**ESSIEN PRECIOUS EKONG**

**15/SCI03/005**

**BCH 404 ASSIGNMENT**

1. **ENUMERATE AND DISCUSS THE PRINCIPAL TECHNIQUES FOR IMMOBOLIZATION OF ENZYMES**

Immobilization is a technical process in which enzymes are fixed to solid supports, creating a heterogeneous immobilized enzyme system. Immobilized form of enzymes mimics their natural mode in living cells where most of them are attached to cellular cytoskeleton, membrane and organelle structure. They are robust and resistant to environmental changes. The techniques for immobilization of enzymes include:

* **ADSORPTION:** This is the easiest technique and includes reversible surface interaction between carrier and enzyme. The forces formed are weak force, mostly electrostatic, for example Van der Waals forces, ionic bond and hydrogen bonding interactions, although hydrophobic bonding can be significant, but although these forces are very weak, but sufficiently large in number to enable reasonable binding. This method is done by mixing the enzymes and a support material with each other in adsorption properties at optimum pH, ionic strength, etc., for a time, after that collect immobilized enzyme and wash it to remove unbound enzymes.

**Advantages of this method include:**

Little or no damage to enzyme /cells.

It is easy, cheap, and fast.

No changes happen to carrier or enzyme/ cells.

It is reversible.

**Disadvantages include:**

Leakage of enzyme/cells from the support

Separation of product is not easy.

Nonspecific binding.

* **COVALENT BINDING:** Covalent binding immobilization method consists of formation of a covalent bond or a strong bond between the enzyme and a carrier. This covalent bond is formed between the functional groups present on the surface of carrier and the surface functional groups of the enzyme. The functional groups on the surface of the enzyme are amino groups (NH2) of arginine or lysine, carboxylic group (COOH) of glutamic acid or aspartic acid, hydroxyl group (OH) of threonine or serine, and sulfhydryl group (SH) of cysteine. Hydrophilicity is one very important factor that affects the choice of specific carrier. Thus, hydrophilic carriers such as polysaccharide polymers are popular materials for enzyme immobilization. For example, cellulose, starch, dextran, and agarose. Also, hydroxyl groups can form hydrogen bonds with water and create an aqueous (hydrophilic) environment in the support. Covalent binding consists of two steps. First, activation of functional groups found on carrier surface by a specific reagent, and the second, adding enzyme to form covalent bond with activated surface of carrier.
* **ENTRAPMENT**: One of the easiest techniques of immobilization is entrapment. There are several major methods of entrapment:
* Ionotropic gelation of macromolecules with multivalent cations (e.g. alginate).
* Temperature-induced gelation (e.g. agarose, gelatin).
* Organic polymerization reaction by chemical/photochemical (e.g. Polyacrylamide).
* Precipitation from an immiscible solvent (e.g. polystyrene).

Entrapment can be accomplished by cross linking the polyionic polymer material with multivalent cations in an ion-exchange reaction after mixing with enzyme to form a structure that traps the enzymes/cells (ionotropic gelation).

* **ENCAPSULATION:** Encapsulation of enzymes as well as cells can be accomplished by wrapping the biological components inside different forms of semi permeable membranes. It is as entrapment in that the enzymes/cells are free in movements, however limited in space. Vast proteins or enzymes cannot go out or enter capsule, however small substrates and products can go freely across the semi permeable membrane. Numerous materials have been utilized to form microcapsules are in range of 10-100 μm in diameter; such as, nylon and cellulose nitrate. Biological cells also may be used as capsules, and a famous example of this is the use of erythrocytes (red blood cells). The membrane of the erythrocytes is normally just permeable to small molecules. However, when erythrocytes are placed in hypotonic solution, they swell, expanding the cell membrane and substantially expanding the penetrability. In this condition, erythrocytes proteins go out of the cell and enzymes can inter into the cell. Returning these erythrocytes swollen to the isotonic solution enables the cell membrane to return to the normal state and the enzymes inside the cell can't leak out. A distinct advantage of this technique is co immobilization.
* **CROSSLINKING:** This method of immobilization depends only on enzyme and it is support- free as it done by joining the enzyme (or the cells) to each other to prepare a large, three-dimensional complex structure, and it can be done chemically or physically. Chemical type of crosslinking normally includes formation of covalent linkage between the cells by means of a bi- or multifunctional reagent, for example glutaraldehyde and toluene isocyanate. However, limiting factors can be used in this method for living cells and many enzymes because of harmful materials.
1. **WHAT IS THE GENERATION TIME OF BACTERIA POPULATION THAT INCREASES FROM 15,000 TO 15,000,000 CELLS IN EIGHT HOURS OF GROWTH?**

t = 10 hours

B = 15,000

b = 15,000,000

G =?

G= t/n

n = 3.3 log b/B

3.3 log 15,000,000/15,000= 3.3 log (1000)

n= 9.9

G= t/n

G= 10/9.9= 1.01 hours

G= 1.01 x 60 minutes

G= 60.6 minutes