The method selected is dependent upon the purpose of the evaluation study and the available instruments and resources. Strengths and weakness of nursing practice should be revealed through this comparison.

MAKE INTERPARTACTIONS

Analysis and interpretation of the data follow as the next component of the model. The purpose here is the identification of discrepancies between the established criteria and current practice. If no variations are discovered, then the remainder of the model is by passed and one begins again with value clarification .It is unlikely, however, that no discrepancies will be found. Judgments are made about strengths, deficiencies and other problems in quality.

COURSE ACTION

Suitable courses of action are then considered. Alternatives intended to resolve discrepancies and reward strengths are identified and examined. Decisions may range from simple actions to complex plans entailing many changes.

CHOOSE ACTION

The last two components of the model consist of the selection and implementation of the best actions. Judgments are made about strengths, deficiencies and other problems in quality; it may be positive or negative

TAKE ACTION

Some actions may need to be performed immediately while others take longer to initiate. The decisions as to which action to choose are influenced by the organization context and available resources. At this point the cycle is repeated and the actions are reassessed to determine if the expected improvements in practice actually occurred or have been maintained.

FACTORS AFFECTING QUALITY ASSURANCE CARE

- ✓ lack of Resources
- ✓ personal problems
- ✓ unreasonable patients and attendants
- ✓ improper maintenance
- ✓ absence of accreditation laws
- ✓ lack of incident review procedures
- ✓ lack of good hospital information system
- ✓ absence of conducting patient satisfaction surveys
- ✓ lack of nursing care records
- ✓ Miscellaneous factors like lack of good supervision, Absence of knowledge about philosophy of nursing care, substandard education and training, lack of policy and administrative manuals.

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