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BCH 404

Assignment

1. Immobilization enzyme is defined as the imprisonment of cell or enzyme in a distinct support or matrix. The support or matrix on which the enzyme are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecules. They are settling advantages such as enhanced reproducibility of the process they are undertaking, more stability of production, high enzyme substrate. In addition, there are some disadvantages of immobilization enzyme e.g (partial) loss of activity, changed kinetics, and diffusion or mass transfer limitations. Enzyme immobilization can be applied in different industries such as industrial production, biomedical applications, food industry, research, detergent industry etc.

   Over the years some support or matrix are used in immobilization of enzyme by holding it permanently or temporarily for a brief period of time. These support are group into three categories; natural polymers (alginate, chitosan and chitin, collagen, starch, pectin etc), synthetic polymers (polyvinyl chloride (PVC), UV activated, Diethylaminoethyl cellulose (DESE cellulose) and polyethylene glycol (PEG). For immobilization we have to decide the support material first, and then the immobilization method, taking into account the intended use and application. They are about 4 or 5 different methods or principle of immobilization of enzyme these are adsorption, covalent binding, entrapment, encapsulation, and cross linking.

i. Adsorption

Using adsorption as immobilization method is one of the oldest and easiest technique and includes reversible surface interaction between carrier and enzyme. There is no permanent bond formation between carrier and the enzyme in this method. The bond formed are weak bond these weak bonds stabilize the enzymes to the enzymes to the support or carrier. The weak bonds involved are; Van der Waals bond, ionic bond and hydrogen bonding interactions. Some advantage of this method are Little or no damage to enzyme /cells, Easy to carry out, no changes happened to carrier or enzyme/ cells and Reversible. Whilst there are some disadvantages such as Desorption of enzymes from the carrier, separation of product is not easy and Efficiency is less. This method is done in four steps;

1) **Static process:** Immobilization to carrier by allowing the solution containing enzyme to contact the carrier without stirring.
2) **Dynamic batch process:** Carrier is placed in the enzyme solution and mixed by stirring or agitation.
3) **Reactor loading process:** Carrier is placed in the reactor, and then the enzyme solution is transferred to the reactor with continuous agitation.
4) **Electrode position process:** Carrier is placed near to an electrode in an enzyme bath and then the current is put on, under the electric field the enzyme migrates to the carrier and deposited on its surface.

ii. Covalent Binding

Covalent binding immobilization method consists of formation of a covalent bond, strong bond, between the enzyme/cell and a carrier. It’s one of the widely used methods of enzyme immobilization. This covalent bond is formed between the functional groups present on the

surface of carrier (amino groups, imino group, hydroxyl groups, carboxyl groups etc) and the surface functional groups of the enzyme (Hydroxyl groups of Serine and Threonine, Imidazole group of Histidine, Alpha amino group at 'N' terminal of enzyme, Alpha carboxyl group at 'C' terminal of enzyme etc). Some carriers or supports commonly used for covalent bonding are Carbohydrates (Cellulose, DEAE cellulose etc), Synthetic agents (Polyacrylamide), Protein carriers **(**Collagen and Gelatin), Amino group bearing carriers **(**amino benzyl cellulose) etc.

Some advantages of covalent binding include strong linkage of enzyme to the support, no leakage or desorption problem and comparatively simple method and one disadvantages could be enzyme inactivation by changes in the conformation when undergoes reactions at the active site, which can be overcome through immobilization in the presence of enzyme's substrate or a competitive inhibitor. This method is done in three steps;

1) **Diazoation:** Bonding between amino group of support and tyrosil or histidyl group of enzyme.
2) **Peptide bond:** Bonding between amino or carboxyl groups of the support and that of the enzyme.
3) **Poly functional reagents:** Use of a bi-functional or multifunctional reagent (glutaraldehyde) which forms covalent bonds between the amino group of the support and amino group of the enzyme.

iii. Entrapment

This is one of the easiest techniques of immobilization. In this method enzymes are physical entrapped inside a porous matrix. The bonds involved in stabilizing the enzyme to the matrix may be covalent or non-covalent. The matrix used will be a water soluble

polymer, the nature of matrix varies with different enzymes. Some commonly used matrixes for entrapment are polyacrylamide gels, cellulose triacetate, agar etc). Advantage of this method includes fast method of immobilization, mild conditions are required etc. The greatest disadvantage of this method is that there is a possibility of leakage of low molecular weight enzymes from the matrix. There are several methods of entrapment;

a) **Inclusion in the gels:** enzymes trapped inside the gels.
b) **Inclusion in fibers:** enzymes supported on fibers made of matrix material.
c) **Inclusion in microcapsules:** Enzymes entrapped in microcapsules formed by monomer mixtures such as polyamine and calcium alginate.

iv. Encapsulation

This type of immobilization of enzymes as well as cells can be accomplished by wrapping the biological components inside different forms of semi permeable membranes (nitro cellulose or nylon). In this method the effectiveness depends upon the stability of enzymes inside the semi permeable membranes. The advantages of encapsulation include cheap and simple, large quantity of enzymes can be immobilized by encapsulation. Disadvantages are there is limitation of pore size, only small substrate molecule are able to cross the membrane.

v. Cross Linking

This method is also known as copolymerization. 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. Unlike other methods, there is no matrix or support involved in this method. Commonly used polyfunctional reagents are glutaraldehyde and diazonium salt. This technique is

cheap and simple but not often used with pure enzymes. This method is widely used in commercial preparations and industrial applications. The greatest disadvantage or demerit of this method is that the polyfunctional reagents used for cross linking the enzyme may denature or structurally modify the enzyme leading to the loss of catalytic properties.

2. t = 10 hours

   B = 15,000

   b = 15,000,000

   G =?

   G = t/n

   n = 3.3log b/B

   ∴ 3.3log $15{,}000{,}000/15{,}000$ = 3.3log(1000)

   n = 9.9

   find number of generation;

   G = t/n

   G = $\frac{10}{9.9}$ = 1.01hrs

   G = 1.01 × 60 minsss

   = 60.6 minutes