Enzymes Immobilization: An Overview Of Techniques, Support Materials And Its Applications

Dr. Sikander Ali, Wajeeha Zafar, Sammia Shafiq, Mehvish Manzoor

Abstract: With the increasing demands of world biotechnology industries, there is a need to enhance the productivity, reaction stability, reusability and shelf life of enzymes. So, novel techniques are required to facilitate large scale and economic formulations. Enzyme immobilization is done in order to meet all the challenges to enzyme activity. It provides an excellent base for increasing availability of enzyme to the substrate with greater turn over a considerable period of time. This can be done by entrapment, support binding, cross linking of enzyme crystals, etc. Several natural and synthetic support materials are used for the immobilization of enzymes. These increase the efficiency of an enzyme to a great extent. Nowadays, immobilized enzymes are preferred over their free counterpart due to their prolonged availability. Immobilized enzymes are widely used in pharmaceutical industries, cosmetic industries, food processing, biofuel production and many other sectors.

Index Terms: enzyme immobilization, techniques, biotransformation, support binding, biosensors, applications**.**

1 INTRODUCTION

Enzymes are the biocatalysts that catalyze many chemical and biochemical reactions. These biocatalysts are universally present in plants, animals and microbial cells. Enzymes are widely used in food industries like baking (Gomes *et al*., 2012), dairy products, starch conversion and beverage processing (Dicosimo*et al.,* 2013). Enzymes are also widely used in textile industries and give a desirable texture to end product. The use of enzymes is also a crucial part of processing in paper and pulp making and detergents industries (Rao*et al.,* 2009). Many industries such as health care & pharmaceuticals and chemical manufacturing are utilizing the catalytic nature of enzymes to enhance their output.Enzymes are also used in waste management sector for purification of polluted water and treatment of solid garbage (Tonini and Astrup, 2011). In the past few years the worth of enzymes in manufacture of biofuels from living/organic matter has increased tremendously. But, sometimes these properties of enzymes and their applications in industry are affected due to their shelflife, recovery and reusability. Enzymes can be immobilized in order to overcome these problems. Biocatalysts are widely used in industrial sectors due to the fact that they can be easily produced, are highly specific in their action and are environmental friendly.

- *Dr. Sikander Ali is working as Associate Professor at Government College University, Pakistan E-mail: alisbiotech@yahoo.com*
- *Wajeeha Zafar is student at Institute of Industrial Biotechnology Government College University, Pakistan. E-mail: wajeehazafar7@gmail.com*
- *Sammia Shafiq is student at Institute of Industrial Biotechnology Government College University, Pakistan. E-mail: sammia239@gmail.com*
- *Mehvish Manzoor is student at Institute of Industrial Biotechnology Government College University, Pakistan. E-mail: mahvish_manzoor@yahoo.com*

On commercial scale utilization the reusability factor of these biocatalysts becomes crucial, and if they fail to do so then they would no longer be economic. To maintain the stability of these biocatalysts during a biochemical reaction is a challenging task. So in order to tackle this challenge, enzymes are made efficient and used again and again by the process of immobilization, despite of the fact that it is a costly method.Enzymes are attached to a specific support material other than substrate or product to make them immobilized. Materials used as support materials for immobilization of enzymes are mostly inert polymers or inorganic compounds. An ideal support material should be affordable and must be inert, physically strong and regenerate able (Singh, 2009). Enzymes can be immobilized by many procedures and some factors govern the functioning of immobilized enzymes.

2 TYPES OF IMMOBILIZATION

Basically there are methods to immobilize an enzyme. These are discussed as under;

2.1 Support binding

In this method the enzyme is bound to a support/carrier material. This can be done by physical, ionic or covalent interactions. However, it is difficult for enzyme to keep fixed to the carrier under industrial conditions of high reactant and product concentrations and high ionic strength if these two are bound by physical interactions(such as hydrophobic and van der Waals interactions). However, ionic interaction is normally stronger than physical binding and covalent binding is even stronger. It has a benefit as it prevents the leakage of enzyme from the surface of support material.

2.2 Entrapment of enzyme by inclusion

This method involves the entrapment of enzyme in a gel lattice (polymer network) such as organic polymer or sol gel.The physical barriersare however not too strong to prevent enzyme leakage entirely. So, covalent binding is also done in addition to entrapment. There is no clear cut

difference between entrapment and support binding, however entrapment involves the the synthesis of the polymeric meshwork catalyzed by the enzyme, whereas in support binding, the enzyme is attached onto a prefabricated support.

2.3 Cross-linking of enzyme aggregates or crystals

This is relatively an advance method of immobilizing an enzyme. This method involves a strategy in which enzymes are immobilized free of any carrier material, i.e. by cross linking enzyme crystals (CLECs), and cross-linking enzyme aggregates (CLEAs) (Sheldon *et al.,* 2006). This method has many advantages such as increased activity of enzyme, high stability and the cost of production and processing is lowered because here no carrier is required.

3 TECHNIQUES USED FOR IMMOBILIZATION

3.1 Adsorption

Enzyme adsorption can be done by hydrophobic bonding or salt linkages between enzyme and carrier materials. For this purpose support can be dipped in enzyme solution for enough time to let it physically adsorbed. There is another way to adsorb enzyme by drying it on the surface of an electrode.This protects enzyme against aggregate formation, denaturation and hydrophobic interactions.Adsorption protects enzymes against aggregation, proteolysis and interaction with hydrophobic interfaces (Spahn and Minteer, 2008).Coconut fibers are able to hold a good amount of water and have a highcation exchange ability and are environmental friendly, so they are used by researchers to immobilize enzyme. Many molecular sieves having sialons on their pore walls are successfully used by scientists for enzyme immobilization. These facilitate us to immobilize an enzyme by hydrogen bonding. Different chemical modifications in currently used support materials can result in even better immobilization. Immobilization of lipase extracted from *Yarrowialipolytica*was done by using octyl-agarose and octadecyl-sepabeads and it resulted in more stability and gave higher yields. Octadecyl-sepabeads are hydrophobic in nature and increase the affinity between enzyme and support material (Cunha *et al.*, 2008). Immobilization of lipase from *Candida rugosa* on biodegradable polymer (3-hydroxybutyrate-co-hydroxyvalerate), resulted in 94% residual activity (Cabrera-Padilla *et al.,* 2011).Ethical issues and production costs can be lowered by using environmental friendly support materials for immobilizing enzymes.Use of biocompatible support material such as mesoporous silica nanoparticles (MSNs) for immobilization results in long-term durability and efficiency of enzyme (Popat*et al*., 2011).

3.2 Covalent binding

Enzymes have different side chain amino acid residues and have reactivity based on different functional groups which is utilized for covalent binding of enzymes to support materials (D'Souza, 1998; Singh, 2009). Silanized silica gel carriers with removed unreacted aldehyde groups are covalently bound to enzymes resulting in highly stable and hyperactive biocatalysts (Lee et al., 2006). Enzymes when covalently bound to mesoporous silica and chitosan, lead to an increase in the half life and heat endurance of enzymes. Covalent binding of electrospunnanofibers to enzyme lead to an increase in surface area and porosity and thus residual activity of enzymes is increased tremendously. Alchohol dehydrogenase is cross linked to attapulgitenanofibres, increasing the enzymes' thermal stability. Different immobilized enzymes obtained by covalent binding to different supports are used in medicines and drugs manufacturing because of their enhanced stability and reusability.

3.3 Affinity immobilization

In affinity immobilization, specific nature of enzyme to its support under different physiological conditions is utilized. Affinity immobilization can be achieved by two ways: either precoupling the template to a ligand to which it has affinity for or conjugating the enzyme to a substance that develops affinity for the template (Sardar*et al.,* 2000).Purification of enzymes is also done by affinity immobilization. Support materials like alkali stable chitosan- coated porous silica beads and agarose-linked multilayered concanavalinA harbor larger amounts of enzymes that result in an increase in stability and efficiency of enzyme (Shi *et al.,* 2003; Sardar and Gupta, 2005).

3.4 Entrapment

Entrapment involves the detention of enzymes in gels or fibers by covalent or non-covalent interactions (Singh, 2009).Alginate–gelatin– calcium crossbreed carriers prevent enzyme leakage, provide increased mechanical stability and efficient encapsulation is achieved. Use of nanoparticles such as electrospunnanofibers as a support material for entrapment have revolutionized the field of enzyme immobilization with their wide-ranging applications in the field of chemistry, biomedicine, biosensors and biofuels (Dai and Xia, 2006; Wang *et al.,* 2009). It has been reported that lipase of Candida rugosa when entrapped in chitosan resulted in prevention of friability and leaching and augmentation of entrapment efficiency and enzyme activity. In addition this support is also non-toxic, biocompatible and has a great affinity to proteins because of its hydrophilic nature.Use of mesoporous silica entrapment support material has also been reported because of its high surface area, uniform pore distribution and high adsorption capacity. Carrageenan entrapped lipases are considered as highly thermostable and organic solvent tolerant enzymes (Jegannathan*et al.,* 2010).

4 DIFFERENT SUPPORTS MATERIALS USED FOR ENZYME IMMOBILIZATION

For the preparation of supported enzyme properties such as enzyme and its supportive material both are important, through both of these properties immobilized enzyme can be prepared. Immobilized enzymes have their own specific biochemical, chemical, Kinetic and mechanical properties. Different type of polymeric materials are used for this purpose such as synthetic organic polymers, biopolymers, smart polymers and some kinds of other supports such as hydrogels and inorganic supports.

4.1 Synthetic organic support materials

Eupergit C which is a type of acrylic resins used as supportive material. This polymer is highly stable both chemically and mechanically because it does not shrink and

swell under highly drastic conditions at a wide range 0 to 14. Eupergit C is a polymer which is porous in nature having pore size 25nm and particle size 170 micro metre (Katchalski-Katzir*et al*., 2000). It has components such as "N,N '-methylene-bi (methacrylamide), glycidyl methacrylate, allylglycidyl ether and methacrylamide". It makes covalent interaction with the amino group of enzyme through its oxirane component and this is most stable at pH 1 to 12 (Figure 2).

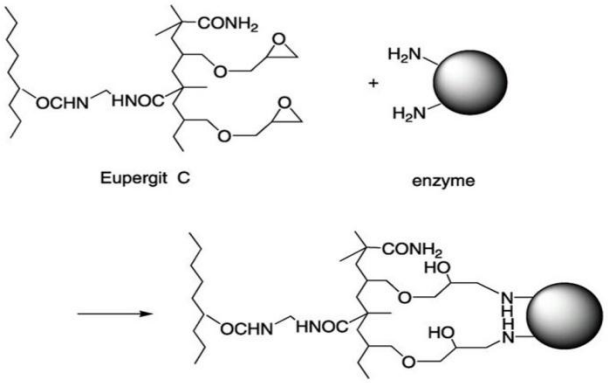

Figure 1: *Immobilization of enzyme on EupergitC.*

Other groups present in the support can bind to the protein to prevent this binding capping of epoxy group is performs to make them inactive. Many reagents are used for capping purpose included "mercaptoethanol, ethanolamine, glycine, etc". Oxirane of high densities groups present in the Eupergit C make enzyme immobilized at different its surfaces. Immobilization of enzyme through Eupergit C above mechanism makes it most successful at industrial level(Katchalski-Katzir*et al*., 2000; Wegman*et al*., 2001Kallenberg*et al.,*2005). In the same case Amberlite XAD-7 is a porous acrylic resins which can be used to immobilize the enzymes through covalent attachment and binding e.g*C. antarctica* lipase is an enzyme widely used is immobilized through Novozym 435 containing macro porous acrylic resins which has abilityto absorb enzymes(Kirk and Christensen 2002). This procedure has a drawback because enzyme has no covalent bindings so in aqueous it can dissolve easily. Lipases taken from other microorganisms e.g "*Humicolalanuginosa*, *Candida Antarctica* and *Rhizomucormiehei"*along with respective support material vary in their hydrophobicity (Petkar*et al*., 2006).Supports having hydrophobic nature are more suitable to immobilized hydrophobic lipase.

4.2 Biopolymers

A wide range of biopolymers such as polysaccharides including cellulose, starch, chitosan(Krajewska, 2004) agarose and some protein in nature such as albumin, gelatin are mainly used as supportive material for enzyme immobilization. At industrial level the immobilized enzyme used for biotransformation(Chibata*et al*., 1992; Tosa*et al.,* 1996). It was used more than 40 years back, by "resolution of racemic acylamino acids" to produce L-amino acids through aminoacylase from *Aspergillusoryzae*. By applying "ionic adsorption" technique on DEAE-Sephadex the enzyme is immobilize. Cellulose is modified by DEAE-Sephadex with "diethylaminoethyl functionalities" and in fixed bed reactor this process is carried out through continuous operation.This process has a wide range of applications e.g. "in the immobilization of a recombinant epoxide hydrolase obtained from *Aspergillusniger"* (Karboune*et al.*, 2005). Through this process retention of activity is observed upto 70% in the resolution of p-chlorostyrene oxide. The immobilized biocatalyst also show maximum activity at high substrate concentrations "(306g/L)" it has recycling ability upto 7 times but carried slowly as compared to free enzyme.

4.3 Hydrogels

There is another way by which enzyme can be immobilized in non-aqueous media for this purpose hydrogels and cryogels are use which can be natural and synthetic. In spite of enzymes whole cells can also be immobilized by polyvinyl alcohol (PVA) cryogels. PVA can be prepared through freeze drying method(Lozinsky*et al*., 2003). On other hand a good and mechanically stable quality of PVA can be prepared by "partial drying of afforded lens-shaped hydrogels (Lentikats) at room temperature". They can be easily separated "(diameter3–5 mm and thickness 200–400mm)" and degraded(Jekel*et al*., 1998)Lentikats as whole cell biocatalyst used to immobilize the whole cells of "Rhodococcus equiA4" through entrapment technique (Figure 3).The whole cell of "*Rhodococcusequi*A4 have nitrile hydratase and amidase activities" (Kubac*et al*., 2006). Though the enzymes with their smaller size can diffuse into the gel matrix and leached into the aqueous media easily. For free enzymes in order to entrap them their size must e increased by cross-linking or any other technique. On other side the immobilization of free enzymes in "PVA hydrogels" organic media is used because it does not allow the enzyme to leach out from the gel matrix.

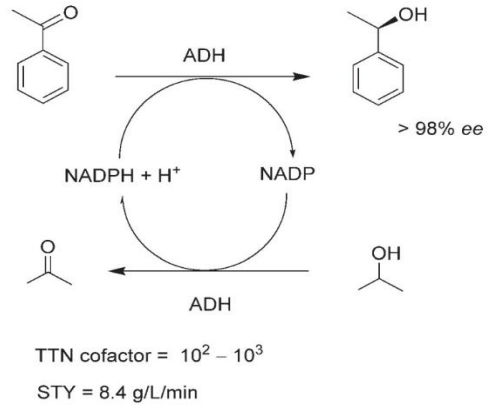

Figure 2: *Alcohol dehaydrogense in Lentikat*

In this process cofactors also used for the immobilization of certain enzymes such as an enzyme alcohol "dehydrogenase (EC 1.1.1.1)" from *Lactobacillus kefir* NADP is used in PVA beads (Metrangolo-Ruiz De Temino*et al*., 2005).Immobilized enzymes have thermal stability with long lasting effects towards organic solvents under specific reaction conditions. There is a new method by which the size of enzyme can be increased to make a complex with polyelectrolyte because enzymes have ampholytic character on the basis of the pH of medium in which they

are present either polycationis and polyanions due to this behavior they form complexes with “ampholytic character”. Dautzenberg and his colleagues used first time this principle for the immobilization of amyloglucosidase (EC 3.2.1.3) by making its complex with sodium polystyrenesulfonate (PSS) it retains its activity upto 45% and over 5 cycles there is no loss in its activity.

4.4 Inorganic supports

Al, silica, (Petri *et al*., 2005)porous crystalline substances and zeolites (Diaz *et al.,* 1996; Yan *et al*., 2002) are some inorganic solids used in the immobilization of enzymes.. Silica granulation is one of the cheapest processes to immobilize the enzyme. It is used for washing purpose because of its strong detergent effects during washing. CaLB lipase is immobilized on silica granules by this granulation technique. Initially, absorb the lipase into the silica powder (agglomeration)(Kirk andChristensen, 2002). They has specific composition can absorb only in organic media and in aqueous media they disintegrates and desorbed. When water is removed these granules synthesize ester under vacuum. CaLB silica granules have activity similar to the Novozym 435 during the synthesis of synthesis of the “skin emollient, isopropyl myristate”. Mesoporous silica’s also known as nanosilica as support material has several advantages as they have “volume (ca. 1mLg^{-}1), diameters of pores (2–40 nm) and surface areas (300–1500 m2g^{-}1)” they are also highly stable under a wide range of different temperature (Figure 4).

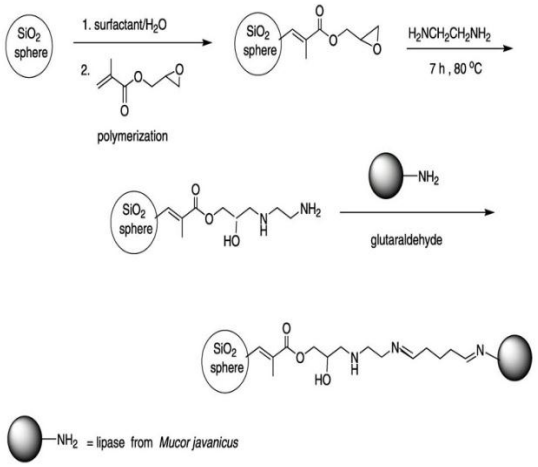

Figure 3: *Immobilization of a lipase on silica nanoparticles*

These support materials have larger pore size so that they can uptake smaller size enzymes into them easily. Either the enzyme can beplaced on the surface of support material or into the surface immobilization through calcined and non-calcined material can be determined. If calcinated material absorbs the enzyme then it will be inside otherwise it will stay on the surface of support. Immobilization of “a-chymotrypsin (EC 3.4.21.2) on sol-gel glass” which is mesoporous in nature through covalent binding involved a mechanism “during reaction modifications at the surface hydroxy groups with 3,3,3-trimethoxypropanal this increase the half-life of immobilized enzyme thousand times more than free enzyme”.

4.4.1 Protein-coated microcrystals technique:

(PCMCs) is a type of inorganic supports used for immobilization purpose. This is used for lyophilized enzyme by adding lyoprotectants and other inorganic salts (Kreiner*et al*., 2001).Protein-coated microcrystals is synthesized by mixing “the aqueous solution of enzyme with any salt such as potassium sulphate, amino acid or any sugar” then it is mixed vigorously and added drop wise in solvent like isopropyl alcohol in result enzyme in the form of micro sized crystals are formed. This process has the significance because this dehydrates the enzyme, lowers the denaturation of enzyme and makes the conformation more active. Such immobilized enzymes can be stored then in organic solvents. This technique is useful for enzymes such as “lipases, oxidoreductases, catalase, soybean peroxidase and horse radish peroxidase”(Kreiner*et al*., 2004).

4.5 Smart polymers

Smart polymers are stimulus responsive which make them unique from other supports. Smart polymer helps in enzyme immobilization through covalent attachment. These polymers are highly responsive during any changes in environment by changing their confirmation. Environment changes included “pH, temperature, ionic strength etc”(Galaev and Mattiasson, 1999; Galaev and Mattiasson, 2004; Roy *et al*., 2004; Roy and Gupta, 2006). Poly-N-isopropylacrylamide (polyNIPAM) is a biocompatible polymer. Poly-N-isopropylacrylamide shows “critical solution temperature” but below the temperature upto 32^{o}C it dissolves into the aqueous media and above this precipitation of immobilized enzymes occurs under soluble conditions this can be used for the process of biotransformation. It leads to minimize “diffusion limitations and loss of activity” which prevent the changes in the conformation of immobilized enzyme support surface. This has an advantage when temperature becomes higher than LCST enzyme precipitates and reaction stop (Figure 5). Enzyme-polyNIPAM conjugates can be produced by two processes as following:

1. **Copolymerization with NIPAM:**
In this mechanism polymerized vinyl groups are introduced into the enzymes.
2. **By reacting NH_3 group with enzyme**:
The enzyme either with a “copolymer of NIPAM containing reactive ester groups or the homopolymer with an N-succinimide ester functions as terminal group”.

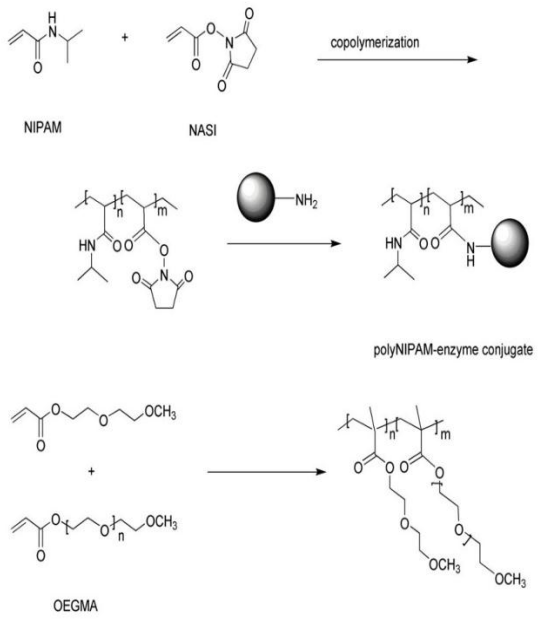

Figure4: *Thermoresponsive polymers for enzyme immobilization*

For example: NIPAM containing highly reactive functional group used for immobilization of an enzyme penicillin G amidase (PA) through condensation process and the resulting enzyme shows hydrolytic activity such as free enzymes (Ivanov*et al*., 2003).

5 APPLICATIONS OF IMMOBILIZEDENZYMES

Immobilized enzymes are used in various fields such as in medicine, antibiotic production, food industry, biodiesel production, bioremediation, drug metabolism etc.

5.1 Use of immobilized enzymes as a biosensor

The devices i.e. electrical, mechanical, chemical which is used to detect biological species is called Biosensors. Various enzymes are used as biosensors. The ideal biosensors respond to the low concentration of analytesand also ability to differentiate among the biological species according to reorganization molecules immobilized to the surface. Biosensor as biomarkers used to detect food pathogens (Leung *et al.,* 2007). Biosensors are extremely specific, highly sensitive and low cost and environmental friendly. Due to all these properties biosensors are most suitable for the detection of biological components. Immobilized enzymes used as biosensor are of great importance due to inability of loss of enzymes, stability is maintained, shelf life of biosensors become high, enzymes respond in less time and it also offers disposable products that is used in stationery (Amine *et al.,* 2006). Immobilized enzymes used as biosensor are the indicators of organophosphorus pesticides, heavy metals, organophosphorus chlorine, glycoalkoids worked on the principle of enzyme inhibition. Normal enzymes function is mostly destroyed by this system so we use immobilized enzymes for this function (Ivanov*et al.,*2003a, b). Malistera and Guascito (2005) explained the application of immobilized glucose oxidase by electropolymerization as a biosensor which is used for the indication of heavy metals. Immobilized urease is used for the indication of mercury. Urease is entrapped between PVC and cellulose triacetate layers on the surface of iridium oxides electrodes. Immobilized polyphenol oxidase is also used as a biosensor. Biosensors are used for determination of atrazine concentration. Biosensors are also used as an indicator of pesticides. (EI.Kaoutit*et al*.,2004).

5.2 Uses in medical field

Several immobilized proteins, antibodies and many enzymes are used for several purposed in medicinal field. Immobilized enzymes play a vital role in medical fields such as for the diagnosis of various diseases. Enzymes based electrodes are used in medicine which is major application of immobilized enzymes. Various immobilized enzymes used as biosensors in medical field due to the low cost, more stability and high reliability (D'Orazio, 2003). Biosensors are used to monitor the metabolites which are indirect indication of the disease and various biological disorders. This method is being studied more to replace diagnostic tools which nowadays being used in labs such as glucose test strips and mass spectroscopy etc. Biosensors based on immobilized enzymes are used for detection of wide range of complex compounds in various samples i.e. blood, serum and urine etc. (Malhotra and Chaubey, 2003). Nowadays, the use of Surface Plasmon Resonance has become popular in health science research and drug discovery. For the low cost and sensitive biosensors SRP are geared toward development. Nanotechnology plays great role in the design of optimized and enhanced surfaces for SPR (Hoa*et al*., 2007).

5.3 Use in Antibiotic production

Various methods are used for antibiotic production but scientists are searching for most established and fine tuned process for antibiotic production such as Beta lactum. Beta lactumacrylase are the enzymes used for the hydrolysis of Penicillin G and cephalosporins C. Penicillin G acrylase, an immobilized enzyme is used in aqueous medium. Mutated acrylases are used in fermentation and semi synthetic beta lactum antibiotics synthesis (Giordano, 2003).Cephalix production from immobilized Penicillin G acrylase is widely studied. 85 percent of cephalix is produced by the conversion of 7-amino-3-Deacetoxy- Cephalosporanic acid to cephalix under optimum conditions (Maladkar, 1994). Cephalexin is produced from immobilized enzyme i.e. cephazolinsynthetase from *E.coli*. The complex physiochemical studies has made it possible to design highly efficient method for cefazolin production (Kurochkina and Nys, 1999).

5.4 Uses in food industry

Processing of food samples can be easily done by the used of immobilized enzymes. Various processes being done in food industry can be completed efficiently by the use of these immobilized enzymes. Multiple immobilized enzymes are mostly more advantageous and appealing. Lactose hydrolysis during whey production can be done by immobilized enzymes. High fructose corn syrups are produced by immobilized glucose isomerase. Amino acid acrylases are the immobilized enzymes used to make fermentation process efficient. Two system i.e. aminoaacrylase and glucose isomerase are immobilized and used which proved to be techno-economically feasible. Various immobilized enzymes are used for different

purposes such as glucoamylases, proteases, and flavor creating enzymes in food industry (Caprio, 2000). D.tagatose has attracted attention due to high benefit effect, can be used as an alternative of sucrose and D. tagatose use for the synthesis of optically active compounds and as an ingradients of detergents, cosmetics , pharmaceuticals. L.arabinose is used for the production of D.tagatose and further transformed to D.galactose as a substrate for industrial feasibility (Oh, 2007). For starch hydrolysis immobilized amylases are used in food industry. These enzymes are immobilized with calcium alginate beads. Uses of immobilized enzymes overcome the problem of gelantinization of starch during the process of hydrolysis (Gangadharan*et al*., 2009).

5.5 Uses in Biodiesel production

Biodiesel production has ability to replace fossil fuels to prevent environmental hazards. In biodiesel production, there is no production of sulphur dioxide, carbon monoxide, and halogens so this process is eco friendly (Iso*et al*., 2001). Apart from that biodiesels are renewable, biodegradable and non-toxic fuel (Antolin*et al*., 2002; Tiwari*et al*., 2007). The process of biodiesel production involves the transesterification of triglycerides by the help by lipases which produces glycerol and fatty acid methyl ester which can easily be purified. Biodiesels are produced from vegetable oils, fats and micro algal oils. Immobilized enzymes can be used for biodiesel production that reduces the cost of production. Lipases used for biodiesels productions are immobilized by various supports which are as follow: ceramics, kaolimites, silica, zeolites (Yagiz*et al*., 2007).

5.6 Bioremediation method

Annually large amount of dyes are being produced which is used in textile, dyeing and printing industry. These dyes are then discharged without treatment in canals and water system. Azo dyes are being transformed anaerobically into highly toxic compounds which proved to be very dangerous and carcinogenic (Akhtar*et al*., 2005b). Use of enzymes for the removal of pollutants is much appealing and has gained much importance. These pollutants can be removed by microbes but they have certain limitations so use of enzymes in this case is more beneficial. Many conventional methods for the degradation of dyes have become outdated due to various limitations (Khan and Husain, 2007b). Peroxidase from bitter gourd is immobilized by cheap support and used for the degradation of dyes used in textile industry even after 10 cycle of usage the enzyme loses only 50% activity (Akhtar et al., 2005). Laccases from number of enzymes are used in textile industry for degradation of various dyes.

5.7 Uses in cosmetics

All over the world people are using variety of cosmetic products for various purposes like cleaning, softening, adoring etc. For the preservation of beauty these cosmetics are used in different forms from decades. World market for cosmetics is about 200 billion euro. Most of them have short life span and need to manufacture continuously. Cosmetics involves different things i.e. fragrances and perfumes, decorative cosmetics, make up products, lip care, nail paints, self tanning), skin care (body milk, lotion, shaving foam, sunscreen, insect repellents), hair care (shampoo, conditioner, and coloring agents) etc. The composition of each cosmetic depends on the product and various chemicals used in their formation. Liquid skin cleaners comprise surfactants (e.g. cocamido propyl betaine, sulfosuccinate, alkylpoly glycoside), refattingagents (e.g. fatty acid poly glycol esters, fatty acids and alcohols, lecithin derivatives), thickeners (e.g. ethoxylatedglycoesters, xanthan gum), skin conditioners (e.g. protein hydrolysates, poly (dimethyl siloxane), polyquaternium). Certain plan extracts are used in cosmetics, vitamins, skin calming agents, various oils also added .Lipases are enzymes which have ability to hydrolyse carboxylic compounds having ester bonds in hydrophobic compounds. In the production of cosmetics lipases play vital role and act as an active ingredient in its formation and as a biocatalysts. As a biocatalyst lipases are very essential for skin care. For superficial cleansing active lipases are the most important (Masunaga and Cosmet, 2002), as they have ability for the mild loosening and removal of dust from the skin and lipases used along with proteases help in breaking down of fat deposits. Lipases are also used in cosmetics for controlled release of active molecules in situ which founds its application in the field of functional perfumes for the development of even fragrance over time. Many techniques are used for the immobilization of lipases. Use of these enzymes in cosmetics formation is considered green, as it is free from petroleum based organic solvents. The biocatalytic process reduces the byproduct formation. These processes are non-toxic, economical and eco-friendly and biocatalytic materials are also biodegradable.

6 CONCLUSION

In conclusion, immobilized enzymes which can be produced easily are highly specific in their action and also environmental friendly are widely used in different fields of science. Immobilized enzymes have their own specific biochemical, chemical, Kinetic and mechanical properties.Immobilization of enzymes can be done by some techniques such as entrapment, support binding, cross linking of enzyme crystals etc and different polymeric materials are used for this purpose such as synthetic organic polymers, biopolymers, smart polymers, hydrogels and inorganic supports.Immobilized enzymes are used in various fields such as in medicine, antibiotic production, food industry, biodiesel production, bioremediation, drug metabolism and many others.

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