Immobilized Enzymes: Techniques and Industrial Applications – A Mini Review

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ABSTRACT

Reports on chemical immobilization of proteins and enzymes first appeared in the 1960s. Since then, immobilized proteins and enzymes have been widely used in the processing of variety of products and increasingly used in the field of medicine. Here, we present a review of recent developments in immobilized enzyme use in medicine. Immobilized enzymes are widely used for variety of applications. Based on the type of application, the method of immobilization and support material can be selected. The immobilized enzymes can be separated from the reaction mixture and reused and also immobilized in order to prevent the enzyme from being exposed to harsh conditions, high temperature, surfactants, and oxidizing agents etc. the immobilized enzymes are also widely used in food industry, pharmaceutical industry, bioremediation, detergent industry, textile industry, etc. Enzyme immobilization improves the operational stability and is also due to the increased enzyme loading which causes the controlled diffusion. Several hundreds of enzymes are immobilized and used for various large scale industries. Immobilization technique reduces the effluent treatment costs and this paper reviews the methods and applications of immobilized enzymes.

INTRODUCTION

An enzyme derived from an organism or cell culture that catalyses metabolic reaction in living organisms and /or substrate conversions in various chemical reactions. The enzymes are the potential catalyst works in mild temperature, pressure, pH, substrate specificity under suitable reaction conditions and for the production of desired products without any intermediate products as contaminations for these advantages enzyme are used in variety of application such as cosmetics, paper industry, textile industry, food industry, pharmaceutical industry, laundry and in detergents etc (Aehle et al., 2007; B M Berna et al., 2006; Costa et al., 2005; Guisan et al., 2009; Sheldon et al.,2007). The biotechnological method of producing enzyme is expensive; hence new methods have been implemented to reduce the cost. The enzymes have various other limitations such as low stability, highly sensitive to the process conditions and these problems can be overcome by the immobilization techniques (Cao et al., 2005; Hernandez et al., 2011; Krajewska et al., 2004). Immobilized enzymes are being used since 1916, when Nelson and Griffin discovered that invertase when absorbed to charcoal has the ability to hydrolyse the sucrose (Nelson et al., 1916). The possibility of immobilized enzyme for its reuse and stability was identified by Grubhofer and Schelth, reported the covalent immobilization of several enzymes (Grubhofer et al., 1953). The repeated assay can be done with the immobilized enzyme which reduces the cost of assay and the reuse of enzyme process is also very simple and it can be attained through ultrafiltration technique. Presently, immobilized proteins/enzymes are used routinely in the medical field, such as in the diagnosis and treatment of various diseases. For example, immobilized antibodies, receptors, or enzymes are used in biosensors and ELISA for the detection of various bioactive substances in the diagnosis of disease states; encapsulated enzymes are also used in bioreactors for the removal of waste metabolites and correction of inborn metabolic deficiency. Moreover, the use of artificial cells as well as the development of controlledrelease drug delivery systems to release encapsulated enzymes or proteins may also be considered a form of immobilized enzyme use.

ENZYME IMMOBILIZATION METHODS

Covalent Binding:

Covalent binding is a conventional method for immobilization; it can be achieved by direct attachment with the enzyme and the material through the covalent linkage (Wong et al., 2008). The covalent linkage is strong and stable and the support material of enzymes includes polyacrylamide, porous glass, agarose and porous silica (Ghous et al., 2001). Covalent method of immobilization is mainly used when a reaction process does not require enzyme in the product, this is the criteria to choose covalent immobilization method. The covalent binding is normally formed between the functional group in the support matrix and the enzyme surface that contains the amino acid residues. The amino acid residues involved in the covalent binding are the sulfhydryl group of cysteine, hydroxyl group of serine and threonine (Chae et al., 1998; Quirk et al., 2001).The attachment between the enzyme and the support material can be achieved either through direct linkage or through the spacer arm. The potentiality of using the spacer arm is that it provides the greater degree of the mobility to the enzymes hence the enzymes show the higher activity when compared to the direct attachment.

Entrapment:
Enzymes are occluded in the synthetic or natural polymeric networks, it is a permeable membrane which allows the substrates and the products to pass, but it retains the enzyme inside the network, the entrapment can be achieved by the gel, fibre entrapping and microencapsulation (Bernfeld et al., 1963).The advantage of entrapment of enzyme immobilization is fast, cheap and mild conditions required for reaction process. The disadvantage is that limitation in mass transfer. The support matrix protects the enzymes from microbial contamination, proteins and enzymes in the micro Environment (Riaz et al., 2009). Microencapsulation method is that the enzyme molecules are capsulated within spherical semipermeable membranes with a selective controlled permeability. This method provides the large surface area between polymeric material and the enzyme. The drawback of this method is inactivation of enzyme during encapsulation.

Adsorption:
This is a simple method of preparing an immobilized enzymes and the materials used for adsorption are activated charcoal, Alumina, Ion exchange resins, this method is cheap and easy for use and the disadvantage is a weak binding force between the carrier and the enzyme (Rosevear et al., 1987). This method comes under carrier bound immobilization and the process of immobilization is reversible. Adsorption is the easiest and oldest immobilization techniques(Brady et al.,2009). The interaction between the enzyme and the surface of the matrix through weak forces by salt linkage, hydrogen bonds, hydrophobic bonds, ionic bonds and van der waals forces. Based on the charges of the matrix and the protein arrangements the strongly bound, but not distorted enzyme will be formed The advantage of enzyme adsorption is minimum activation step and as a result of minimum activation, no reagents required. It is cheap and easy way of immobilization.

Affinity Binding:
The immobilization of enzyme linked to the matrix through the specific interactions. The Two methods are being followed in affinity immobilization. The first method is the activation of the support material which contains the coupled affinity ligand, so that the enzyme will be added. The advantage of this method is the enzyme is not exposed to any harsh chemicals conditions. The second method, the enzyme modified to another molecule which has the ability to bind towards a matrix (Porath et al., 1992).

Metal Linked immobilization:
In metal linked enzyme immobilization, the metal salts are precipitated over the surface of the carriers and it has the potential to bind with the nucleophilic groups on the matrix. The precipitation of the ion on the carrier can be achieved by heating. This method is simple and the activity of the immobilized enzymes is relatively high (30-80%). The carrier and the enzyme can be separated by decreasing the pH, hence it is a reversible process (Yücel et al., 2001). The matrix and the enzyme can be regenerated, by the process.

APPLICATION OF THE IMMOBILIZED ENZYMES

Biomedical Application:
Immobilized enzymes are used in medicine from 1990 (Ofagain et al., 1992; Tischer et al., 1992)., immobilized enzymes are used for diagnosis and treatment of diseases in the medical field. The inborn metabolic deficiency can be overcome by replacing the encapsulated enzymes (i.e, enzymes encapsulated by erythrocytes) instead of waste metabolites, the RBC acts as a carrier for the exogenous enzyme drugs and the enzymes are biocompatable in nature, hence there is no immune response (Johnson et al.,1998).The enzyme encapsulation through the electroporation is a easiest way of immobilization in the biomedical field and it is a reversible process for which enzyme can be regenerated (Lizano et al., 1998).The enzymes when combined with the biomaterials it provides biological and functional systems.

Food industry Application:
In food industry, the purified enzymes are used but during the purification the enzymes will denature. Hence the immobilization technique makes the enzymes stable. The immobilized enzymes are used for the production of syrups. Immobilized beta-galactosidase used for lactose hydrolysis in whey for the production of bakers yeast. The enzyme is linked to porous silica matrix through covalent linkage. This method is not preferably used due to its cost and the other technique developed by Valio in 1980, the enzyme galactosidase was linked to resin (food grade) through cross linking. This method was used for the various purposes such as confectionaries and icecreams .

Biodiesel Production:
Biodiesel is monoalkyl esters of long chain fatty acids. Biodiesel is produced through triglycerides (vegetable oil, animal fat) with esterification of alcohol (methanol, ethanol) in the presence of the catalyst. The production of catalyst is a drawback of high energy requirements, recovery of glycerol and side reaction which may affect the pollution. Hence the biological production of liquid fuel with lipases nowadays has a great consideration with a

rapid improvement.Lipase catalyses the reaction with less energy requirements and mild conditions required. But the production of lipase is of high cost, hence the immobilization of lipase which results in repeated use and stability. The immobilization of lipase includes several methods entrapment, encapsulation, cross linking, adsorption and covalent bonding. Adsortion method of immobilization is widely used in recent years when compared to covalent bond, entrapment and cross linking (Jegannathan et al., 2008). In the biological production of biodiesel the methanol inactivates the the lipase, hence the immobilization method is an advantage for the biodiesel production (Shimada et al., 2002). The low cost of lipase, candida sp as origin is of more industrial use (Tan et al., 2010).

Textile Industry:

The enzymes derived from microbial origin are of great interest in textile industry. The enzymes such as cellulase, amylase, liccase, pectinase, cutinase etc and these are used for various textile applications such asscouring,biopolishing, desizing, denim finishing, treating wools etc. Among these enzymes cellulase has been widely used from the older period to till now. The textile industries now turned to enzyme process instead of using harsh chemical which affects the pollution and cause damage to the fabrics.The processing of fabrics with enzymes requires high temperatures and increased pH, the free enzymes does not able to withstand the extreme conditions. Hence, enzyme immobilization for this process able to withstand at extreme and able to maintains its activity for more than 5-6 cycles. PolyMethyl Methacrylateis linked with cellulose covalently. In this method the nanoparticle is synthesized with cellulase as core particle Endoglucanase is a component of Cellulase enzyme, Endoglucanase is microencapsulated with Arabic Gum is a natural polymer with the biodegradable property is used as a matrix for encapsulation of endoglucanase. Encapsulation of endoglucanase prevented it to retain its activity in the presence of detergents.

CONCLUSION

Enzyme immobilization is widely exploited technique in various industries food industry, pharmaceutical industry, bioremediation, detergent industry, textile industry etc. This method is used due to its technical and economical advantage. Large number of enzymes have been immobilized and used in various large scale processes. This Stabilization method can lower the cost of the enzymes. Enzyme immobilization provides operational stability to enzymes

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