Lec.7 Enzyme technology

Enzyme technology: it is technology includes enzyme production, extraction, and purification. Enzymes are used in various fields such as food industry, production of energy, improve the environment, and others.

Enzymes are complex protein molecules found in living cells and act as catalysts in reactions leading to chemical changes in the substrates on which they run.

There are enzymes in animal cells, plant cells and microorganisms, but it's produced rapidly by **microorganisms** for the following reasons:

1) Easy of handling and development of microorganisms.
2) The possibility of controlling the conditions of production and continue throughout the year and avoid environmental and seasonal changes.
3) The possibility of improving its production, either by dealing with their genes or the environment in which they grow.
4) Usually have a shorter fermentation time and nutrient media is inexpensive.

Types of microbial enzymes

Cells of micro organisms create many enzymes help it in the growth and metabolism, there are two types of it:

1- **Constitutive enzymes**: are usually formed to be used by the cells and is rarely found in the medium.

2- **Adaptive enzymes:** formed depending on the substrates found in the medium, because it is formed in order to dismantle the compound in order to exploit it, for example *Pseudomonas* used for analysis complex chemical compounds in the environment like pollutants in oil or pesticides in soil and water.

Another division of enzymes is:

1- **Intracellular enzymes:** enzymes secrete inside the cell or composed in the cell membrane and not secrete out of cells.

2- **Extracellular enzymes:** enzymes secrete extracellular, and they include most of the enzymes that are commercially produced by microbial fermentation.

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 & 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/down streaming processing is difficult/ expensive	Purification/downstreaming processing is easier/cheaper

Types of fermentation cultures

Selecting the fermentation medium is an important factor in reducing the cost of production, speed up the growth of the microbe, increase production and improve the quality. The medium must contain a source of energy, carbon, nitrogen and some growth promoters such as vitamins and amino acids. There are two types of it:

1- **Surface culture:** for the production of some mold enzymes where it is used where the Semi solid medium which is less expensive and can be used as a high proportion of dry matter in the medium, but drawbacks for it is contaminated and volatilization in fungal spores making it difficult to produce more one enzyme.

2- **Submerged culture**: which is more common because they not need a place which is less exposed to contamination and more application of automatic control, but the costs are high.

Examples of some of the microbial enzymes and their uses

The biocatalysts used in multivarious ways in different field. Trevan (1987) has grouped the applications into four broad categories:

(i) **Therapeutic uses**. These enzymes, such as **streptokinase** produce by *Streptococcus pyogenes* and it is used in the treatment of blood clots, **L-Asparaginase** produce by *E.coli* , **L-glutaminase** produce by *Achromobacter* spp. used in the treatment of leukemia and **β-galactosidase** produce by *Lactobacilli* spp. used in the treatment of lactose intolerance.

(ii) **Analytical uses.** Like Glucose oxides, used for detecting glucose in blood, and urease used for measurement urea in body fluids.

(iii) **Manipulative uses.** Like lysozyme used for disrupts mucopeptide of bacterial cell wall, and nuclease used for genetic manipulation.

(iv) **Industrial uses.** Like amylases, proteases and others as below:

Amylases

About 85% of the activity of the enzyme alpha-amylase product by *Bacillus subtilis* bears high temperatures. However, while the amylase enzymes produced by the fungus *Aspergillus oryzae* lose more than 90% of its activity at high temperatures. These enzymes are used in the food, chemical and pharmaceutical industry.

Protease

Produced by bacterial species such *as Bacillus subtilis & Bacillus licheniformis.* These enzymes are used in leather tanning and textile industry. Protease can be divided according to their **mechanism of action** to:

Serine proteases,Thiol proteases, Metal protease, **Acid protease**.

Or divided on the basis of **the optimum pH** for its work to:

1 - Acid protease: its properties similar to enzymes of animal **Renin and pepsin**, and not be sensitive to material associated with Chelating agents and heavy elements.

2 - Alkaline protease: its properties similar to the animal enzymes **Trypsin and Chymotrypsin** and the most common enzyme are Subtilisin.

3 - Neutral protease: mostly have metal component and containing zinc in the composition and inhibits their work by materials associated with Chelating agents, least stable type.

Glucose isomerase

This enzyme can be isolated from strains of *Bacillus & Arthrobacter* and can be used for the desalination factor of corn starch. It is extracted from cells, so it's intracellular enzyme.

• Cellulases

Produced by bacterial species such as *Bacillus, Clostridium* or fungus *Aspergillus, Trichoderma* and use in the food industry or for the analysis of cellulose residues to produce alcohol or proteins.

• Dextranase and Pectinase

Dextranase produced by *Aspergillus or Bacillus,* and used in the food industry and the dental industry. **Pectinase** is produced by *Rhizopus & Aspergillus,* and can be used as a leaching

clarification of juices and others.

• Alkene oxidase enzyme

Produced by fungi *Oudmansiella, Caldariomyces*. It can be used for plastic, which biodegradable.

Thermally stable DNA polymerase

Produced by *Thermus aquaticus*, heat-resistant enzyme can be used in diagnostic medicine and in molecular biological researches through the use of device PCR.

Immobilized enzymes

Immobilized enzymes and cells means determining movement or installed by linking them to the certain surfaces. Considers these enzyme systems are modern and used for industrial and medical application. It has the following features:

• The possibility of re-use of the enzyme again and reduce costs, especially for expensive enzymes.
• Increase the stability of the enzyme with the possibility of the use of multiple enzymes.
• Greater control over the production process.
• Facilitate the purification process of immobilized enzyme from the medium and the product will be more pure.

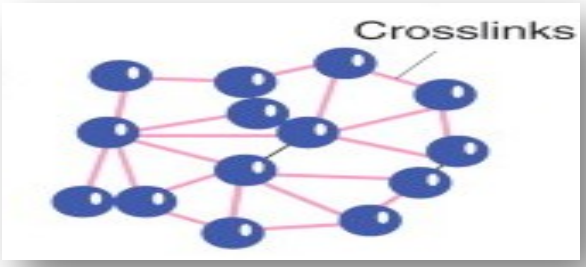
Advantages of immobilization	Disadvantages of immobilization
1. Easier to separate enzyme and products	1. Immobilisation may alter shape of enzyme
2. Allows catalysis in unfavourable media	2. May alter catalytic ability
3. Increases stability and can be manipulated easily	3. Enzyme may become detached
4. Allows continuous production/enzyme used for longer	4. Expensive
5. Enzyme can be recovered and reused	
6. Enzyme does not contaminate product/no purification required	

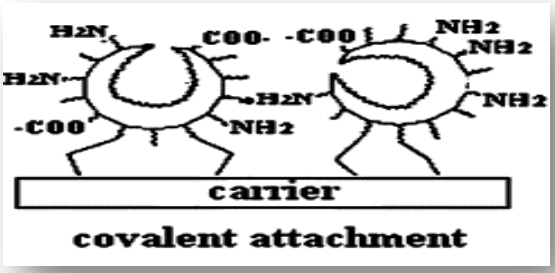
Techniques for immobilized enzymes and cells

There are a number of chemical and physical methods used to immobilized enzymes and cells, and the most common are:

1- Covalently-linked enzymes.

Its consist of covalent bonds between the functional group of the enzyme and the effective groups on the supported material, such as agarose, cellulose, glass, etc. It affected by concentration of the enzyme, concentration of supporting material, Ionian concentration, pH and temperature.



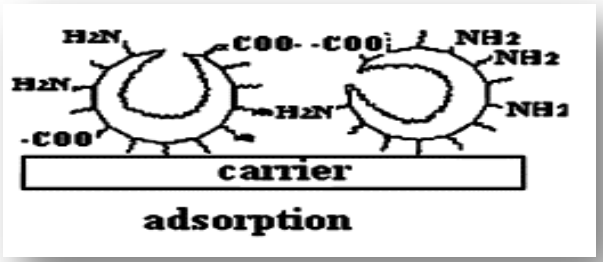


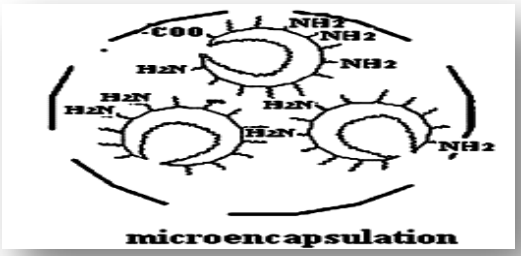
2-Cross-linked enzymes

Enzymes are linking with each other by materials carry effective functional groups, such as gluteraldehyde. Positives are: ease and strong of the link.

3-Adsorbed enzymes

Enzymes or cells correlate on solid surfaces by ionic or hydrogen or hydrophobic bonds , which is relatively weak, so it has affected by environmental conditions such as the pH, ionic forces and temperature. Positives are: a simple and inexpensive. Materials most commonly used are ion exchangers such as, organic polymers, mineral salts and silicon materials.





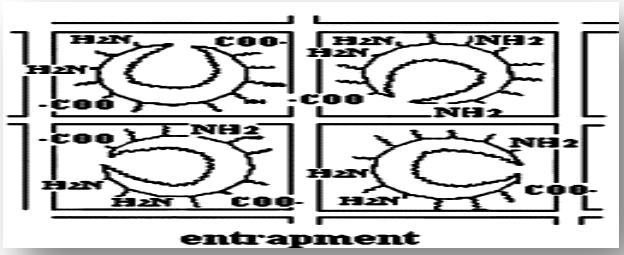
3- Encapsulated enzymes

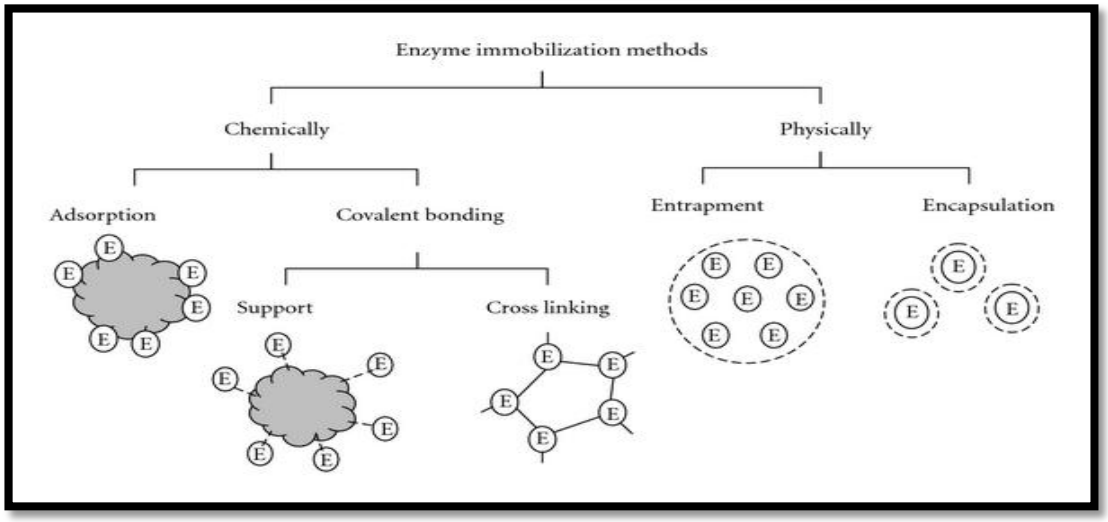
The enzymes are surrounded by semi- osmotic membrane allows the passage of supporting material particles to engage and interact with the enzyme. Manufactures membranes used nylon, resins or cellulose, and these methods easy and cheap and give

a big surface area for interaction, but do not give great protection and high stability of the enzyme.

4- Entrapment enzymes

The enzymes here within the confines of a network of fiber or polymeric materials, and materials used for this purpose as polyacrylamide, cellulose, acetate, collagen, calcium alginate and others. positives are: easy way and did not need to complex conditions or requirements of many , either drawbacks are: the enzyme that may leak from the gel particles or break during use compression.





Immobilization of whole cells

Restricted cells may be used for certain materials as a metabolic enzyme possesses integrated systems can take advantage of them to get the required materials. The benefits of this method are:

• Do not need to extract the enzymes and the provision of cost, effort and time spent in these steps.

• Can be used as one system or multiple enzymes or integrated metabolic pathway.