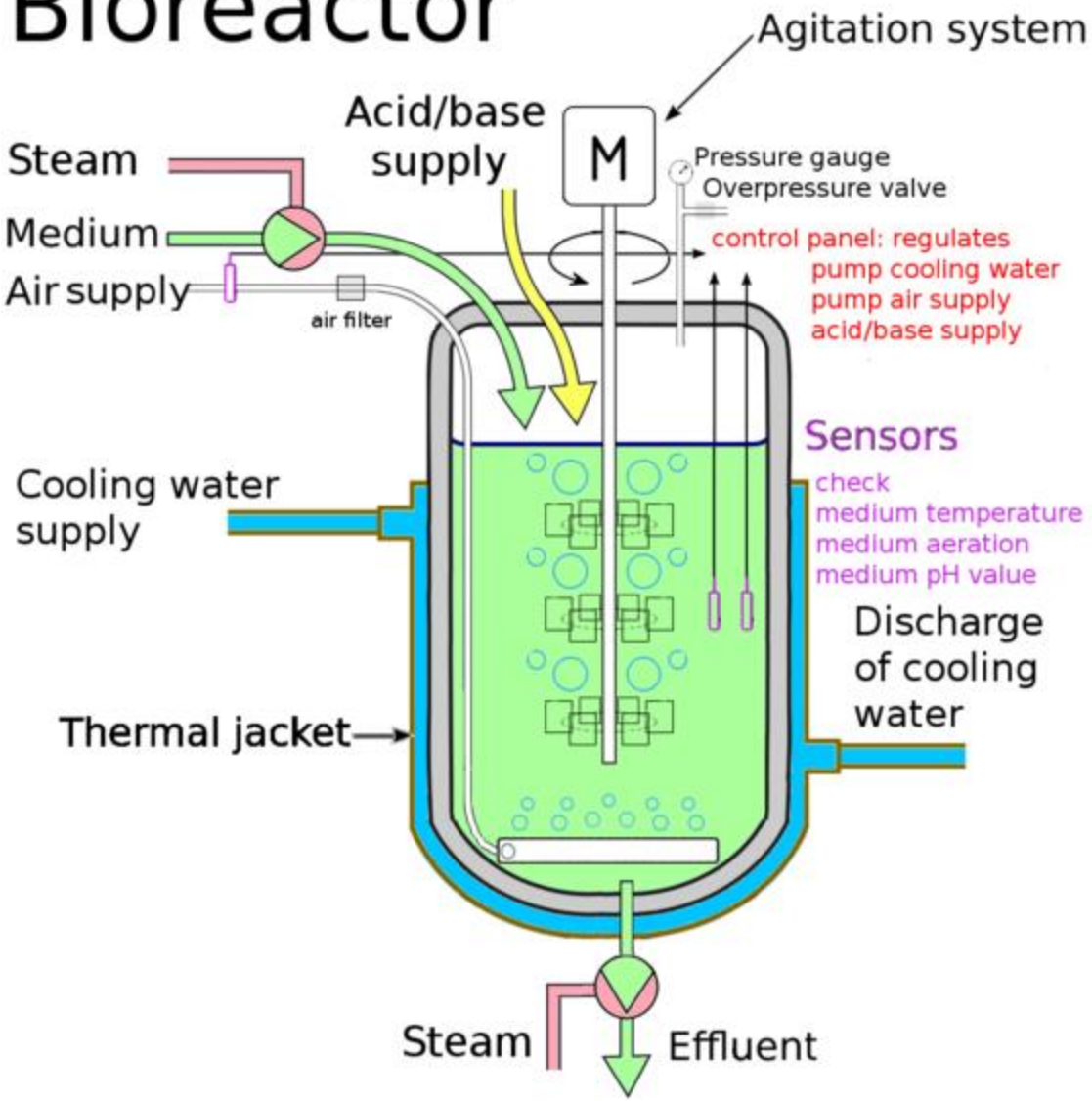
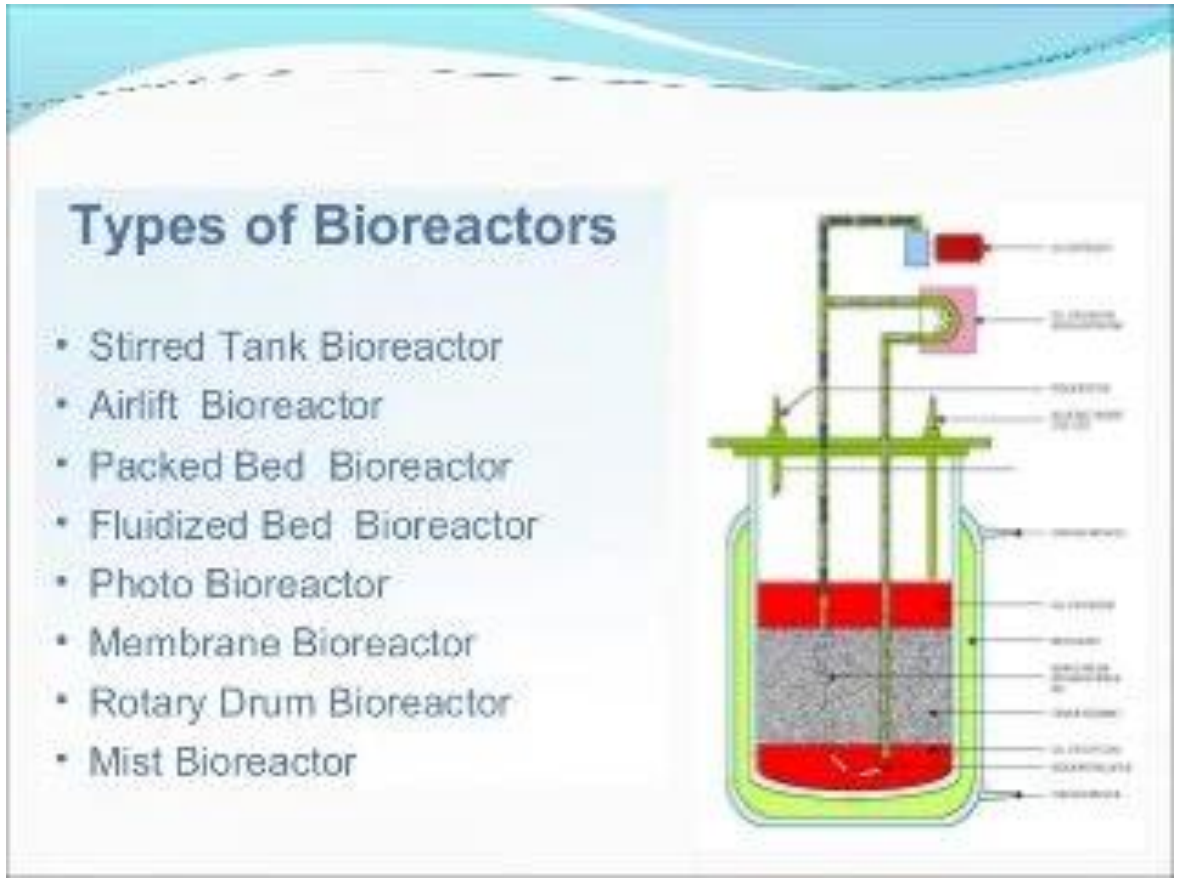
Bioreactor

- A bioreactor is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Bioreactors are extensively used for food processing, fermentation, waste treatment, etc. On the basis of the agent used, bioreactors are grouped into two broad classes:
  - those based on living cells and,
  - those employing enzymes.
- But in terms of process requirements, they are of the following types:
  - aerobic,
  - anaerobic,
  - solid state, and
  - immobilized cell bioreactors.
- All bioreactors deal with heterogeneous systems having two or more phases, e.g., liquid, gas, solid.
- Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other.

- A bioreactor should provide for the following:
  - agitation (for mixing of cells and medium),
  - aeration (aerobic fermenters; for O2 supply),
  - regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc.,
  - sterilization and maintenance of sterility, and
  - withdrawal of cells/medium (for continuous fermenters).
- Modem fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.
- The size of fermenters ranges from 1-21 laboratory fermenters to 500,000 1 or, occasionally, even more; fermenters of upto 1.2 million litres have been used. Generally, 20-25% of fermenter volume is left unfilled with medium as "head space" to allow for splashing, foaming and aeration. The fermenter design varies greatly depending on the type of fermentation for which it is used.

Bioreactor


Agitation system
Acid/base supply
M
Steam
Pressure gauge
Overpressure valve
Medium
control panel: regulates
pump cooling water
pump air supply
acid/base supply
Air supply
air filter
Sensors
check
medium temperature
medium aeration
medium pH value
Cooling water supply
Discharge of cooling water
Thermal jacket
Steam
Effluent


Types of Bioreactors
• Stirred Tank Bioreactor
• Airlift Bioreactor
• Packed Bed Bioreactor
• Fluidized Bed Bioreactor
• Photo Bioreactor
• Membrane Bioreactor
• Rotary Drum Bioreactor
• Mist Bioreactor

Immobilized Cell Bioreactors

Bioreactors of this type are based on immobilized cells. Cell immobilization is advantageous when

- the enzymes of interest are intracellular,
- extracted enzymes are unstable,
- the cells do not have interfering enzymes or such enzymes are easily inactivated/removed and
- the products are low molecular weight compounds released into the medium.

Under these conditions, immobilized cells offer the following advantages over enzyme immobilization:

- enzyme purification is not needed,
- high activity of even unstable enzymes,
- high operational stability,
- lower cost and
- possibility of application in multistep enzyme reactions.

Cell immobilization may be achieved in one of the following ways.

- Cells may be directly bound to water insoluble carriers, e.g., cellulose, dextran, ion exchange resins, porous glass, brick, sand, etc., by adsorption, ionic bonds or covalent bonds.
- They can be cross linked to bi- or multifunctional reagents, e.g., glutaraldehyde, etc.
- Polymer matrices may be used for entrapping cells; such matrices are polyacrylamide cell, κ-carrageenan (a polysaccharide isolated from a seaweed), calcium alginate (alginate is extracted from seaweed), poly glycol oligomers, etc.

CELL IMMOBILIZATION TECHNIQUES

Immobilization of whole cells has been defined as the physical confinement or localization of intact cells to a certain defined region of space with preservation of some, or most, catalytic activity. Some of the advantages of whole-cell immobilization in comparison with enzyme immobilization are the higher stability and enzyme activity, multivariate enzyme applications, and the lower cost.

There are five different types of techniques for cell immobilization:

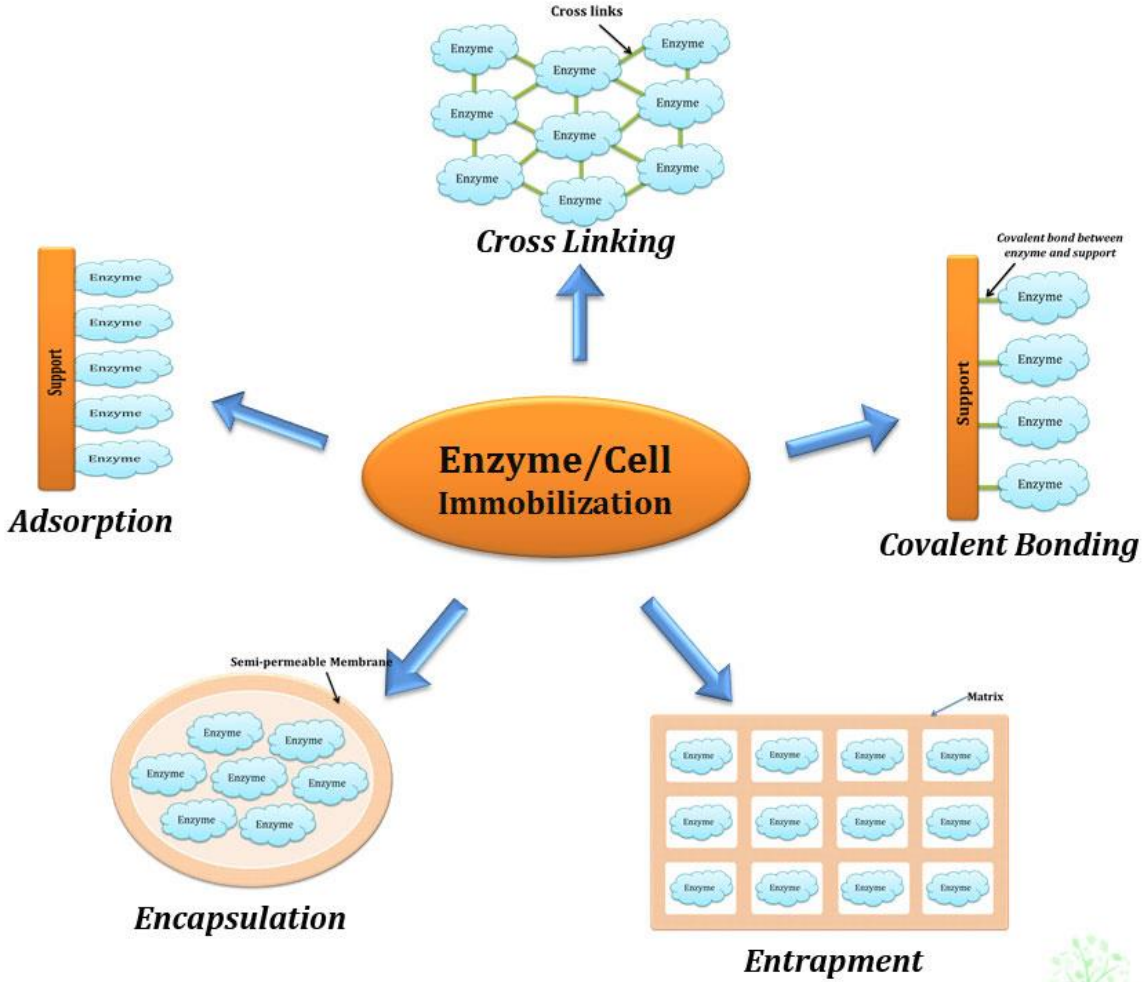
Adsorption:
it is one of the simplest & oldest methods of cell immobilization..This method is based on physical adsorption of enzyme protein on the surface of water insoluble carriers. Enzymes can be adsorbed physically on a surface active adsorbent by contacting an aqueous solution of an enzyme with an adsorbent.

Covalent Bonding:
this is the most widely used method for immobilization.This method involves formation of covalent bonds between the chemical groups in enzyme & the chemical groups on the support or carrier.

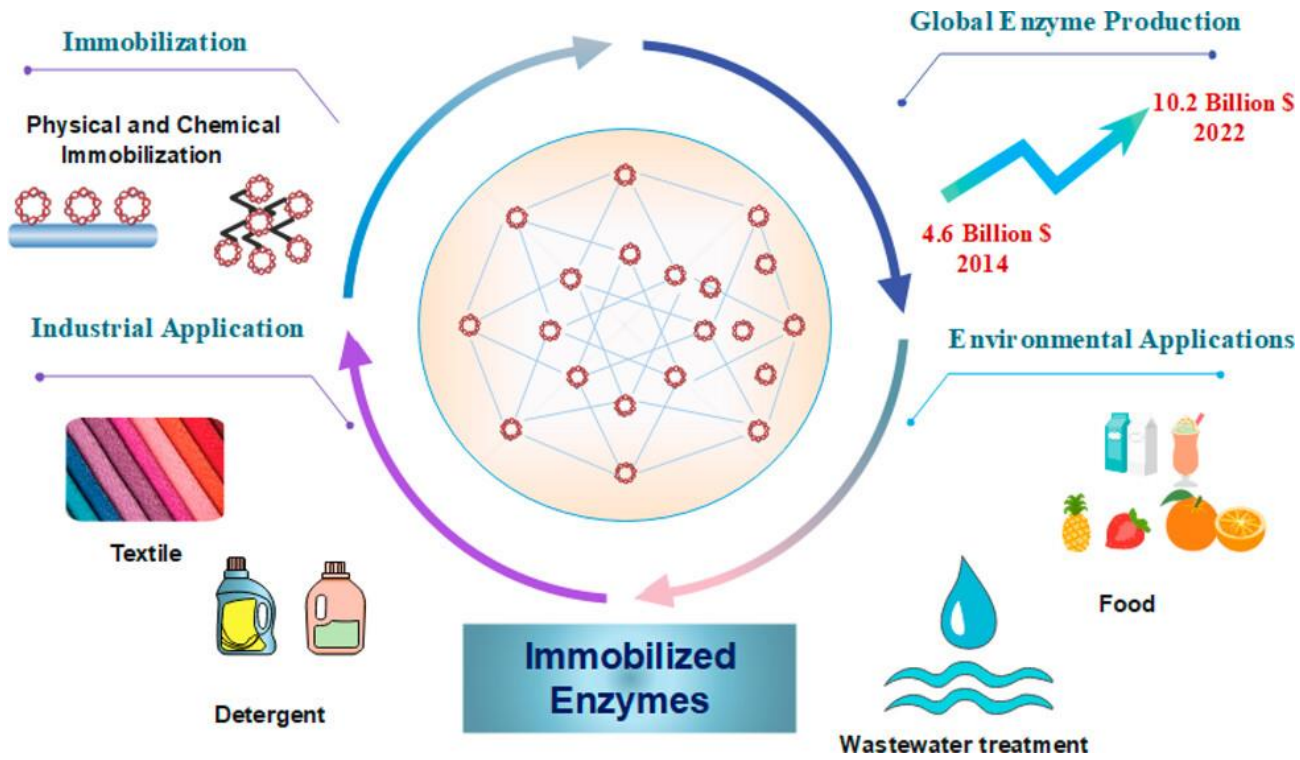
Entrapment:
In this method the cells are not directly attached to the support surface.The cells are simply trapped inside the polymer matrix. Entrapment is carried out by mixing the biocatalyst into a monomer solution followed by polymerization initiated by a change in temperature or by a chemical reaction.

Cross-linking:
In this method enzymes are directly linked by covalent bonds between various groups of enzymes through multifunctional reagents. In this method there is no matrix or support involved.

Encapsulation:
In this method immobilization is done by enclosing the enzymes in a membrane capsule.The capsule is made up of semi-permeable membrane like nylon.



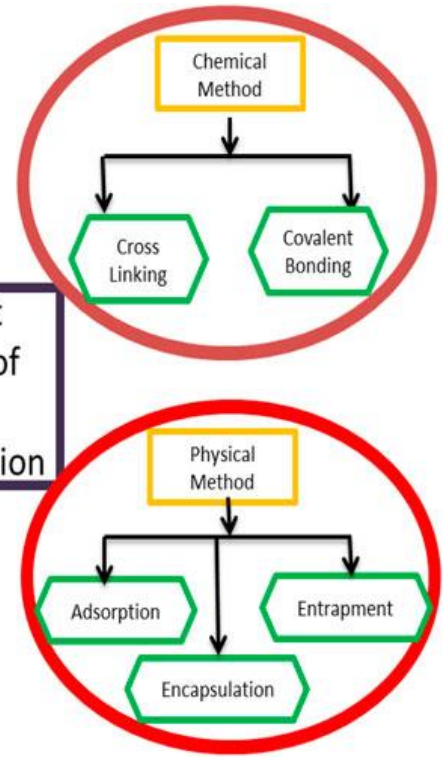
Enzyme/Cell Immobilization Methods



Characteristics

- Low cost
- Easy preparation
- Strong binding force
- Highly stable
- Commercial potential
- Ecofriendly

Different methods of enzyme immobilization



Applications of immobilization

1. **Industrial production:** Industrial production of antibiotics, beverages, amino acids etc. uses immobilized enzymes.
2. **Biomedical applications:** Immobilized enzymes are widely used for diagnosis and treatment of many diseases such as inborn disorder.
3. **Food industry:** Enzymes like pectinases and cellulases immobilized on suitable carriers are successfully used in the production of jams, jellys and syrups from fruits and vegetables.
4. **Research:** The use of immobilized enzyme allow researcher to increase the efficiency of different enzymes such as different proteases for cell and organelle lysis.
5. Biodiesel production from vegetable oils.
6. **Textile industry:** Scouring, bio polishing and desizing of fabrics.
7. **Waste water management:** Treatment of sewage and industrial effluents.
8. **Detergent industry:** Immobilization of lipase enzyme for effective dirt removal from cloths.

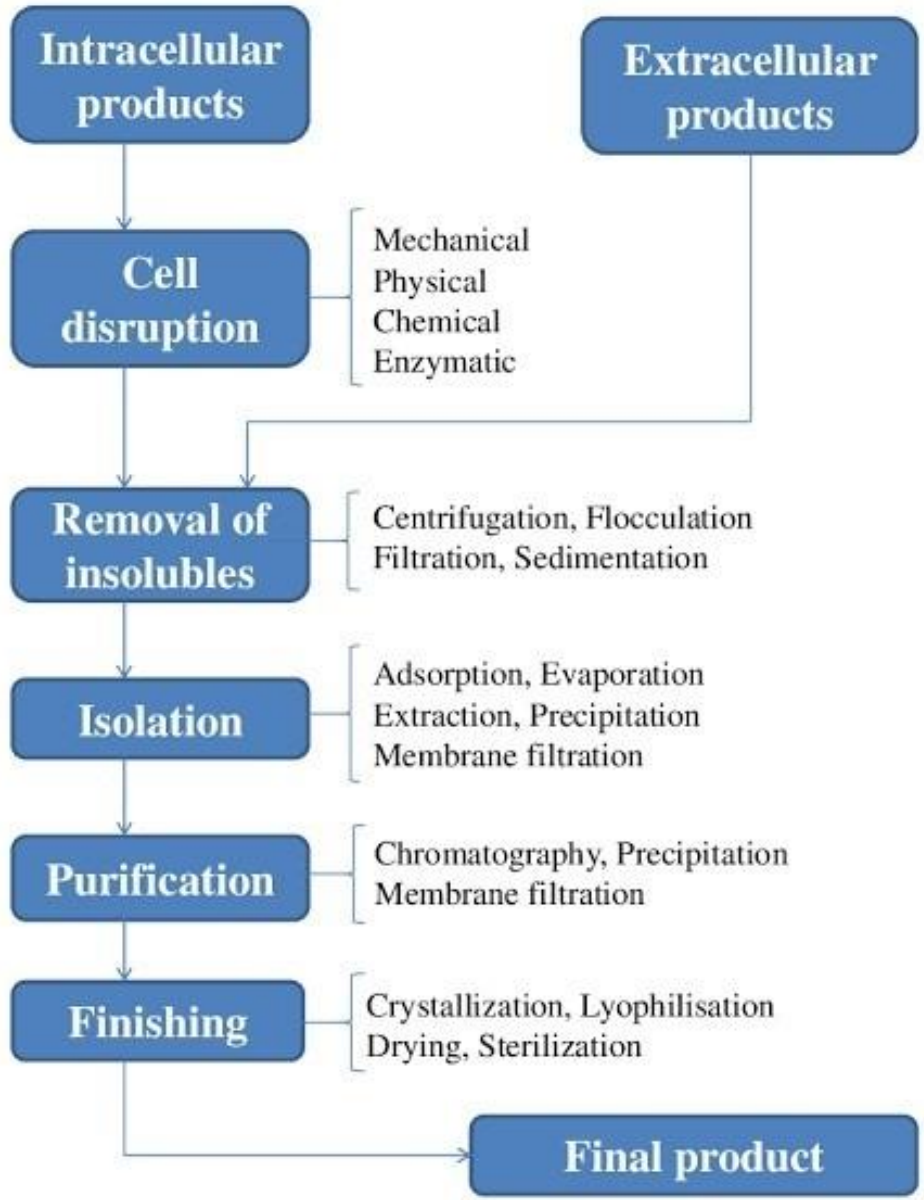
Bioreactor Media

- The medium composition is as critical to product yields as high producing strains of microorganisms. The medium not only provides the nutrients needed for microbial growth but also for the metabolite production.
- The organisms vary greatly in their nutrient requirements from autotrophs, which produce all the biochemicals required from simple inorganic nutrients deriving their energy from oxidation of some inorganic component of the medium to the difficult organisms like lactic acid bacteria, which require many organic compounds for their growth.
- The various media may be grouped into two broad categories: (i) synthetic and (ii) complex.
    - A **synthetic or chemically defined medium** is desirable for various studies, but product yields from such media are generally low.
    - The **complex media** contain undefined constituents like soybean meal, molasses, corn steep liquor, etc., and give much higher yields of metabolites.
- **Carbon source** can be simple (e.g., sugar, alcohol) or complex carbohydrates, proteins, molasses, potatoes, sweet potatoes, etc.
- In many processes, **precursors** need to be provided, e.g., phenylacetic acid for penicillin G, inorganic cobalt for vit. B12.
- **Buffers** are also added to prevent drastic changes in pH, and anti foam would often be needed when complex media are used.
- For much fermentation, e.g., antibiotic production, medium suited for rapid cell growth is unsuitable for product formation. In such cases, specialized media for production have to be devised.

Downstream Processing

- The various processes used for the actual recovery of useful products from a fermentation or any other industrial process is called downstream processing. The cost of downstream processing (DSP) is often more than 50% of the manufacturing cost, and there is product loss at each step of DSP.
- Therefore, the DSP should be efficient, involve as few steps as possible (to avoid product loss), and be cost-effective. The various steps in DSP are as follows:
    - separation of particles,
    - disintegration of cells,
    - extraction,
    - concentration,
    - purification and
    - drying.

Downstream process



Separation of Particles - The first step in DSP is the separation of solids, usually cells, from the liquid medium. This is generally achieved as follows.

Filtration: It is used for the separation of filamentous fungi and filamentous bacteria, e.g., streptomycetes, and often for yeast flocks. The various techniques of filtration employed are, surface filtration, depth filtration, centrifugal filtration, cross flow filtration, and rotary drum vacuum filtration.

Centrifugation: It may be used to separate bacteria and usually protein precipitates. But difficulties arise due to small differences in the densities of the particles and the medium. In addition, equipment cost, power consumption, temperature, etc. are the other disadvantages.

Flocculation and Floatation: Flocculation, i.e., sticking together of cells, can be induced by inorganic salts, mineral hydrocolloids are organic polyelectrolytes. Since sedimentation rate of a particle increases with size, flocculated cells can be recovered by centrifugation. In cases, where flocculation is not effective, very fine gas bubbles can be created by sparging, release of overpressure or electrolysis. The gas bubbles adsorb to and surround the cells, raising them to the surface of medium in form of foam (floatation); long chain fatty acids or amines promote stable foam formation.

The cells collected in the foam are readily recovered. Flocculation and floatation are used for the most efficient recovery of microbial biomass in some single cell protein production systems.

Important uses of industrial microorganisms

Product	Micro-organism	Use
Vitamin B12	*Streptomyces*	vitamin supplements
Lactic Acid	*Lactobacillius delbrueckii*	chemical reagents
Citric Acid	*Aspergillus niger*	food preservative
Ethanoic Acid	*Acetobacter sp.*	vinegar, solvent
Pectinases Micriorganism	*Aspergillus sp.*	degrading pectin
Ethanol	*Saccharomyces*	chemical reagents, drinks
Penicillin	*Penicillin chrysogenum*	antibiotic

List of enzymes used for commercial activities

Enzyme	Source	Application	Use in the Form
α - Amylase	1. Bacillius licheniformis 2. B.amylolique faciens	Hydrolysis of starch to dextrans	Extracellular, soluble
Glucoamylase	Aspergillus niger	Dextran hydrolysis to glucose	Extracellular, soluble
Xylose (Glucose) isomerase	Bacillus coagulans*	Pure glucose to equilibrium mixture of glucose + fructose	Immobilized whole cells
Alkaline Protease	B. licheniformis, B.subtilis	Detergents (protein digestion)	Extracellular, soluble
Neutral Protease	B.amylolique faciens, B.subtilis	Protein digestion in brewing substances	Extracellular, soluble
Acid Protease, e.g., rennet	A. niger**	Milk coagulation and cheese flavour enhancement	Extracellular, soluble
Pectinase	A. niger, B.subtilis	Pectin hydrolysis in fruit juices	Extracellular, soluble
Glucanase	A. niger, B.subtilis	In fruit juices	Extracellular, soluble
Cellulase	A.niger, Trichoderma reesei	Cellulose hydrolysis in fruit juices	Extracellular, soluble
Lipase	Rhizopus Species	Detergents, lipid hydrolysis	Extracellular, soluble
Lactase	A.niger	Milk lactose hydrolysis to glucose	Immobilized on glass beads

Industrial application of microorganisms

Industry	Applications	Enzymes	Source
Baking & Milling	• Reduction of bulk viscosity • Acceleration of fermentation • Increase in loaf (unoccupied) volume • Improvement of crumb (fragments) softness • Maintenance of freshness	Amylase	Fungal
	Improvement of dough texture, reduction of mixing time, increase in loaf volume	Protease	Fungal/ Bacteria
Brewing	Mashing (Crushing, Squashing, Smashing)	Amylase	Fungal/ Bacteria
	Chill proofing	Protease	Fungal/ Bacteria
	Improvement of fine filtration	β-glucanases	Fungal/ Bacteria
Cereals	Precooked baby foods, breakfast foods	Amylase	Fungal
Chocolate & Cocca	Manufacture of syrup	Amylase	Fungal/ Bacteria
Coffee	Coffee bean fermentation	Pectinase	Fungal
	Preparation of coffee concentrates	Pectinase	Fungal
		Hemicellulase	
Confectionery	Manufacture of soft Centre candies	Invertase	Fungal/ Bacteria
		Pectinase	
Corn syrup	• Manufacture of high maltose syrups	Amylase	Fungal
	• Production of low D.E. syrup	Amylase	Bacteria
	• Prod. of low glucose from corn syrup	Amyloglucosidase	Fungal
	• Manufacture of fructose syrup	Glucose isomerase	Bacteria
Dairy	Manufacture of protein hydrolysates	Protease	Fungal/ Bacteria
	Stabilization of evaporated milk	Protease	Fungal
	Production of whole milk concentrates, ice-cream & frozen desserts	Protease	Yeast