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.

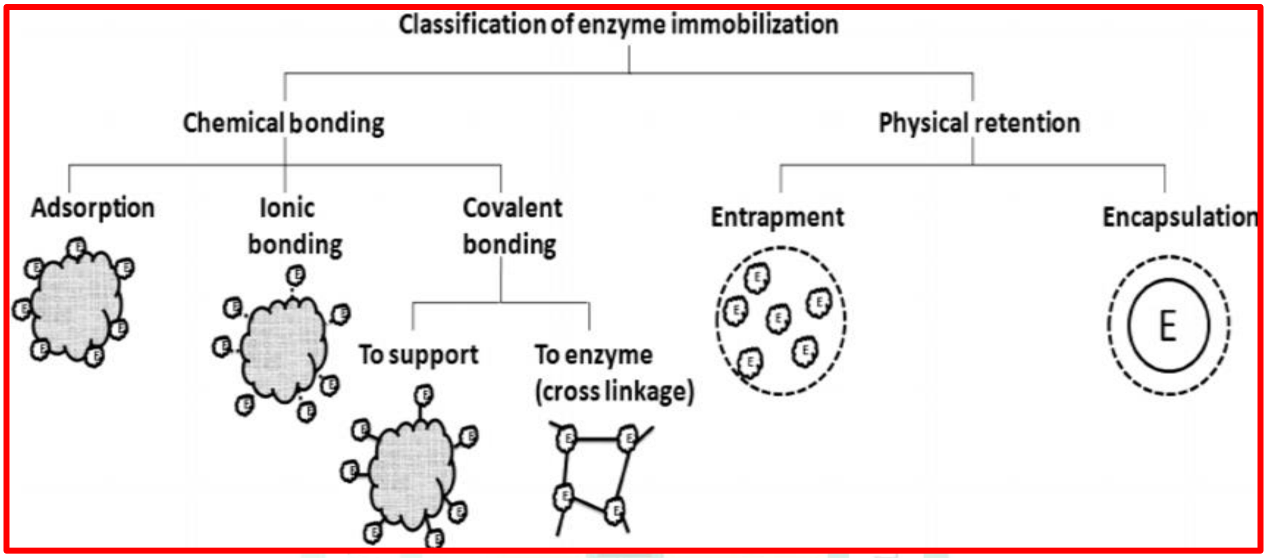

Figure: Immobilization methods of enzymes.

Although preparing enzyme in this way is difficult.

The advantages of using immobilized enzymes are:

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.

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 monosaccharaides 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, methane, Co_2 and hydrogen. However immobilized lactase can be used to break down the lactose in milk making it suitable for lactose in tolerant people to drink.

Bio separation ((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 bio separation is usually product, the second methods used in isolation or purification product.