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ASSIGNMENT

1. Enumerate and discuss the principal techniques for immobilization of enzymes

Techniques for the immobilization have been broadly classified into four categories, namely, entrapment, covalent binding, cross-linking, and adsorption and the combination of one or more of these physical techniques together with chemical conjugation techniques.

ENTRAPMENT

One of the easiest techniques of immobilization is entrapment. In recent years, calcium alginate has attraction as an immobilization support material. It has been utilized for immobilization of variety of cell types, sub-cellular organelles, multi-component systems, and enzymes. The physicochemical characteristics of this matrix in gel form have an important effect on the reactions of entrapped biologically active material in the gel. Critical parameter in selecting a matrix is pore size. The difference between entrapment technique and adsorption and covalent binding is that however the enzyme is restricted in movement by the structure of a gel lattice but it is free in solution. The pore size of a gel lattice is controlled to ensure that the structure become tight enough to prevent loose of enzyme or cells, it also allow free movement of the substrate and product. The support acts as a barrier to mass transfer, and although this have serious reaction kinetics implications, but it can prevent interaction between harmful cell, proteins, and enzymes and immobilized biocatalyst. There are several methods of entrapment

which includes: **Ionotropic gelation of macromolecules with multivalent cations (e.g. alginate)** and in this method 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. Another method of entrapment is **temperature-induced gelation (e.g. agarose, gelatin)** Change in temperature is a simple way of gelation by phase transition utilizing 1-4% solutions of gelation. κ-carrageen a polymers that can easy form gels by ionotropic gelation and by temperature-induced phase transition, which has form a greater degree of flexibility in gelation frameworks for immobilization. Other methods include precipitation from an immiscible solvent (e.g. polystyrene) and organic polymerization reaction by chemical/photochemical (e.g.Polyacrylamide).

COVALENT BINDING

Covalent binding immobilization method consists of formation of a covalent bond, strong bond, between the enzyme/cell and a carrier. This covalent bond formed between the functional groups present on the surface of carrier and the surface functional groups of the enzyme. These functional groups on the surface of the enzyme such as 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. Many factors affect the choice of specific carrier, and research work has demonstrated that hydrophilicity is one of the most important factors for keeping up enzyme activity. Thus, hydrophilic carriers such as polysaccharide polymers are popular materials for enzyme immobilization. For example, cellulose, starch, dextran (sephadex), and agarose (sepharose). The sugar residues in these polymers contain ideal functional groups, hydroxyl groups, for covalent bond formation. Also,

hydroxyl groups can form hydrogen bonds with water and create an aqueous (hydrophilic) environment in the support. The supports are usually used in bead form. There are other popular supports for immobilization of enzymes is porous silica and porous glass. Porous silica contains small spherical particles of silica fused together having micro cavities and small channels. The reactions involved in covalent binding may include: Isourea linkage formation, peptide bond formation, Diazo linkage formation and alkylation formation.

There are two steps involved in covalent binding: First one, 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. Normally the activation reaction is designed to make strong electrophilic (electron deficient) functional groups on the carrier. In the coupling reaction, these activated groups will react with strong electron donating nucleophiles, such as the amino group (NH2), functional groups of certain amino acids on the surface of most enzymes, to form strong covalent bond.

CROSS LINKING

This method of immobilization depend 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, threedimensional 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 diisocyanate. However, limiting factors can be used in this method for living cells and many enzymes because of harmful materials. To minimize the close problems that can be found because of crosslinking of single enzyme, both albumin and gelatin have been used. This technique uses a bi- or multifunctional compounds,

which serve as the reagent for intermolecular cross-linking of the biocatalyst. Crosslinking methods are done under relatively severe conditions in comparison with those of physical adsorption or encapsulation. Hence, in the previous cases, conformational change of the enzyme structure and partial destruction of the active site may occur.

ADSORPTION

Using adsorption as immobilization method 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 done by mixing the enzyme(s) 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. Desorption may be happen as a result of physical factors, for example, flow rate, agitation, particle-particle Collisions.

There are some advantages and disadvantages of adsorption as a method of enzyme immobilization.

Advantages

1. Little or no damage to enzyme /cells.
2. Easy, cheap, and fast.
3. No changes happened to carrier or enzyme/ cells.
4. Reversible.

Disadvantages

1. Leakage of enzyme/cells from the support
2. Separation of product is not easy.
3. Nonspecific binding.

2. What is the generation time of bacteria population that increases from 15,000 to 15,000,000 cells in 10 hours of growth?

Generation time=?

B=15,000

b=15,000,000

t=10 hours

$G= \frac{t}{n}$

$n=3.3\log\frac{b}{B}$

$n=3.3\log\frac{15,000,000}{15,000}$

$n=3.3\log1000$

$n=3.3\log10^3$

$n=3x3.3\log10$

n=3x3.3x1

n=9.9

$G = \frac{t}{n}$

$G = \frac{10}{9.9}$

G=1.01 hours

G=1.01 x 60 minutes

G=60.6 minutes