## **Lec.7 Biotechnology Dr. Sahira Nsayef**

|  |
| --- |
|  |

**Enzyme Technology**

Enzyme technology 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:**

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

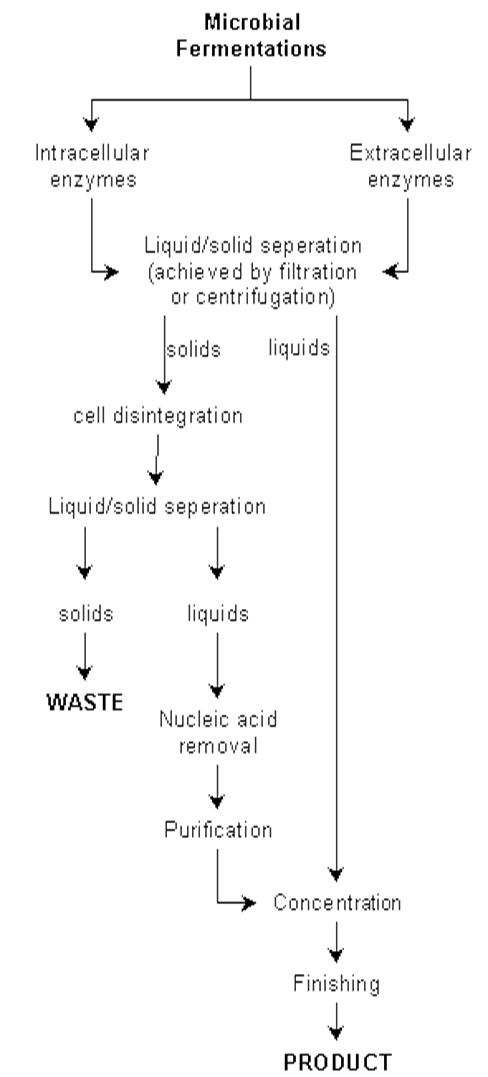
**Isolating the Enzyme**

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 | *Aspergillusniger* | 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.

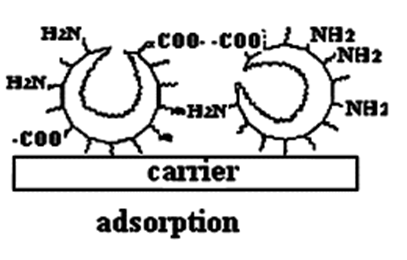
3- Enzyme may become detached.

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 electrostaticforces such as vanderwaal forces, ionic bridges and hydrogen bonds.

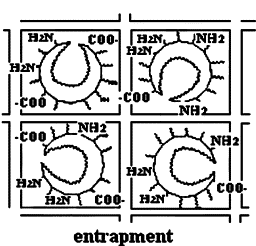


**2)Entrapment**

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.

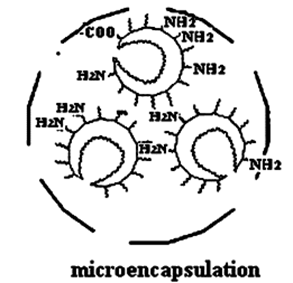
**1. Inclusion within a cross linked gel:**

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



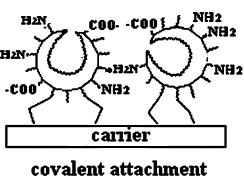
**2. Microencapsulation:**

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.



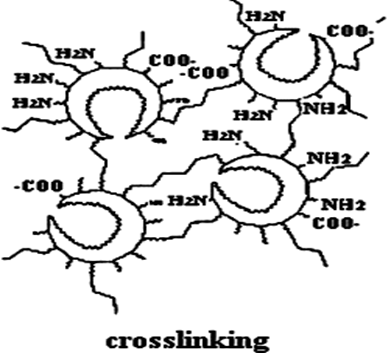
**3)Covalent binding**

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.



**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.

****

