Enzymes of Biotechnology

Definition:-

"Enzyme are biocatalyst which is used for living cell." Enzyme are isolated from micro-organism. 1st discovered in USA by Takadiastase (fungal amylase) in Pharmaceutical industry.

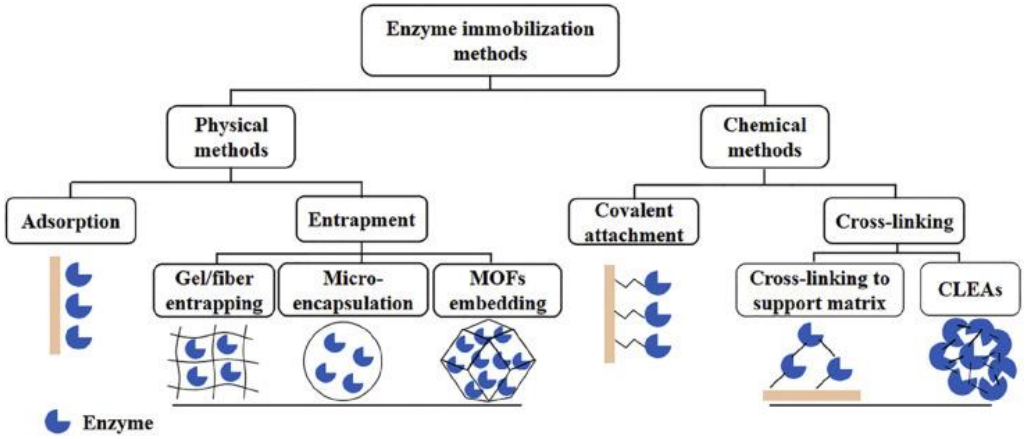
Advantages:-i.Food industry
ii.Paper industry
iii. Textile industry
iv. Washing powder
v. Improvement in environment & scientific research

- Methods of Enzyme Immobilization:-

Definition:-

"It is confining or anchoring the enzyme in or an inert action supportfor their stability and functional reuse called enzyme immobilization."

a) Adsorption Method
b) Entrapment Method
c) Covalent Method
d) Cross-linking



a) Adsorption Methods:-

Adsorption is the most economical and simple method to immobilize enzymes by adsorbing them on to charged or neutral surfaces of inert substrate.

Adsorption involves the physical binding of enzyme on inner active support.

The support materials maybe Inorganic or Organic.

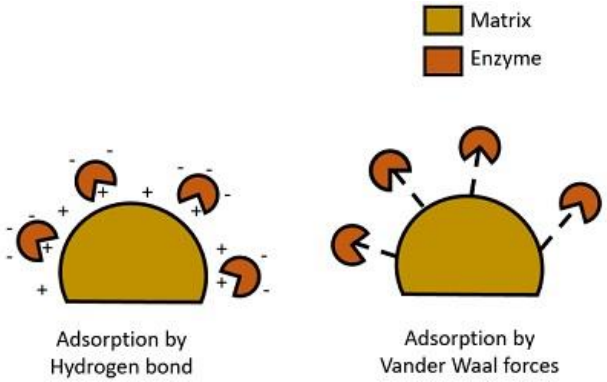
i. Inorganic support materials Eg:- Silica gel, Alumina, Calcium phosphate gel, glass.

ii. Organic support materials Eg:- Starch, Carboxymethylcelluose(CMC), DEAE cellulose, DEAE Sephadex.

Adsorb enzyme can be easily removed by minor changes in ph, ionic strength &Temperature.

Types:-

1. Vander Waal's Forces
2. Hydrogen Bounding



Advantages:-a. No pore diffusion limitation
b. Easy to carry out
c. No reagent are required
d. Minimum activation steps involved
e. Comparatively cheap method of immobilization

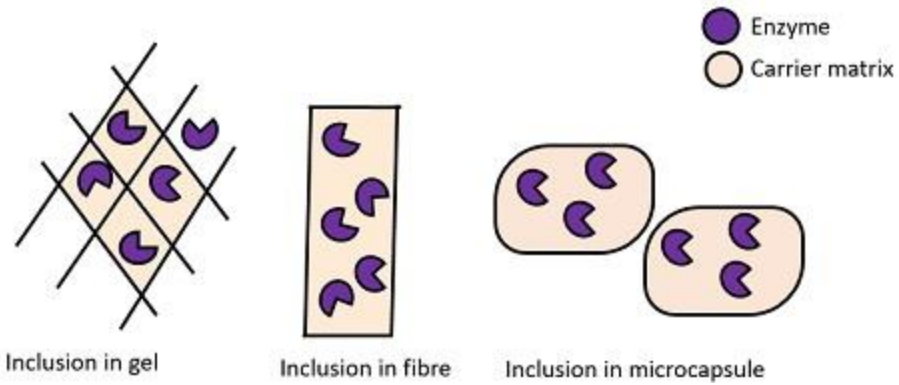
Disadvantages:-a. Less Efficicent
b. Desorption of enzymes from carrier

b) Entrapment Method:-

Entrapment is a phenomenon in which the enzyme molecules are held or entrapped within appropriate fibers or gels. Example of enzymes:- Agar, Gelatin, Polyacrylamide gels, Cellulose triacetate, Alginate.

Types:-

1. Inclusion in Gels
2. Inclusion in Fibre
3. Inclusion in Micro-capsule



1. Enzyme Inclusion in Gels:-Enzymes trapped inside the gels.
2. Enzyme Inclusion in Fibre:-Enzymes supported on fibres made of matrix material.
3. Enzyme Inclusion in Micro-capsule:-Enzymes entrapped in microcapsules formed by monomer mixture such as polyamide and calcium alginate.

Advantages:-a. Fast method of immobilization
b. Cheap (low enzymes available)
c. Easy to practice at small scale

Enzymes of Biotechnology

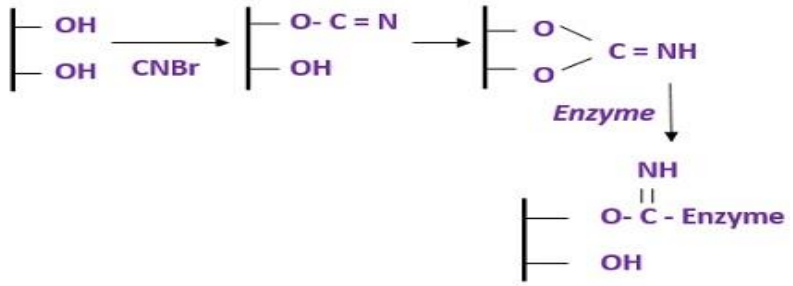
Disadvantages:-a. Leakage of enzyme
b. Pore diffusion limitation
c. Not much success in industrial process

c) Covalent Method:-

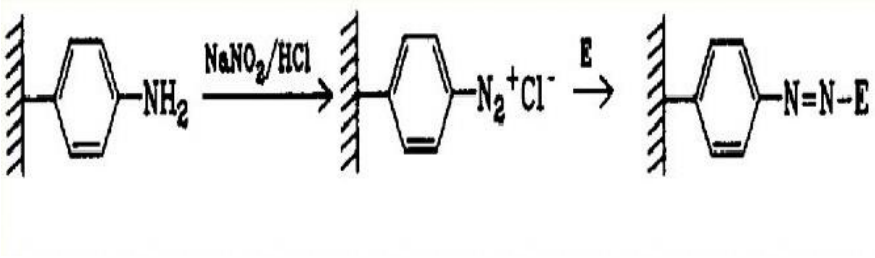
Covalent bonding which can be achieve by chemical group of enzyme & chemical group of support.

Reactions:-

1.Cyanogen Bromide Activation-

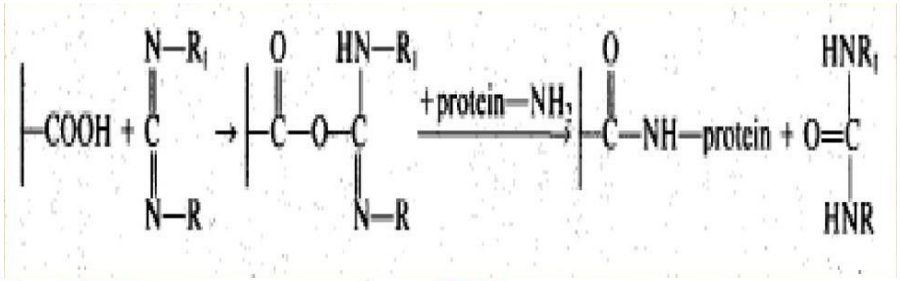


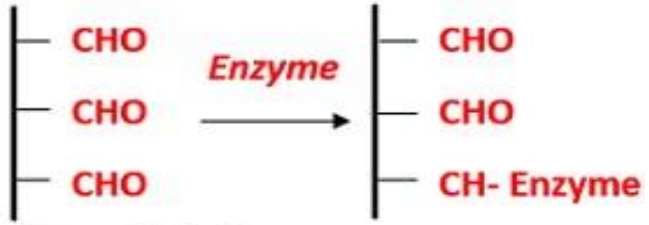
2.Diazotation-



3.Peptide Bond Formation-

4.Activation by Bifunctional/Polyfunctional reagent-





Glutaraldehyde

Advantages:-a. Strong linkage of enzyme to the support
b. No leakage or desorption problem
c. Comparatively simple method
d. Wide applicability

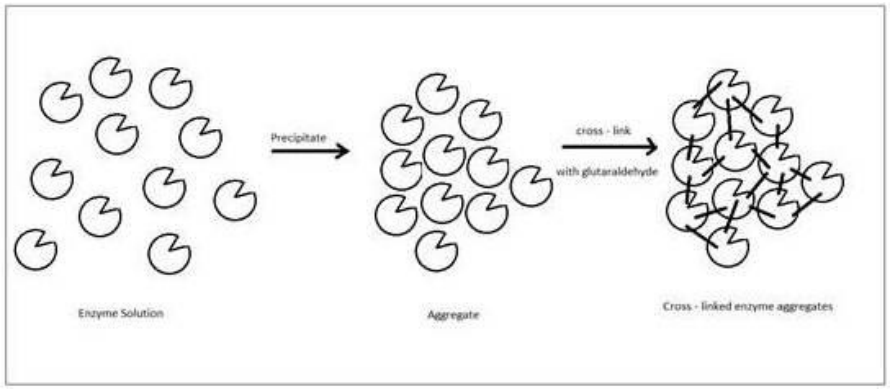
Disadvantages:-
Only small amounts of enzymes may be immobilized(0.02 grams per gm matrix.)

d) Cross-linking:-

This method is also called as copolymerization.In this method of immobilization enzymes are directly linked by covalent bonds between various groups of enzymes via polyfunctional reagents.cross-linked

gels include **gelatin, collagen, agarose, and agar agar**.

Advantages:-Cheap and Simple method, large quantity of enzymes can be immobilized by encapsulation.
Disadvantages:-Pore size limitation, Only small



substrate is able to cross themembrane.

- Applications of Enzyme Immobilization:-i. Immobilization cell are widely used in industrial, analytical.
  ii. Production of high fructose syrup.
  iii. It is used in affinity chromatography & purification of product.
  iv. Used in production of amino acids.
  v. Production of other organic compounds.
  vi. Usedin the production of Steroid.

References

- U.Satyanarayan ,Biotechonlogy, Books & Allied Pvt.Ltd, 10th edition, 2016, Page no :- 281-292.
- AshutoshKar&K.Sambamurthy, Pharmaceutical Biotechnology, New Age International Publishers Pvt.Ltd, 2nd edition , Page no:- 309-319.

PREPAPRED BY :- Mr.KirveYash Santosh

Roll No:- (34)

Guided by :- Dr. Kiran Mahajan

DEPARTMENT:- Pharmaceutics

SUBJECT:- Pharmaceutical Biotechnology

CLASS:- Third year B.pharm

ACADEMIC YEAR:- 2021-2022

BIOSENSOR

INTRODUCTION: -

A Biosensor is an analytical device containing an immobilized biological material (enzyme, antibody, nucleic acid, hormone, organelle or whole cell) which can specifically interact with an analyte and produce physical, chemical or electrical signals that can be measured. An analyte is a compound (e.g. glucose, urea, drug, pesticide) whose concentration has to be measured. Biosensors basically involve the quantitative analysis of various substances by converting their biological actions into measurable signals.

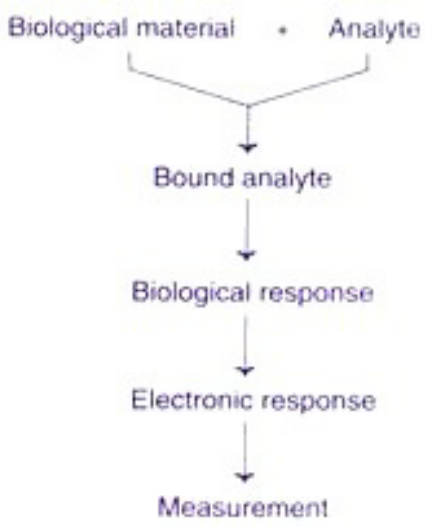
DEFINITION: -

It is an analytical device which contains immobilized biological material (enzyme, antibodies, nucleic acid, hormones, organelles or whole cell) which can specifically interact with analyte and produces physical, chemical or electrical signal that can be measured called as the Biosensor.

PRINCIPLE OF BIOSENSOR: -

The desired biological material is immobilized by conventional methods (physical or membrane entrapment ,non covalent or covalent binding).This immobilized biological material is in intimate contact with the transducer. The analyte binds to the biological material to form a bound analyte which in turn produces the electronic response that can be measured.

In some instances, the analyte is converted to a product which may be associated with the release of heat, gas, electrons or hydrogen ions, The transducer can convert the product linked changes into electrical signals which can be amplified and measured.



TYPES: -

The different types of biosensors are

classified based on the sensor device as well as the biological material that is discussed below.

A. Electrochemical Biosensor: -

Generally, the electrochemical biosensor is based on the reaction of enzymatic catalysis that consumes or generates electrons. Such types of enzymes are named Redox Enzymes. The substrate of this biosensor generally includes three electrodes such as a counter, reference, and working type.

Electrochemical biosensors are classified into four types: -

a) Amperometric Biosensors

b) Potentiometric Biosensors

c) Impedimetric Biosensors

d) Voltametric Biosensors

a) Amperometric Biosensor: -

An amperometric biosensor is a self-contained incorporated device based on the amount of the current ensuing from the oxidation offering exact quantitative analytical information. The simple

amperometric. biosensor infrequent usage includes the “Clark oxygen” electrode

b) Potentiometric Biosensor: -

This type of biosensor provides a logarithmic reply by means of a high energetic range. These biosensors are frequently complete by monitor producing the electrode prototypes lying on a synthetic substrate, covered by a performing polymer with some enzyme is connected.

c) Impedimetric Biosensor: -

The EIS (Electrochemical impedance spectroscopy) is a responsive indicator for a broad range of physical as well as chemical properties. A rising trend towards the expansion of Impedimetric biosensors is being presently observed.

d) Voltametric Biosensor: -

This communication is the base of a new voltametric biosensor to notice acrylamide. This biosensor was built with a carbon glue electrode customized with Hb (hemoglobin), which includes four prostatic groups of the hem (Fe). This type of electrode shows a reversible oxidation or reduction procedure of Hb (Fe).

B) Piezoelectric Biosensors: -

These sensors are a collection of analytical devices which work on a law of “affinity interaction recording”. The platform of a piezoelectric is a sensor element that works on the law of oscillations transform due to a collection jump on the surface of a piezoelectric crystal.

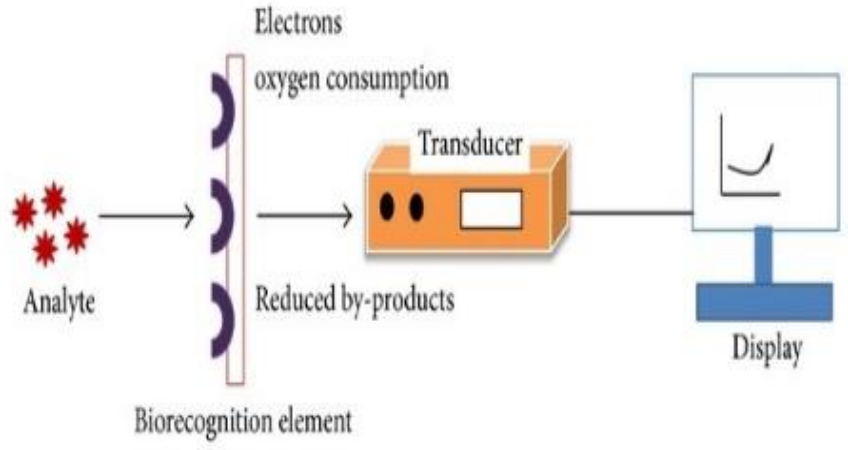
C) Thermometric Biosensor: -

There are various types of biological reactions which are connected with the invention of heat, and this makes the base of thermometric biosensors. Thermometric-biosensor is used to measure or estimate serum cholesterol.

D) Optical Biosensor: -

The Optical biosensor is a device that uses an optical measurement principle. They use fiber optics as well as optoelectronic transducers. The term optrode represents a compression of the two terms optical & electrode.

CONSTRUCTION AND WORKING OF BIOSENSORS:- The combination of biological sensitive element and a transducer will convert the biological material into a corresponding electrical signal. Depending on the type of enzyme, the output of the transducer will be either current or voltage. If the output is voltage, then well and good.



REFERENCE: - A book of biotechnology by U.Satyanarayana. Page.No. 297 - 304.

PREPARED BY: - Jadhav Omkar (27)

GUIDED BY: - Dr. Mahajan Kiran

SUBJECT: - Pharmaceutical Biotechnology.

DEPARTMENT:- Pharmaceutics

ACADEMIC YEAR: - 2021-2022.

CLASS: - T.Y.B.Pharm