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IMMOBILIZATION OF ENZYMES IN THEIR ECONOMIC REUSE-A REVIEW

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ABSTRACT

Enzyme Immobilization has attracted much attention in recent years with proposed applications besides in industrial process these are basis for making of biotechnological products with applications in diagnostics, bioaffinity chromatography, and biosensors. The term immobilized enzymes refers to enzymes physically confined in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously. Immobilization stabilizes the structure of the enzyme, thereby allowing their applications even under harsh environmental conditions of pH, temperature, thermal denaturation and its economic reuse under stabilized conditions. Here the authors discuss various techniques of immobilization and consequences of enzyme immobilization such as catalytic activity, thermal stability and kinetics of immobilized enzymes. The major components of an immobilized enzyme system are the enzyme, the matrix, and the mode of attachment. The enzyme can be attached to the support by interactions ranging from reversible physical adsorption, stable covalent bonds, ionic linkage or entrapment.

KEYWORDS: Biotechnology, Biosensors, Entrapment, Bio-catalyst, Enzyme immobilization.

I. INTRODUCTION

Immobilization of enzymes helps in their economic reuse and in the development of continuous bioprocesses. Biocatalysts can be immobilized either using the isolated enzymes or the whole cells (Guisan et al.,2009; Nisha et al.,2012). Immobilization often stabilizes structure of the enzymes, thereby allowing their applications even under harsh environmental conditions of pH, temperature, organic solvents, and in the fabrication of biosensors. In the future, development of techniques for the immobilization of multi-enzymes along with cofactor regeneration and retention system can be gainfully exploited in developing biochemical processes involving complex chemical conversions (Brady et al.,2009; Amine et al.,2006). Biotechnology is currently considered as a useful alternative to conventional process technology in industrial and analytical fields. This is mainly because, unlike the chemical catalysts, the biocatalyst has the advantages of accomplishing complex chemical conversions under mild environmental conditions with high specificity and efficiency (Jegannathan et al.,2008; Shimada et al.,2002; D'Orazio et al.,2003). Biological systems help in ingredient substitution, processing aid substitution, more efficient processing, less undesirable products, increased plant capacity, increased product yields, and improved or unique products. The variety of chemical transformations catalyzed by enzymes has made these catalysts a prime target of exploitation by the emerging biotech industries (Yucel et al.,2011; Riaz et al.,2009). Despite these advantages, the use of enzymes in industrial applications has been limited by several factors, mainly the high cost of the enzymes, their instability, and availability in small amounts. Also the enzymes are soluble in aqueous media and it is difficult and expensive to recover them from reactor effluents at the end of the catalytic process. This restricts the use of soluble enzymes to batch operations, followed by disposal of the spent enzyme-containing solvent. Over the last few decades, intense research in the area of enzyme technology has provided many approaches that facilitate their practical applications (Caprio et al.,2000). Among them, the newer technological developments in the field of immobilized biocatalysts can offer the possibility of a wider and more economical exploitation of biocatalysts in industry, waste treatment, medicine, and in the development of bioprocess monitoring devices like the biosensor.

Immobilization means associating the biocatalysts with an insoluble matrix, so that it can be retained in proper reactor geometry for its economic reuse under stabilized conditions. Immobilization thus allows, by essence, to decouple the enzyme location from the flow of the liquid carrying the reagents and products. Immobilization helps in the development of continuous processes allowing more economic organization of the operations,

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automation, decrease of labor, and investment/capacity ratio. Immobilized biocatalysts offer several other advantages; notable among them is the availability of the product in greater purity. Purity of the product is very crucial in food processing and pharmaceutical industry since contamination could cause serious toxicological, sensory, or immunological problems. The other major advantages include greater control over enzymatic reaction as well as high volumetric productivity with lower residence time, which are of great significance in the food industry, especially in the treatment of perishable commodities as well as in other applications involving labile substrates, intermediates or products (Hartmier et al.,1988). Biocatalysts can be immobilized using either the isolated enzymes or the whole cells or cellular organelles. Immobilization of whole cells has been shown to be a better alternative to immobilization of isolated enzymes (Tampion et al.,1987; Bena et al.,2006). Doing so avoids the lengthy and expensive operations of enzyme purification, preserves the enzyme in its natural environment thus protecting it from inactivation either during immobilization or its subsequent use in continuous system. It may also provide a multipurpose catalyst, especially when the process requires the participation of number of enzymes in sequence. The major limitations which may need to be addressed while using such cells are the diffusion of substrate and products through the cell wall, and unwanted side reactions due to the presence of other enzymes. The cells can be immobilized either in a viable or a nonviable form. Immobilized nonviable cell preparations, which are normally obtained by permeabilizing the intact cells, for the expression of intracellular activity are useful for simple processes that require single-enzyme with no requirement for cofactor regeneration, like hydrolysis of sucrose or lactose. On the other hand immobilized viable cells, which serve as 'controlled catalytic biomass', have opened new avenues for continuous fermentation on heterogeneous catalysis basis by serving as self-proliferating biocatalysts (Tanaka et al.,1990; Spahn et al.,2008). Most of the enzymes used at industrial scale are normally the extracellular enzymes produced by the microbes. This has been mainly due to their ease of isolation as crude enzymes from the fermentation broth. Moreover, the extracellular enzymes are more stable to external environmental perturbations compared to the intracellular enzymes. However, over 90% of the enzymes produced by a cell are intracellular. The economic exploitation of these, having a variety of biochemical potentials, has been limited in view of the high cost involved in their isolation. Also, compared to extracellular enzymes, the intracellular enzymes are more labile. Delicate and expensive separation methods are required to release the enzymes undamaged from the cell, and to isolate them. This increases the labor and the cost of the enzyme. These problems could now be obviated by the use of permeabilized cells as a source of enzyme. Permeabilization of the cells removes the barrier for the free diffusion of the substrate/product across the cell membrane, and also empties the cell of most of the small molecular weight cofactors, etc., thus minimizing the unwanted side reactions. Side reactions, which can occur due to the presence of other enzymes in a cell, can also be minimized by inactivating such enzymes prior to or after immobilization (Tischer et al.,1999). Such permeabilized cells, which are often referred to as nonviable or non-growing cells, can be exploited in an immobilized form as a very economical source of intracellular enzyme for simple bioconversions like hydrolysis, isomerization and oxidation reactions that do not need a cofactor-regeneration system. The decision to immobilize cells either in a viable or nonviable form is very important and depends on their ultimate application.

II. ENZYME IMMOBILIZATION METHODS

A large number of techniques and methods are now available for the immobilization of enzymes or cells on a variety of natural and synthetic supports (Hassan Mohamed et al.,2016; Hemalatha et al.,2016). The choice of the support as well as the technique depends on the nature of the enzyme, nature of the substrate and its ultimate application. Therefore, it will not be possible to suggest any universal means of immobilization. It can only be said that the search must continue for matrices which provide facile, secure immobilization with good interaction with substrates, and which conform in shape, size, density and so on to the use for which they are intended. Care has to be taken to select the support materials as well as the reagents used for immobilization, which have GRAS status, particularly when their ultimate applications are in the food processing and pharmaceutical industries. Macromolecular, colloidal, viscous, sticky, dense or particulate food constituents or waste streams also limit the choice of reactor and support geometries. Commercial success has been achieved when support materials have been chosen for their flow properties, low cost, non-toxicity, maximum biocatalysts loading while retaining desirable flow characteristics, operational durability, ease of availability, and ease of immobilization. The variety of techniques and supports investigated for the immobilization, have been reviewed in a number of articles (Sikander ali et al.,2017; Hernandez et al.,2011).

Techniques for immobilization have been broadly classified into four categories, namely entrapment, covalent binding, cross-linking and adsorption. A combination of one or more of these techniques has also been investigated. It must be emphasized that in terms of economy of a process, both the activity and the operational

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stability of the biocatalysts are important. They determine its productivity, which is the activity integrated over the operational time.

Entrapment

Entrapment has been extensively used for the immobilization of cells, but not for enzymes. The major limitation of this technique for the immobilization of enzymes is the possible slow leakage during continuous use in view of the small molecular size compared to the cells. A number of synthetic polymers have also been investigated. Notable among them are the photo-cross linkable resins, polyurethane pre-polymers (Mosbach et al.,1987), and acrylic polymers like polyacrylamide (Mc Gregor et al.,1989). Among these, the most widespread matrix made from monomeric precursors is the polyacrylamide gel. Polyacrylamide may not be a useful support for use in food industry in view of its toxicity, but can have potentials in the treatment of waste and in the fabrication of analytical devices containing biocatalysts. One of the major limitations of entrapment technique is the diffusional limitation as well as the steric hindrance, especially when the macromolecular substrates like starch and proteins are used. Diffusional problems can be minimized by entrapment in fine fibers of cellulose acetate or other synthetic materials [or] by using an open pore matrix (Fukui et al.,1987). Recently, the development of so-called hydrogels and thermo-reactive water-soluble polymers, like the albumin-poly (ethylene glycol) hydrogel have attracted attention in the field of biotechnology. In the area of health care, they offer new avenues for enzyme immobilization. Such gels with a water content of about 96% provide a microenvironment for the immobilized enzyme close to that of the soluble enzyme with minimal diffusional restrictions (Galev et al.,1993).

Covalent binding

Covalent binding is an extensively used technique for the immobilization of enzymes, though it is not a good technique for the immobilization of cells. The functional groups extensively investigated are the amino, carboxyl, and the phenolic group of tyrosine. Enzymes are covalently linked to the support through the functional groups in the enzymes (Wong et al.,2008), which are not essential for the catalytic activity. It is often advisable to carry out the immobilization in the presence of its substrate or a competitive inhibitor so as to protect the active site. The covalent binding should also be optimized so as not to alter its conformational flexibility. Some of these problems however, can be obviated by covalent bonding through the carbohydrate moiety when a glycoprotein is concerned. A number of industrially useful enzymes are glycoprotein's wherein the carbohydrate moiety may not be essential for its activity. In general, functional aldehyde group can be introduced in a glycoprotein by oxidizing the carbohydrate moiety by peroxide oxidation without significantly affecting the enzyme activity (Quirk et al.,2001). The enzyme could then be covalently linked to a support containing an alky amine group through Schiff's base reaction. Enzymes like glucose oxidase, peroxidase, invertase, etc. have been immobilized using this technique (D'Urso et al.,1996). Covalent binding has been extensively investigated using inorganic supports. Enzymes covalently bound to inorganic supports have been used in the industry (Gangadharan et al.,2009). Enzymes have also been bound to synthetic membranes, thus integrating bioconversion and downstream processing. Large-scale processes using such an approach have been demonstrated for the preparation of invert sugar using invertase.

Cross-linking

Biocatalysts can also be immobilized through chemical cross-linking using homo- as well as hetero bi-functional cross-linking agents. Among these, glutaraldehyde which interacts with the amino groups through a base reaction has been extensively used in view of its GRAS status, low cost, high efficiency, and stability (Nakajima et al.,1993). The enzymes or the cells have been normally cross-linked in the presence of an inert protein like gelatin, albumin, and collagen. Studies from our laboratory have shown the use of raw hen egg white as an economic, easily available novel proteonic support rich in lysozyme for the immobilization of enzyme or nonviable cells either in a powder, bead or highly porous foam (Marolia et al.,1993) form, using glutaraldehyde as the cross-linker. The unique feature of this support is the large concentration of lysozyme naturally present in hen egg white which gets co-immobilized, thus imparting the bacteriolytic property to the support (Marolia et al.,1994). Adsorption followed by cross-linking has also been used for the immobilization of enzymes. The technique of cross-linking in the presence of an inert protein can be applied to either enzymes or cells. The technique can also be used for the immobilization of enzymes by cross-linking the cell homogenates. Osmotic stabilization of cellular organelles (D'Souza et al.,1997) or halophilic cells prior to immobilization using cross-linkers has also shown promise.

Adsorption

This is perhaps the simplest of all the techniques and one which does not grossly alter the activity of the bound enzyme. In case of enzymes immobilized through ionic interactions, adsorption and desorption of the enzyme depends on the basicity of the ion-exchanger. Moreover, a dynamic equilibrium is normally observed between

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the adsorbed enzyme and the support which is often affected by pH as well as the ionic strength of the surrounding medium. This property of reversibility of binding has often been used for the economic recovery of the support. This has been successfully adapted in industry for the resolution of racemic mixtures of amino acids, using amino acid acylase. A variety of commercially available ion exchangers have been investigated for this purpose. One of the techniques, which have gained importance more recently, is the use of polyethylenimine for imparting polycationic characteristics to many of the neutral supports based on cellulose or inorganic materials (Cao et al.,2005). Enzymes with low pI, like invertase, urease, glucose oxidase, catalase, and other enzymes (Bahuleker et al.,1991), have been bound through adsorption followed by cross-linking on polyethylenimine-coated supports.

Immobilization of enzymes through hydrophobic interaction has also shown promise. One of the important features of this technique, which is of great significance, is that, unlike ionic binding, hydrophobic interactions are usually stabilized by high ionic concentrations, thus enabling the use of high concentrations of substrates as desired in an industrial process without the fear of desorption. Other types of strong interactive binding techniques have also been reported for the reversible immobilization of enzymes. A typical example is the immobilization of soybean b -amylase on phenyl-boronate agarose, which can be reversed for the recovery of the support using sorbitol. Varieties of bio-specific interactions have also been investigated for the reversible immobilization of enzymes by adsorption. Enzymes like acetyl chorine esterase, ascorbic acid oxidase, invertase, peroxidase, glucose oxidase, etc. have been immobilized by bio-specific-reversible immobilization on lectin-bound supports and invertase, using polyclonal anti-invertase antibodies (Saleemudin et al.,1992).

Traditional enzyme immobilization procedures involve isolation of the enzyme, followed by use of several steps for the immobilization. Costs of enzyme purification, the immobilization procedure, bioreactor operational stability, and bioreactor regeneration are the major factors that determine the cost of a bioreactor process. Development of techniques for the simultaneous isolation and immobilization of enzymes from crude extracts has obviated these problems. Some typical examples include the immobilization of a streptavidin-b -galactosidase fusion protein expressed in *Escherichia coli* and bioselectively adsorbed from a crude cell lysate to biotin which is covalently immobilized on controlled pore glass (Jafri et al.,1995), and the simultaneous purification and immobilization of D-amino acid oxidase from *Trigonopsis variabilis* cell lysate adsorbed on phenyl sepharose.

Techniques for the adhesion of whole cells on polymeric surfaces are also currently gaining considerable importance. The major advantage for the cells immobilized through adhesion is reduction or elimination of the mass transfer problems associated with the commonly used gel entrapment method. The technique of immobilization usually being followed is the microbial colonization by recycling of the cell suspension along with nutrients, such that a bio-film is gradually formed. This often results in the immobilization of cells in a viable form for use in heterogeneous fermentations (Huang et al.,1996). Useful techniques have been developed also for the immobilization of nonviable cells to be used as an enzyme source for simple chemical conversions. Notable among them include treating the cells or the support with trivalent metal ions like Al^{3+} or Fe^{3+} or charged colloidal particles, and use of polycationic polymers like chitosan. Novel techniques have been developed to adhere cells strongly on a variety of polymeric surfaces including glass, cotton cloth, cotton threads, and other synthetic and inorganic surfaces using polyethylenimine. This technique has also been recently used for the simultaneous filtration and immobilization of cells from a flowing suspension, thus integrating downstream processing with bio-processing. This technique may have future potentials for the immobilization of cells for food applications. Polyethylenimine is nontoxic and the United States of America Food and Drug Administration have permitted its use as a direct food additive under the Food Drug and Cosmetic Act. (Oh et al.,2007).

Table. 1. Comparison of different enzyme immobilization techniques

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No

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Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

III. KINETICS OFIMMOBILIZED ENZYMES

The kinetic behavior of a bound enzyme can differ significantly from that of the same enzyme in free solution. The properties of an enzyme can be modified by suitable choice of the immobilization protocol, whereas the same method may have appreciably different effects on different enzymes (Safonov et al.,1978). These changes may be due to conformational alterations within the enzyme due to the immobilization procedure, or the presence and nature of the immobilization support

Immobilization can greatly affect the stability of an enzyme. If the immobilization process introduces any strain into the enzyme, this is likely to encourage the inactivation of the enzymes under denaturing conditions (e.g. higher temperatures or extremes of pH). However where there is an unstrained multipoint binding between the enzyme and the support, substantial stabilization may occur. Additional stabilization is derived by preventing the enzyme molecules from interacting with each other and the protection that immobilization affords towards proteolytic and microbiological attack. This latter effect is due to a combination of diffusional difficulties and the camouflage to enzymic attack produced by the structural alterations. In order to achieve maximum stabilization of the enzymes, the surfaces of the enzyme and support should be complementary with the formation of many unstrained covalent or non-covalent interactions. Often, however, this factor must be balanced against others, such as the cost of the process, the need for a specific support material, and ensuring that the substrates are not sterically hindered from diffusing to the active site of the immobilized enzyme in order to react at a sufficient rate (Alejandro et al.,2003).

The kinetic constants (e.g. K_m, V_{max}) of enzymes may be altered by the process of immobilization due to internal structural changes and restricted access to the active site. Thus, the intrinsic specificity ($k_/K_m$) of such enzymes may well be changed relative to the soluble enzyme. An example of this involves trypsin where the freely soluble enzyme hydrolyses fifteen peptide bonds in the protein pepsinogen but the immobilized enzyme hydrolyses only ten. The apparent value of these kinetic parameters, when determined experimentally, may differ from the intrinsic values. This may be due to changes in the properties of the solution in the immediate vicinity of the immobilized enzyme, or the effects of molecular diffusion within the local environment. The relationship between these parameters is shown below.

Intrinsic parameters of the soluble enzyme

↓

Intrinsic parameters of the immobilized enzyme

↓

Apparent parameters due to partition and diffusion

IV. IMMOBILIZATION OF ENZYMES FOR THE FABRICATION OF BIOSENSORS

Various techniques have been used for the immobilization of biocatalyst for biosensor applications (Mulchandani et al.,1998). The choice of the support and the technique for the preparation of membranes has been dictated by the low diffusional resistance of the membrane coupled with its ability to incorporate optimal amount of enzyme per unit area. In this respect, stable membranes have been prepared by binding glucose oxidase to cheese cloth in the fabrication of a glucose biosensor. Enzymes entrapped inside the reversed micelle have also shown promise in the fabrication of biosensors (Gomesh et al.,2004). Cross-linked enzyme crystals (CLCs) described above provides their own support and so achieves enzyme concentration close to the theoretical packing limit in excess of even highly concentrated enzyme solutions. In view of this, CLCs are particularly attractive in biosensor applications where the largest possible signal per unit volume is often critical (Vianello et al.,2006).

Sensors based on small transducer or thinner enzyme immobilized membranes (miniature biosensors) are also emerging. The development of molecular devices incorporating a sophisticated and highly organized biological information processing function is a long-term goal of bioelectronics. For this purpose, it is necessary in the future to develop suitable methods for micro-immobilizing the proteins/enzymes into an organized array/pattern, as well as designing molecular structures capable of performing the required function. A typical example is the micro-immobilization of proteins into organized patterns on a silicon wafer based on a specific binding reaction between strepatavidin and biotin combined with photolithography techniques (Nguyen et al.,1997). Immobilized

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enzymes have also been used for various other analytical purposes. A recent development has been in obtaining a stable dry immobilized enzyme, like acetylcholine esterase, on polystyrene micro-titration plates for mass screening of its inhibitors in water and biological fluids (Murai et al.,1997).

V. INDUSTRIAL APPLICATIONS OF IMMOBILIZED ENZYMES

The first industrial use of an immobilized enzyme is amino acid acylase by Tanabe Seiyaku Company, Japan, for the resolution of racemic mixtures of chemically synthesized amino acids. Amino acid acylase catalyses the deacetylation of the L forms of the *N*-acetyl amino acids leaving unaltered the *N*-acetyl-d amino acid that can be easily separated, racemized and recycled. Some of the immobilized preparations used for this purpose include enzyme immobilized by ionic binding to DEAE-sephadex and the enzyme entrapped as micro droplets of its aqueous solution into fibers of cellulose triacetate by means of fiber wet spinning developed by Snam Progetti. Rohm GmbH has immobilized this enzyme on macro porous beads made of flexi glass-like material (Yu et al.,1982).

By far, the most important application of immobilized enzymes in industry is for the conversion of glucose syrups to high fructose syrups by the enzyme glucose isomerase (Sharma et al.,1980). It is evident that most of the commercial preparations use either the adsorption or the cross-linking technique. Application of glucose isomerase technology has gained considerable importance, especially in non-tropical countries that have abundant starch raw material. Unlike these countries, in tropical countries like India, where sugarcane cultivation is abundant, the high fructose syrups can be obtained by a simpler process of hydrolysis of sucrose using invertase. Compared to sucrose, invert sugar has a higher humectancy, higher solubility and osmotic pressure. Historically, invertase is perhaps the first reported enzyme in an immobilized form. A large number of immobilized invertase systems have been reported (DiCosimo et al.,2013).

One of the major applications of immobilized biocatalysts in dairy industry is in the preparation of lactose-hydrolyzed milk and whey, using b -galactosidase. A large population of lactose intolerant can consume lactose-hydrolyzed milk. This is of great significance in a country like India where lactose intolerance is quite prevalent (Konechy et al.,1984). Lactose hydrolysis also enhances the sweetness and solubility of the sugars, and can find future potentials in preparation of a variety of dairy products. Lactose-hydrolyzed whey may be used as a component of whey-based beverages, leavening agents, feed stuffs, or may be fermented to produce ethanol and yeast, thus converting an inexpensive byproduct into a highly nutritious, good quality food ingredient (Kubal et al.,1990). The first company to commercially hydrolyse lactose in milk by immobilized lactase was Centrale del Latte of Milan, Italy, utilizing the Snamprogetti technology. The process makes use of a neutral lactase from yeast entrapped in synthetic fibres (Sprossler et al.,1983). Specialist Dairy Ingredients, a joint venture between the Milk Marketing Board of England and Wales and Corning, had set up an immobilized b -galactosidase plant in North Wales for the production of lactose-hydrolyzed whey. Unlike the milk, the acidic b -galactosidase of fungal origin has been used for this purpose. A major problem in the large-scale continuous processing of milk using immobilized enzyme is the microbial contamination which has necessitated the introduction of intermittent sanitation steps. A co-immobilizate obtained by binding of glucose oxidase on the microbial cell wall using Con A has been used to minimize the bacterial contamination during the continuous hydrolysis of lactose by the initiation of the natural lacto-peroxidase system in milk (D'Souza et al.,1989).

The immobilized enzymes are widely used in medicine as food additive and in food industry like backing (Gomes et al.,2012). One of the major applications of immobilized enzymes in pharmaceutical industry is the production of 6-aminopenicillanic acid (6-APA) by the deacylation of the side chain in either penicillin G or V, using penicillin acylase (penicillin amidase). More than 50% of 6-APA produced today is enzymatically using the immobilized route. One of the major reasons for its success is in obtaining a purer product, thereby minimizing the purification costs. The first setting up of industrial process for the production of 6-APA was in 1970s simultaneously by Squibb (USA), Astra (Sweden) and Riga Biochemical Plant (USSR). Currently, most of the pharmaceutical giants make use of this technology. A number of immobilized systems have been patented or commercially produced for penicillin acylase which make use of a variety of techniques either using the isolated enzyme or the whole cells (Wiseman et al.,1985; Poulsen et al.,1985). This is also one of the major applications of the immobilized enzyme technology in India. Similar approach has also been used for the production of 7-aminodeacetoxy-cephalosporanic acid, an intermediate in the production of semi synthetic cephalosporin.

Biodiesel has ability to replace fossil fuels to prevent environmental hazards. Apart from that biodiesel is renewable, biodegradable and non-toxic fuel (Tiwari et al.,2007). It has mono-alkyl esters of long chain fatty acids. Biodiesel is produced through triglycerides with esterification of alcohol in the presence of the catalyst, which can easily be purified. It is also produced from vegetable oils, fats and micro algal oil; immobilized

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enzymes can be used as catalyst for biodiesel production that reduces the cost of production by repeated use of catalyst. Lipases used for biodiesel production are immobilized by various supports (Yagiz et al.,2007). In the biological production of biodiesel the methanol inactivates the enzyme lipase; hence the immobilization method is an advantageous for the industrial use (Tan et al.,2010).

VI. CONCLUSION

An important factor determining the use of enzymes in a technological process is their expense. Several hundred enzymes are commercially available at prices of about £1 mg^{-1}, although some are much cheaper and many are much more expensive. As enzymes are catalytic molecules, they are not directly used up by the processes in which they are used. Their high initial cost, therefore, should only be incidental to their use. However due to denaturation, they do lose activity with time. If possible, they should be stabilized against denaturation and utilized in an efficient manner. When they are used in a soluble form, they retain some activity after the reaction which cannot be economically recovered for re-use and is generally wasted. This activity residue remains to contaminate the product and its removal may involve extra purification costs. In order to eliminate this wastage, and give an improved productivity, simple and economic methods must be used which enable the separation of the enzyme from the reaction product. The easiest way of achieving this is by separating the enzyme and product during the reaction using a two-phase system; one phase containing the enzyme and the other phase containing the product. The enzyme is imprisoned within its phase allowing its re-use or continuous use but preventing it from contaminating the product; other molecules, including the reactants, are able to move freely between the two phases. This is known as immobilization and may be achieved by fixing the enzyme to, or within, some other material. The term 'immobilization' does not necessarily mean that the enzyme cannot move freely within its particular phase, although this is often the case. A wide variety of insoluble materials, also known as substrates (not to be confused with the enzymes' reactants), may be used to immobilize the enzymes by making them insoluble. These are usually inert polymeric or inorganic matrices.

Immobilization of enzymes often incurs an additional expense and is only undertaken if there is a sound economic or process advantage in the use of the immobilized, rather than free (soluble), enzymes. The most important benefit derived from immobilization is the easy separation of the enzyme from the products of the catalyzed reaction. This prevents the enzyme contaminating the product, minimizing downstream processing costs and possible effluent handling problems, particularly if the enzyme is noticeably toxic or antigenic. It also allows continuous processes to be practicable, with a considerable saving in enzyme, labor and overhead costs. Immobilization often affects the stability and activity of the enzyme, but conditions are usually available where these properties are little changed or even enhanced. The productivity of an enzyme, so immobilized, is greatly increased as it may be more fully used at higher substrate concentrations for longer periods than the free enzyme.

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