BAKARE TOYOSI AKEEM

16/sci03/004

BCH 404 (INDUSTRIAL BIOCHEMISTRY)

Assignment

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

Immobilized enzymes are defined as "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, which can be used repeatedly and continuously". Immobilization enzyme is defined as the imprisonment of cell or enzyme in a distinct support or matrix. The support or matrix on which the enzymes 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. Enzyme immobilization can be applied in different industries such as industrial production, biomedical applications, food industry, research, detergent industry etc. Techniques for the immobilization have been broadly classified into four categories, namely, entrapment, covalent binding, cross-linking, and adsorption. Sometimes these techniques can be combined with chemical conjugation techniques.

Adsorption

Adsorption as immobilization method is the easiest technique and oldest method which 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. 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. Some advantages of this method are that it is reversible, little or no damage to enzyme or cells, it is easy to carry out and no changes happened to carrier or enzyme or cells. Whilst there are some disadvantages such as Desorption of enzymes from the carrier, separation of product is not easy and Efficiency is less.

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 areCarbohydrates (Cellulose, DEAE cellulose etc), Synthetic agents (Polyacrylamide),Protein carriers **(**Collagen and Gelatin), Amino group bearing carriers **(**amino benzyl cellulose) etc. 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. 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 undergoesreactions at the active site, which

can be overcome through immobilization inthe presence of enzyme's substrate or a competitive inhibitor. This method is done in two steps;

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 (NH_2), functional groups of certain amino acids on the surface of most enzymes, to form strong covalent bond.

Entrapment

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). It is the easiest method of immobilization. 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. 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 bymonomer mixtures such as polyamine and calcium alginate.

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. This technique uses a bi- or multifunctional compounds, which serve as the reagent for intermolecular cross-linking of the biocatalyst. 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 andsimple but not often usedwith pure enzymes. This method is widely used in commercial preparations and industrial applications. Limiting factors can be used in this method for living cells and many enzymes because of harmful materials.

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

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

G = t/n

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

G = 1.01 × 60 min

G = 60.6 minutes