Enzyme bioechnology

Enzyme biotechnology is the study of industrial enzymes and their uses.

The advantages and disadvantages of using enzymes are directly related to their properties:

Advantages	Disadvantages
They are specific in their action and are therefore less likely to produce unwanted by-products	They are highly sensitive to changes in physical and chemical conditions surrounding them.
They are biodegradable and therefore cause less environmental pollution	They are easily denatured by even a small increase in temperature and are highly susceptible to poisons and changes in pH. Therefore the conditions in which they work must be tightly controlled.
They work in mild conditions, i.e. low temperatures, neutral pH and normal atmospheric pressure, and therefore are energy saving	The enzyme substrate mixture must be uncontaminated with other substances that might affect the reaction.

There are two types of enzymes:

- **Intracellular enzymes**, which are produced inside the cell.
- **Extracellular enzymes,** which are produced outside the cell.

Table comparing intra- and extra-cellular enzymes:

Extracellular enzymes	Intracellular enzymes
Easier to isolate	More difficult to isolate
No need to break cells – secreted in large amounts into medium surrounding cells	Cells have to be broken apart to release them
Often secreted on their own or with a few other enzymes	Have to be separated out from cell debris and a mixture of many enzymes and other chemicals
More stable	Often stable only in environment inside intact cell
Purification/downstreaming processing is easier/cheaper	Purification/downstreaming processing is difficult/expensive

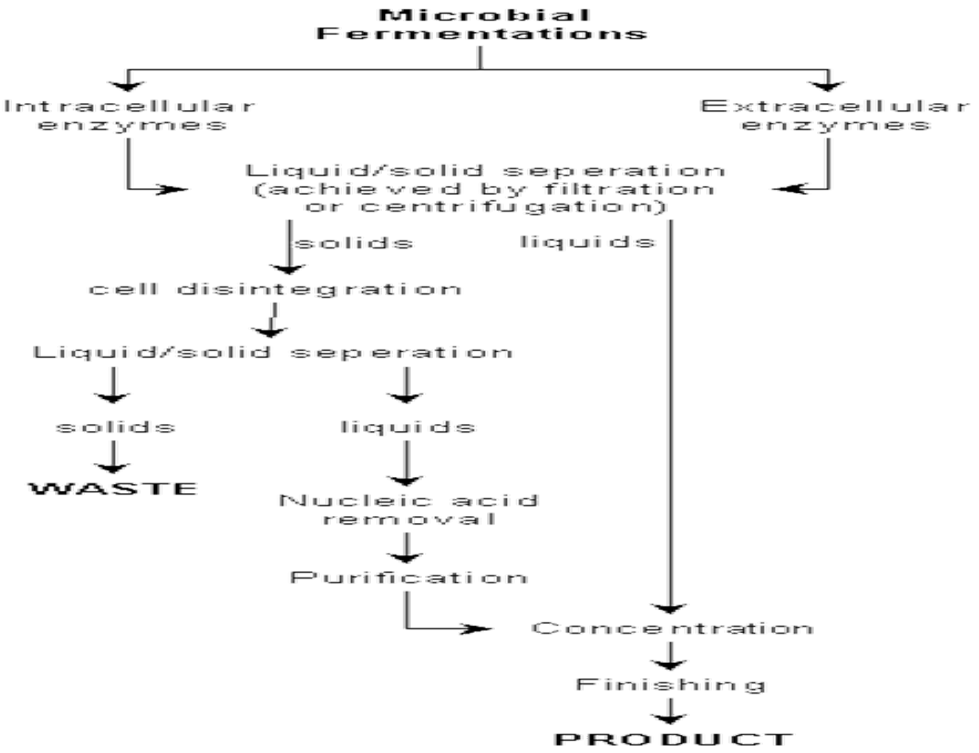
Isolating the Enzyme

Pure enzymes are needed for commercial use; therefore microbes must be grown in aseptic conditions, free from contaminants. It is necessary to prevent contamination with other bacteria since:

• There may be competition for nutrients.

• The required enzyme may not be produced as readily.

• The end-product may be contaminated and unsafe.



Uses of enzymes

Depending on the applications of enzymes,they are grouped into four broad categories:

1- Therapeutic uses

Enzyme	**source**	**Application**
streptokinase	*Streptococcus pyogenes*	Removal of fibrin clots
L-asparaginase	*E.coli*	Cancer chemotherapy
L-glutaminase	*Achromobacter* spp.	Treatmentof leukemia
β-galactosidase	*Lactobacilli* spp.	Treatment of lactose intolerance

2- Analytical uses

Enzyme	**source**	**Application**
Glucose oxidase	*Aspergillus niger*	Detection of glucose in blood
urease	Jack beans	Measurement of urea in body fluids

3- Manipulative uses.

Enzyme	**source**	**Application**
lysozyme	Hen egg white	disrupts mucopeptide of bacterial cell walls
nuclease	bacteria	genetic manipulation

4- Industrial uses.

The industrial use of enzymes (using the whole microbe)

Industry	Microbe
Brewing and baking	*Saccharomyces cerevisiae*
Vinegar production	*Acetobacter*
Yoghurt production	*Lactobacillus*

The industrial use of enzymes (not using the whole microbe)

Enzyme	source	Application
alpha-amylase	*Bacillus* spp.	Conversion of starch to glucose or dextrans in food industry
proteases	*Bacillus* spp.	Laundry aid
Glucose isomerase	*Streptomyces* spp.	Production of high fructose syrups
rennin	bacteria	Cheese making

Improving the Enzyme: Immobilization

Immobilization: The process whereby the movement of enzymes, cells, organelles, etc. in space is completely or severely restricted usually resulting in a water-insoluble form of the enzyme."

Immobilized enzyme: An enzyme fixed by physical or chemical means to a solid support to confine a reaction of interest to a particular site**.**

Advantages of enzyme immobilization:

1- Enzyme can be recovered and reused.

2- Immobilized enzymes are usually more stable.

3- Ability to stop the reaction rapidly by removing the enzyme from the reaction solution.

4- Product is not contaminated with the enzyme, no purification required.

5- Easier to separate enzyme and products.

6- Allows development of a multienzyme reaction system.

7- Reduces effluent disposal problems.

Disadvantages of enzyme immobilization:

1- Immobilization may alter shape of enzyme.

2- May alter catalytic ability.

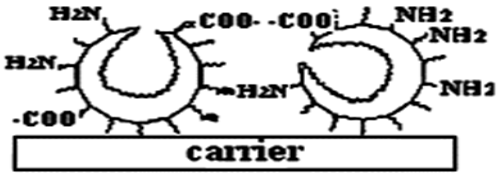
3- Enzyme may become detached.

4- Expensive.

There are four main methods available for immobilizing enzymes:

1) **Adsorption**

This method is based on the physical adsorption of enzyme protein on the surface of water-insoluble carriers such as glass or alginate beads. The bond between the enzyme and carrier molecule involves electrostatic forces such as vanderwaal forces, ionic bridges and hydrogen bonds.



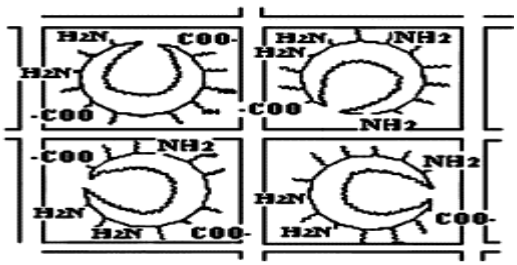
adsorption

2)Entrapment

In entrapment the enzymes or cells are simply trapped inside the polymer matrix. Polymers like polyacrylamide, collagen, cellulose acetate, calcium alginate or carrageenan etc are used as the matrices.

1. Inclusion within a cross linked gel:

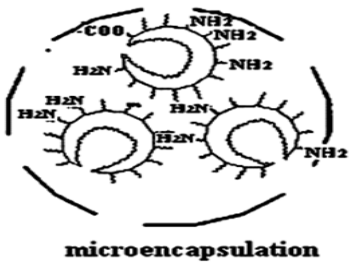
In this entrapment method, a highly cross-linked gel is formed as a result of the polymerization which has a fine "wire mesh" structure.



entrapment

2. Microencapsulation:

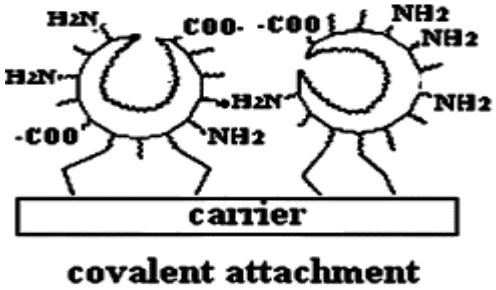
This entrapment involves the formation of spherical particle called as “microcapsule” in which a liquid or suspension of biocatalyst is enclosed within a semi permeable polymeric membrane.



microencapsulation

3)Covalent binding

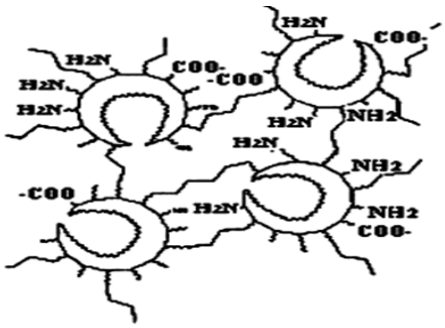
This method involves the formation of covalent interactions between the functional groups present on the support surface and those present on the amino acid residues on the enzyme surface.



covalent attachment

4)Cross linking

This method is based on the formation of covalent bonds between the enzyme molecules leading to three dimensional cross linked aggregates. The most common reagent used for cross-linking is glutaraldehyde.



crosslinking