Immobilized enzyme

Recently, methods have been developed where enzymes are attached to insoluble materials that act as a support for the enzyme. The enzyme can then be help in place during the reaction, removed after words and used again. This is called immobilization of the enzyme. Sometimes entire microbial cells are immobilized.

Immobilized whole cells are useful because, as it is not necessary to stant with a pure enzyme, the process is cheaper and quicker. Whole cells are immobilized in the same way as purified enzymes. They are being used increasingly for complex cultures, such as waste treatment, nitrogen fixation, the synthesis of steroids, semi synthetic antibiotic and other medical products. There are different methods for immobilizing enzyme. They can be: (Fig.)

1- Adsorbed onto an insoluble matrix, such as collagen, (a)

2- Held inside a gel, such as silica gel. (b)

3- Held within a semi-permeable membrane, (c)

4- Trapped in a microcapsule, such as polyacrylamide or alginate beads, (d)

5- This processes all involve a physical bonding of the enzyme. They are not easy to carry out and generally result in low enzyme activity. Alternative enzyme can be chemically bounded to the support medium, (e). Where enzyme activity is high.

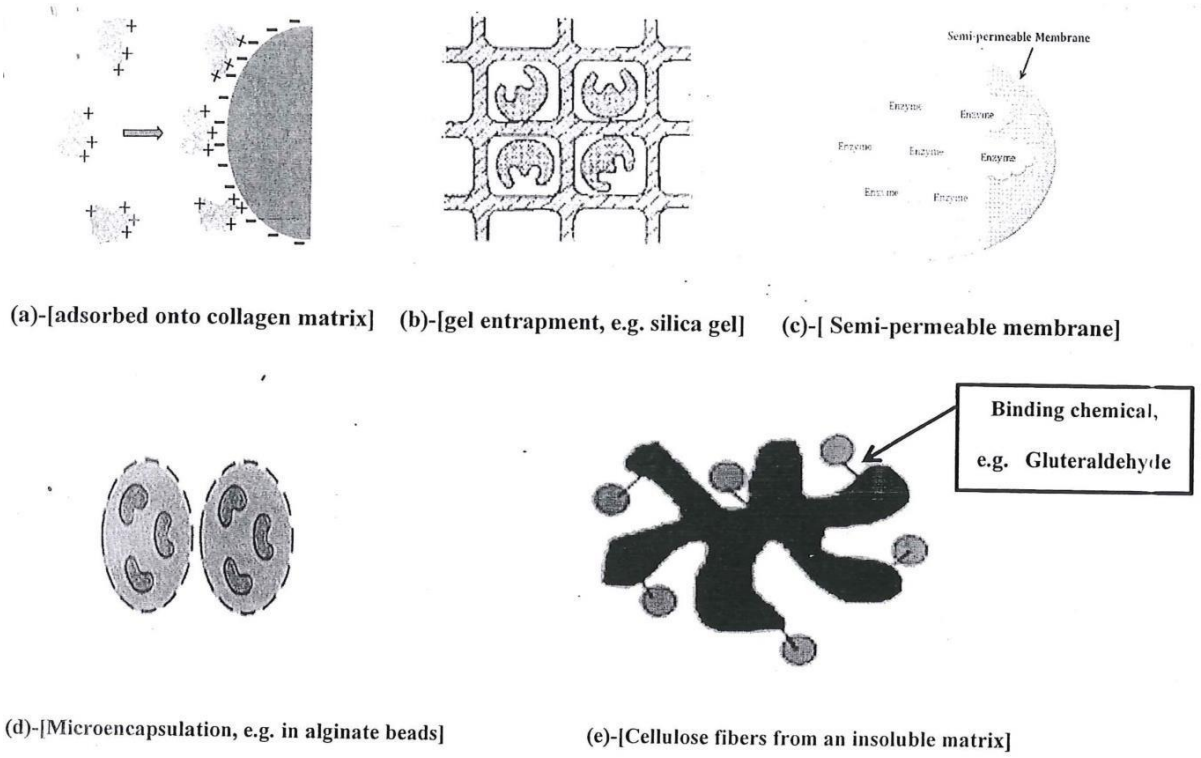


Figure: Immobilization methods of enzymes.

Although preparing enzyme in this way is difficult.

<u>The advantages of using immobilized enzymes are:</u>

1 - Enzymes can be reused again, which is particularly useful when the enzyme is expensive or difficult to produce.

2- The product will not be contaminated by the enzyme, because the enzyme is held in a matrix.

3- The matrix protects the enzyme with a physical condition, so that it is more stable at extremes of temperature and pH.

4- These properties make immobilized enzymes very suitable for continues culture.

5- Immobilized enzymes can be controlled more accurately.

6- Immobilized whole cells mean that several enzymes can participate in the process.

Immobilized enzyme technology is still developing quickly and there are likely to be many new applications for immobilized enzymes in industry, medicine and waste treatment.

The production of lactose free milk :

In many parts of the world, milk is an important part of the adult human diet. It contains the disaccharide sugar lactose which is digested to the monosaccharides glucose and galactose by the enzyme lactase, present in the small intestine, there are many adults who lack this enzyme. These people are said be lactose intolerant. If they drink even a small amount of milk, they suffer severe abdominal cramps, wind and diarrhea.

Because the lactose does not get digested in the small intestine, it passes through to the colon where bacteria feed on it and produce fatty acids, methan, Co2 and hydrogen. However immobilized lactase can be used to break down the lacose in milk making it suiltable for lactose in tolerant people to drink.

Bioseparation ((Downstream processing)) for Biotechnology-Products purification :

Large amounts of biochemical products can be made by different types of cells such as animal cells, plant cells and microbial cells. After these biochemicals are made, they must be separated and purified. These separations are difficult and frequently cost more than the initial manufacture of the biochemicals.

Cell Disruption :

Bioseparation usually begin with the separation of biomass from broth by using the filtration or centrifugation method. In many cases, the desired product is in the broth (extracellular product).

Antibiotics are commonly in the broth; so extracellular enzymes, many polysaccharides, and most amino acid. In all these cases the separated broth can be treated to isolate and purify the product. The biomass is discarded or sold as a byproduct. In some cases, the products of interest, are. not in the broth but are in the biomass. In many cases the product, such as lipids, antibiotics, enzymes are trapped in the biomass: it is (intra cellular products) releasing this trapped material usually involves rupturing the cell wall. The methods of cell rupture have largely been developed in biochemistry. These methods, listed in table:

Table: Cell disintegration (rupture) technology.

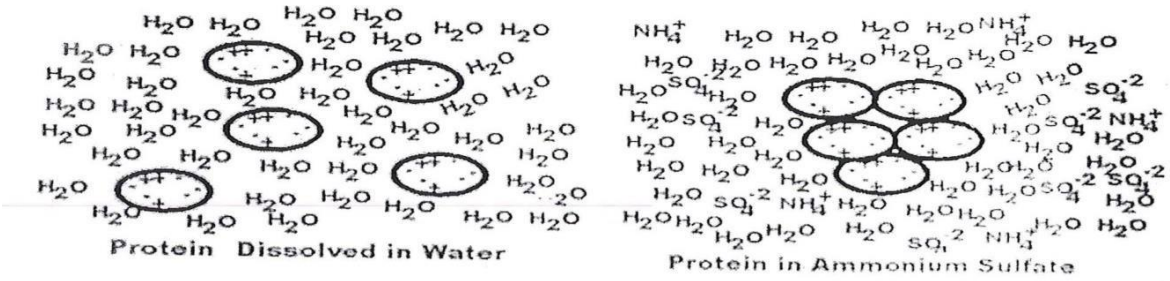
Method	Technique	Principle	Examples
Chemical	Osmotic shock	Osmotic rupture of membrane	Rupture of red blood cells
	Enzyme digestion	Cell wall digestion	Bacteria treated with egg lysozyme
	Solubilization	Detergents solubilize cell membrane	Bile salts acting on E.coli
	Lipid dissolution	Organic solvent dissolves in cell wall	Toluene disruption of yeast
	Alkali treatment"	Saponification of lipids solubilizes membrane	
Mechanical	Homogenization	Cells forced through small hole are broken	Large scale treatment of cell suspension
	Ultrasonication	Cells broken with ultrasonic cavitation	Cell suspension at least on small scale

After the insoluble are removed, the second step in a bioseparation is usually product, the second methods used in isolation or purification product.

Precipitation by Ammonium sulfate $(NH_4)2SO_4$:

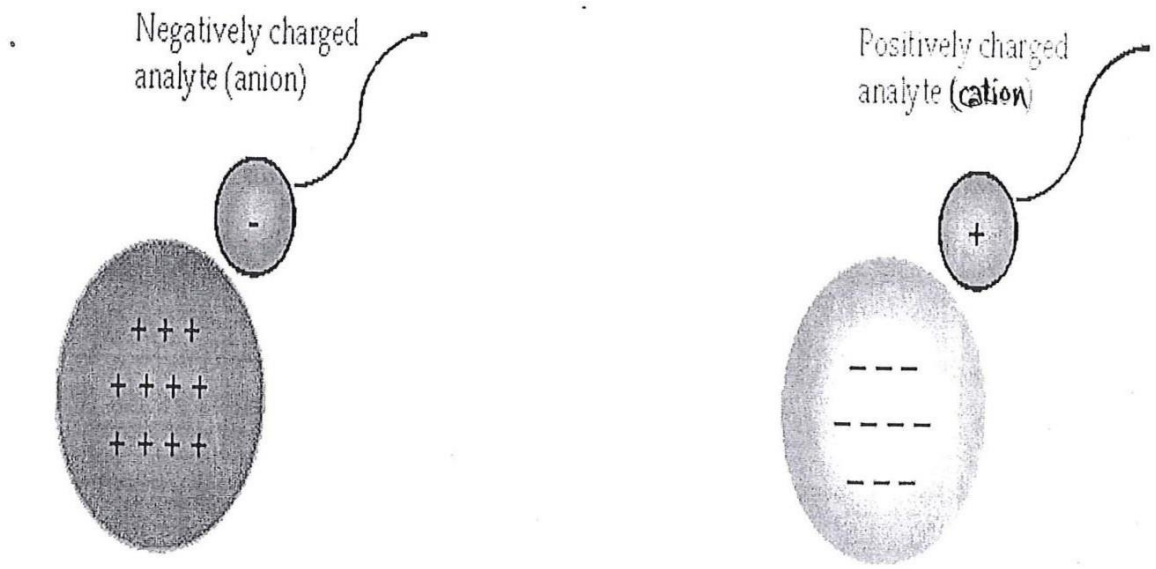
Ammonium sulfate is a good salt to precipitate the protein (product), also this method called salting out. The principle of this method depend on the formation of the complex between the protein molecule and salt, and this complex is insoluble and cause precipitation.

Ammonium sulfate precipitation



Ion exchange chromatography:

Ion exchange chromatography can be broadly defined as the separation of compounds on an insoluble matrix containing labile ions capable of exchanging with ions in the surrounding media, and this method depend on the charge of the protein molecule (product). Ion exchange materials are either anionic exchanger such as DEAE-cellulose (Diethyl amino ethyl cellulose) or cationic exchanger such as CMC (Carboxy methyl cellulose).



anion exchanger stationary phase particle

cation exchanger stationary phase particle

Gel filtration chromatography:

Molecules of product can also be separated on the basis of differences in their size [molecular weight (M.W.)] by passing them down a column containing swollen particles of a gel such as Sephadex G 100, G200 or Sepharose 2B, 4B and 6B, or biogel. The large molecules leaving the column first and the smaller ones last. Fig.

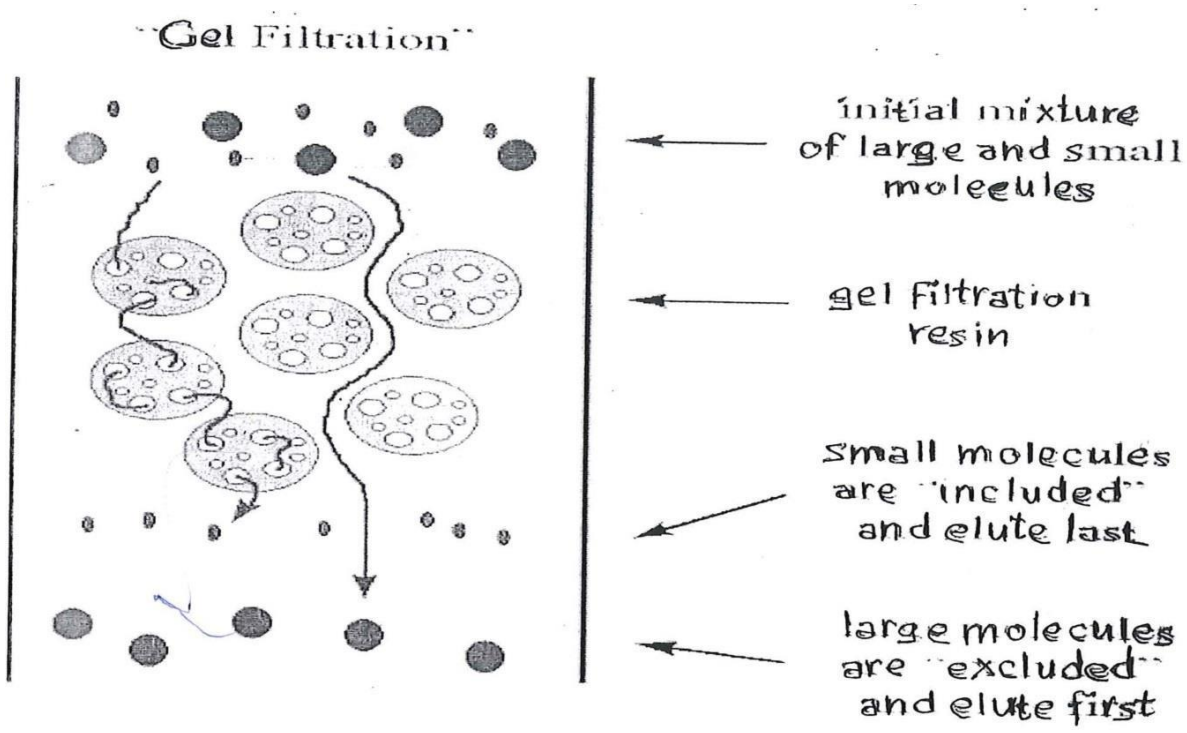
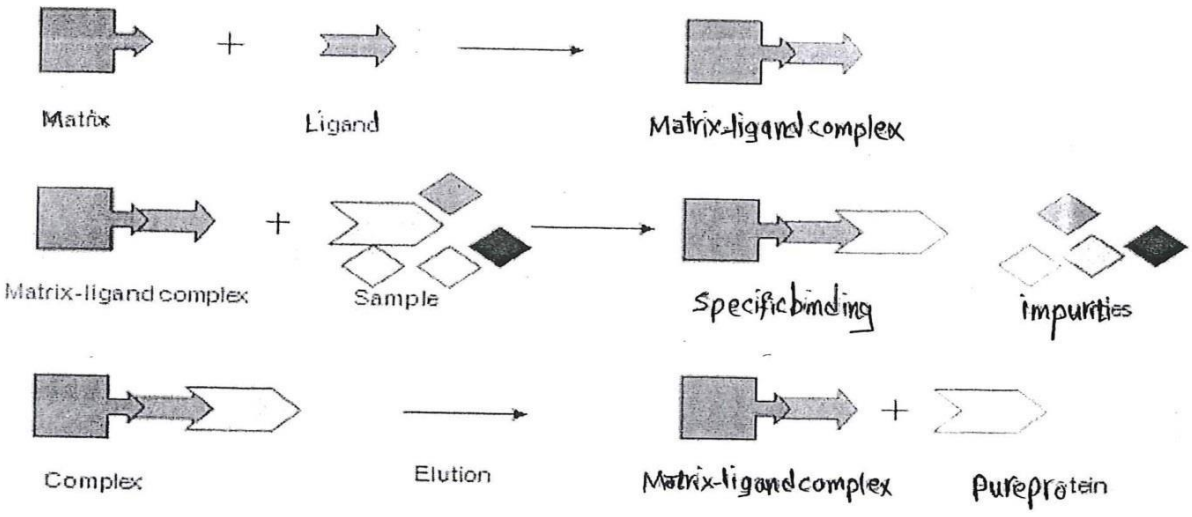


Figure: Gel filtration chromatography.

Affinity chromatography:

This method depends on specific chemical interactions between the product (solute) and resin of column. Affinity adsorption is much more selective between the product and ligand (in column) Fig.



The support is bound to the ligand by covalent or ionic bound ligands do react selectively.