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Recent Advances and Applications of Immobilized Enzyme Technologies: A Review

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Abstract: Immobilized enzymes have been widely used in the processing of variety of products. New strategies are continuously emerging for the formation of diverse immobilized enzymes having superior efficiency and usage. Immobilized enzymes have biomedical and industrial applications and for this reason, this area has continued to develop into an ever-expanding and multidisciplinary field during the last couple of decades. This study is a review of the latest literatures on different enzymes immobilized on various supporting materials. Immobilized enzymes for multiplicity of applications ranging from wine, sugar and fish industry through organic compounds removal from wastewaters to sophisticated biosensors for both in situ measurements of environmental pollutants and metabolite control are reviewed. Generally speaking, immobilized enzymes have immense potential in the analysis of clinical, industrial and environmental samples. Immobilized enzymes and proteins have been tremendously used in antibiotic production, drug metabolism, food industry, biodiesel production and bioremediation. The success of the vast usage of immobilized enzymes lies in the fact that they prove to be environmental friendly, cheaper and easy to use when compared to other parallel technologies.

Key words: Immobilized enzymes, immobilization supports, biosensor, bioreactor, biodiesel, antibiotic production, bioremediation

INTRODUCTION

Enzyme immobilization can be defined as the attachment of free or soluble enzymes to different types of supports resulting in reduction or loss of mobility of the enzyme. Selection of an immobilization strategy greatly influences the properties of biocatalyst. The varying levels in activity and diffusion limitations occurring with immobilization are mainly dependent on the properties of support material and the immobilization method. Support materials play an important role in the usefulness of an immobilized enzyme as it should be low-cost and provide adequate large surface area together with the least diffusion limitation in the transport of substrate and product for enzymatic reactions (Krajewska, 2004). In order to fully retain the biological activity, enzymes should be attached onto surfaces without affecting their conformational and functional properties.

Generally, the choice of a suitable immobilization strategy is determined by the physico-chemical properties of both supporting surface and the enzyme of interest. The use of free enzymes as compared to their immobilized forms show some significant drawbacks such as thermal instability, susceptibility to attack by proteases, activity inhibition, high sensitivity to several denaturing agents and the difficulty of separating or reusing the free catalyst at the end of the reaction from the reaction mixture. Many of these restrictions can be resolved by using enzymes in immobilized forms (Khan *et al*., 2006). Enzymes may be immobilized by a variety of methods which may be broadly classified as physical where weak interactions between support and enzyme exist and chemical where covalent bonds are formed with the enzyme (Kirk *et al*., 2002; Cherry and Fidantsef, 2003; Sheldon, 2007). To the physical methods belong: containment of an enzyme within a membrane reactor; adsorption (physical, ionic) on a water-insoluble matrix; inclusion (or gel entrapment); microencapsulation with a solid membrane; microencapsulation with a liquid membrane and formation of enzymatic Langmuir-blodgett films.

The chemical immobilization methods include: covalent attachment to a water-insoluble matrix; crosslinking with use of a multifunctional, low molecular weight reagent and co-crosslinking with other neutral substances, e.g., proteins (Cao, 2005; Khan *et al.,* 2005a). Numerous other methods which are combinations of the ones listed or original and specific of a given support or enzyme have been devised. However, no single method and support is best for all enzymes and their applications. This is because of the widely different chemical characteristics and composition of enzymes, the different properties of substrates and products and the different

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uses to which the product can be applied. Besides, all of the methods present advantages and drawbacks. Adsorption is simple, cheap and effective but frequently reversible, covalent attachment and crosslinking are effective and durable but expensive and easily worsening the enzyme performance and in membrane reactor-confinement, entrapment and microencapsulations diffusional problems are inherent. Consequently as a rule the optimal immobilization conditions for a chosen enzyme and its application are found empirically by a process of trial and error in a way to ensure the highest possible retention of activity of the enzyme, its operational stability and durability (Khan *et al*., 2005b; Krajewska, 2009).

New designs of immobilization supporting materials with tailorable pore size are being studied more and more and in parallel, structure and surface characteristics of the target enzymes are worked out. These all studies have enabled more precise control of the immobilization of vast number of enzymes. The synthesis of vast number of immobilized enzymes has been applied in different fields for the benefit of humanity.

Uses of immobilized enzymes: During the initial years of the development of the field of immobilized enzymology, researchers used to find only the advantage of the immobilized enzymes in comparison to their soluble/free counterparts. Advantages of immobilized versus soluble enzymes included comparative studies in pH profile, various denaturing agents organic solvents, temperature, etc. Now recently during the last couple of decades, immobilized enzyme technology has advanced into and ever-expanding and multidisciplinary fields to analyze clinical, industrial and environmental samples. Here, we present recent developments and used of immobilized enzymes in different fields such as in medicine, antibiotic production, drug metabolism, food industry, biodiesel production, bioremediation, etc.

Use of immobilized enzymes as biosensors: Biosensors are electrical, optical, chemical or mechanical devices with the capability to detect biological species selectively. They are often modified with biological entities to enhance their selectivity. Examples of biological recognition molecules include enzymes, antibodies and oligonucleotides. The ideal biosensor not only has to respond to low concentrations of analytes but also must have the ability to discriminate among species according to the recognition molecules that are immobilized on its surface. Biosensors have wide applications including biomarker detection for medical diagnostics and pathogen and toxin detection in food and water (Leung *et al*., 2007). Analytical technology based on biosensors is an extremely broad field which impacts on many major industrial sectors such as the pharmaceutical, healthcare, food and agricultural industries as well as environmental monitoring. Because of their exceptional performance, capabilities which include high specificity and sensitivity, rapid response, low cost, relatively compact size and user-friendly operations, these properties of biosensors make them an important tool for detection of various chemical and biological components (Amine *et al*., 2006). The development of biosensors based on immobilized enzymes came out to solve several problems such as loss of enzyme, maintainace of enzyme stability and shelf life of biosensors and additionally to reduce the time of enzymatic response and offer disposable devices which can be easily used in stationary or in flow system.

Biosensors based on principle of enzyme inhibition have by now been applied for a wide range of significant analytes such as Organophosphorus Pesticides (OP) organochlorine pesticides, derivatives of insecticides, heavy metals and glycoalkaloids. The choice of enzyme/analyte system is based on the fact that these toxic analytes inhibit normal enzymatic function. Typically, the percentage of inhibited enzyme (1%) that results after exposure to inhibitor is quantitatively related to the inhibitor (i.e., analyte) concentration (Ivanov *et al*., 2003a, b).

Malitesta and Guascito (2005) have described the application of biosensors based on glucose oxidase immobilized by electropolypmerization for heavy metal determination. Similarly, urease has been entrapped in both Polyvinyl Chloride (PVC) and cellulose triacetate layers on the surface of pH-sensitive iridium oxide electrodes and used for the determination of mercury. The immobilization of polyphenol oxidase during the anodic electropolypmerization of polypyrrole has been also reported.

The biosensor has been used for the determination of atrazine concentration in low ppm level. The determination of pesticides with the help of biosensors have become increasingly important in recent years because of the widespread use of these compounds (El-Kaoutit *et al*., 2004).

As in the literature reports, the development and uses of biosensors for the detection of various compounds as pesticides, heavy metals, toxins, etc., is subject of considerable interest, particularly in the area of food and environmental monitoring (Table 1).

USE OF IMMOBILIZED ENZYMES IN MEDICINE

Presently, immobilized proteins/enzymes are used routinely in the medical fields, for the diagnosis and

Table 1: Survey of immobilized enzymes used as biosensors for the detection of some special compounds, pesticides and heavy metals

Enzymes	Inhibitors	Immobilization matrix	Samples	References
Biosensors for the determination of pesticides				
Acetylcholinesterase	Paraoxon	Mutiwell carbon nanotubes	Real water sample	Joshi *et al.*, 2005
Acetylcholinesterase	Paraoxon	Entrapment in TCNQ-graphite	Orange juice	Schulze *et al.*, 2002
Acetylcholinesterase	Paraoxon,	Entrapment in a PVA-SbQ	Spiked	Bachmann *et al.*, 2000
Variants	Carbofuran	Polymer	River water sample	
Butyrylcholinesterase	Chloropyrifos-methyl, coumaphos, carbofuran	Cross linking with BSA in GA vapour	Spiked grape juice	Ivanov *et al.*,2003a,b
Choline oxidase	Pirimiphos-methyl	PB-SPE surface	Durum wheat	Del Carlo *et al.*, 2005
Parathion hydrolase	Parathion	CPE surface	Spiked river water	Sacks *et al.*, 2000
Catalase	Azide	Gelatin with GA	Fruit juices	Sezginturk *et al.*, 2005
Biosensors for the determination of heavy metals				
Urease	Hg^{2+}, Cu, Cd	Entrapment in sol-gel matrix	Tap and river water	Tsai and Doong, 2005
Urease	$Hg(NO_3)_2$, phenyl mercury $HgCl_2$, $Hg_2(NO_3)_2$,	Entrapment in sol-gel film	Water samples	Doong and Tsai, 2001
Glucose oxidase	Hg^{2+}	Cross-linking with GA and BSA	Spiked water	Mohammadi *et al.*, 2002
Glucose oxidase	Chromium (IV)	Cross-linking with GA and covering with alanine membrane	Soil sample	Zeng *et al.*, 2004
Biosensors for the determination of other chemical components				
Butyrylcholinesterase	α-chaonine, α-solanine, solanide	Cross-linking with GA vapour	Potatoes	Korpan *et al.*,2002
Butyrylcholinesterase	α-chaconine, α-solanine,	Cross-linking with BSA and GA vapour	Agriculture	Dzyadevych *et al.*, 2003
Butyrylcholinesterase	Tomatine	Cross-linking with BSA and GA	Tomatoes	Dzyadevych *et al.*, 2006
Tyrosinase	Benzoic acid	Mixture of graphite, tyrosinase and teflon	Mayonnaise sauce, cola soft drinks	Morales *et al.*,2002
Acetylcholinesterase	Anatoxin-a	Entrapment in PVA-SbQ	Fresh water	Devic *et al.*, 2002
Glutathione S transferase	Captan	Entrapment in sodium alginate gel	Contaminated water	Choi *et al.*,2003

BSA: Bovine Serum Albumin, GA: Glutaraldehyde, PVA-SbQ: Polyvinyl Alcohol bearing Styryl pyridium group, PB-SPE: Prussian Blue Screen Printed Electrode, TCNQ: 7,7,8,8-tetracyanoquinone diaminomethane,

treatment of various diseases. Immobilized proteins as antibodies, enzymes, receptors have revolutionized the medical fields in terms of time, manpower, accuracy and reliability.

Enzyme-based electrodes represent a major application of immobilized enzymes in medicine. The high specificity and reactivity of an enzyme towards its substrate are properties being exploited in biosensor technology. Biosensors used in clinical applications possess advantages such as reliability, sensitivity, accuracy, ease of handling and low-cost compared with conventional detection methods. These characteristics in combination with the unique properties of an enzyme render an enzyme based biosensor ideal for biomedical applications (D'Orazio, 2003). Recently, numerous clinical trials and intensive research efforts have indicated that continuous metabolic monitoring holds great potential to provide an early indication of various body disorders and diseases. In view of this, the development of biosensors for the measurement of metabolites has become an area of intense scientific and technological studies for various research groups across the world. These studies are driven by the need to replace existing diagnostic tools such as glucose test strips, chromatography, mass spectroscopy and Enzyme Linked Immunosorbent Assays (ELISA) with faster and cost effective diagnostic devices that have the potential to provide an early signal of metabolic imbalances and assist in the prevention and cure of various disorders like diabetes and obesity (Vaddiraju *et al.*, 2010). In recent years however, intensive research has been undertaken to decentralize such tests so that they can be performed virtually anywhere and under field conditions. Hence, the development of portable, rapid and sensitive biosensor technology with immediate on-the-spot interpretation of results are well suited for this purpose. The importance of biosensors results from their high specificity and sensitivity which allow the detection of a broad spectrum of analytes in complex sample matrices (blood, serum, urine or food) with minimum sample pretreatment (Malhotra and Chaubey, 2003). Now-a-days, the use of Surface Plasmon Resonance (SPR) biosensors is increasingly popular in fundamental biological studies, health science research, drug discovery, clinical diagnosis and environmental and agricultural monitoring. SPR allows for the qualitative and quantitative measurements of biomolecular interactions in real-time without requiring a labeling procedure. Today, the development of SPR is geared toward the design of compact, low-cost and sensitive biosensors. Nano-technology is also increasingly used in the design of biologically optimized and optically enhanced surfaces for SPR (Hoa *et al.*, 2007).

USE OF IMMOBILIZED ENZYMES FOR ANTIBIOTIC PRODUCTION

Competition with well established, fine tuned chemical processes for antibiotics production is a major

Table 2: Survey of the immobilized enzymes used for antibiotic production

Enzymes	Immobilization support	Antibiotic produced	References
Penicillin acylase from *E. coli*	Polyacrylamide gel	Cephalexin	Illanes *et al.*, 2007
Acylase from *E. coli*, *A. turbidans* and *K. citrophila*	Glyoxyl agarose	β-lactam antibiotics	Hernandez-Justiz *et al.*, 1999
Penicillin G acylase	Nylon hydrolon membrane	Cephalexin	Schroen *et al.*, 2001
Penicillin G acylase from *E. coli*	Silica gel	6-APA	Massolini *et al.*, 2001
Penicillin acylase	Polyacrylamide gel	cephalexin	Aguirre *et al.*, 2002
Acetyl xylan estrase	CLEAs using glutaraldehyde	Desacetyl β-lactam	Montoro-Garcia *et al.*, 2010
Penicillin G acylase from *E. coli*	Polyacrylic beads Cephalosporanic acid	7-amino-3-deacetoxy	Pan and Syu, 2005
Penicillin G acylase from *B. badius*	Cross-linked Enzyme Aggregates (CLEA) with glutaraldehyde	6-aminopenicillanic acid	Rajendhran and Gunasekaran, 2007
Penicillin G acylase from *P. rettgeri*	Methacrylic polymers	β-lactam antibiotics (cephalexin)	Senerovic *et al.*, 2006
cephalosporin-C deacetylase from *B. subtilisin*	Anion-exchange resin, KA-890, using glutaraldehyde	deacetyl 7-aminoceph-alosporanic acid	Takimoto *et al.*, 2004
Penicillin G acylase	Eupergit	6-APA	Abian *et al.*, 2003
Penicillin acylase	Poly-N-isopropylacrylamide	Cephalexin	Ivanov *et al.*, 2003a, b
Penicillin G acylase from *E. coli*	Nylon membrane chemically grafted with butylmethacrylate	Cephalexin	Travascio *et al.*, 2002
Penicillin acylase from *S. lavendulae*	Eupergit C.	6-aminopenicillanic acid	Torres-Bacete *et al.*, 2000
D-amino-acid oxidase from *T. variabilis*	(Poly) acrylamide gel and MnO_2	Glutaryl-7-amino-cephalosporanic acid	Vikartovska-Welwardova *et al.*, 1999
Cefazolin synthetase from *E. coli*		Cefazolin	Kurochkina and Nys, 1999

challenge for the industrial implementation of the enzyme synthesis of biologically important antibiotics such as β-lactam. Enzyme based routes are acknowledged as an environment friendly approach, avoiding organic chloride solvents and working at room temperature. Among different alternatives, the kinetically controlled synthesis, using immobilized Penicillin G Acylase (PGA) in aqueous environment with the simultaneous crystallization of the product is the most promising one (Giordano *et al.*, 2006).The β-lactam acylase is traditionally used for the hydrolytic processing of penicillin G and cephalosporin C. New and mutated acylase can be used for the hydrolysis of alternative fermentation products as well as for the synthesis of semisynthetic β-lactam antibiotics.

The yield of hydrolysis and synthesis has been greatly improved by process design including immobilization of the enzyme and the use of alternative reaction media. Significant advances have also been made in the resolution of racemic mixtures by means of stereo-selective acylation/hydrolysis using β-lactam acylases (Sio and Ouax, 2004).

Enzymatic production of cephalexin using immobilized penicillin G acylase has also been studied in greater details. Conversion of 7-Amino-3-Deacetoxy-Cephalosporanic Acid (7-ADCA) to cephalexin by Immobilized Penicillin G Acylase (IMPGA) have been investigated and it has been observed that under optimized conditions, IMPGA can attain 85% conversion of 7-ADCA to cephalexin. Furthermore, IMPGA can be reused for about 10 cycles (Maladkar, 1994). Production of cefazolin by immobilised cefazolin synthetase from *E. coli* as a biocatalyst has been shown possible. The complex of the physico-chemical studies makes it possible to design a highly efficient technological process for production of cefazolin (Kurochkina and Nys, 1999). Table 2 shows some of the latest references for the synthesis of various antibiotics by different enzymes immobilized on different supports.

USE OF IMMOBILIZED ENZYMES IN FOOD INDUSTRY

Immobilized enzymes are of great value in the processing of food samples and its analysis. The extent of lactose hydrolysis whey processing, skimmed milk production, etc. has been greatly enhanced by using respective enzymes as immobilized forms. The production of high fructose corn syrups has been greatly facilitated by the use of immobilized glucose isomerase. Similarly in Japan, the fermentation industry proved its processing efficiency for amino acids through the use of immobilized amino acid acylase. A relatively new concept is the use of a single matrix for immobilizing >1 enzymes to enhance food processing. Immobilized multi-enzyme systems offer many attractive advantages however such a process also raises some interesting questions about kinetics. Two systems, amino acylase and glucose isomerase have been demonstrated to be techno-economically feasible. Immobilization of other enzymes such as glucoamylase, lactase, protease and flavor modifying enzymes has received some attention recently for food processing (Carpio *et al.*, 2000). D-tagatose has attracted a great deal of attention in recent years due to its health benefits and similar properties to sucrose. D-tagatose can be used as a low-calorie sweetener as an intermediate for synthesis of other optically active compounds and as an additive in detergent, cosmetic and pharmaceutical

Table 3: Survey of the immobilized enzymes used in food industry

Enzyme and source	Immobilization support	Food substrate	References
β-galactosidase and amyloglucosidase	Bone powder	Lactose, whey, whey permeates, skimmed milk	Carpio *et al.*, 2000
Pectinase from *A. aculeatus*	Anion exchange resin	Pectin	Sarioglu *et al.*, 2001
Laccase from *P. oryzae, B. cinerea*	Silica gel	Wine, fruit juice and beer processing	Minussi *et al.*, 2002
Trypsin	Cellulose	β-lactoglobulin	Yamamoto *et al.*, 2005
Cardosin A (protease)	Agarose glutaraldehyde support	α-lactalbumin	Barros *et al.*, 2003
Pectin lyase	Alginate beads	Esterified pectin	Busto *et al.*, 2006
Tyrosinase	Polyacrylic acid carbon nanotubes	Phenolics in red wine	Kim *et al.*, 2010
β-galactosidase	Organic and inorganic supports	Removal of lactose from milk	Husain, 2010
Lipase from *C. rugosa*	Calcium alginate beads	Oil and grease	Jeganathan *et al.*, 2006
Pectinase	Anion-exchange resin	Pectin solution	Sarioglu *et al.*, 2001
β-galactosidase from	Duolite A-568	Muscat wine	Gueguen *et al.*, 1997
C. molischiana Glucoamylase	Chitin	Starch and hydrolyzed mannose starch	Freire and Sant'Anna, 1990

Table 4: Survey of the immobilized lipases used biodiesel production

Source of lipase	Immobilization support	Substrate	References
T. lanuginosus	Polyurethane foam	Canola oil and methanol	Dizge and Keskinler, 2008
C. antarctica	Ceramic beads	Waste cooking oil	Al-Zuhair *et al.*, 2009
P. fluorescens, P. cepacia, M. juvanicus, C. rugosa, R. niveus	Porous kaolinite	Safflower oil	Iso *et al.*, 2001
P. expansum	Silica gel (resin D4020)	Waste oil	Li *et al.*, 2009
T. lanuginosus	Microporous polymeric matrix	Sunflower, soyabean, waste cooking oil	Dizge *et al.*, 2009
C. rugosa	Chitosan	Rapeseed oil	Shao *et al.*, 2008
Lipase	Hydrotalcite and zeolite	Waste cooking oil	Yagiz *et al.*, 2007
S. cerevisiae	Mg-Al hydrotalcite	Refined rape oil	Zeng *et al.*, 2009
C. antartica and *Candida* sp.	Acrylic resin and textile membrane		Tan *et al.*, 2010
C. rugosa	Activated carbon	Palm oil	Moreno-Pirajan and Giraldo, 2011
P. cepacia	Polymeic matrix	Sapium sabiferum	Li and Yan, 2008
Rhizopus	Polyurethane foam	Soyabean oil	Hama *et al.*, 2007
P. depacia	Hydrophobic sol-gel support	Soyabean oil	Noureddini *et al.*, 2005
Lipase	Magnetic Nanoparticles	Triolein and ethanol	Dussan *et al.*, 2010
Rhizomucor	Zeolites	Waste oil	Macario *et al.*, 2008
Candida sp.	Fixed bed reactor	Waste cooking oil	Chen *et al.*, 2009
C. rugosa	Calcium alginate beads	Oil and grease	Jeganathan *et al.*, 2006
Rhizopus oryzae	Biomass support particles	Jatropha oil	Tamalampudi *et al.*, 2008
Fusarium heterosporum	Biomass support particles	Rapeseed oil	Koda *et al.*, 2010
Commercial lipase	Macroporous polypropylene	Vegetable oil	Salis *et al.*, 2008
Thermomyces lanuginosus	Aldehyde lewatit	Ethanol and soyabean oil	Rodrigues *et al.*, 2010

formulations. Biotransformation of D-tagatose has been produced using several biocatalyst sources. Among the biocatalysts, L-arabinose isomerase has been mostly applied for D-tagatose production because of the industrial feasibility for the use of D-galactose as a substrate (Oh, 2007).

Calcium alginate beads have been used very efficiently as an effective supports for immobilization of alpha amylase for starch hydrolysis. Studies have also proved immobilization as an important technique for continuous and repeated use of enzymes in industrial application and also rapid separation of the enzyme from the reaction medium, thus improving their economic feasibility.

Compared to the free enzyme, the higher activity of the immobilized enzyme at higher temperatures and the ability to hydrolyze raw starch such as that of potato would help overcome problems related to gelatinization of starch during hydrolysis (Gangadharan *et al.*, 2009). Table 3 shows the processing of various food substrates using respective immobilized enzymes.

USE OF IMMOBILIZED ENZYMES FOR BIODIESEL PRODUCTION

Biodiesel has gained importance in the recent past for its ability to replace fossil fuels which are likely to run out within a century. Especially, the environmental issues concerned with the exhaust gases emission by the usage of fossil fuels also encourage the usage of biodiesel which has proved to be eco-friendly far more than fossil fuels. Biodiesel fuel does not produce sulfur oxide, halogens, carbon monoxide and minimize the soot particulate (Iso *et al.*, 2001).

Biodiesel fuel (fatty acid methyl esters) produced by transesterification of triglycerides has attracted considerable attention as a renewable, biodegradable and nontoxic fuels (Antolin *et al.*, 2002; Tiwari *et al.*, 2007). Recently, lipase-catalyzed transesterification has become more attractive for biodiesel production since the glycerol can be removed easily and the purification of fatty acid methyl esters is simple (Dizge and Keskinler, 2008). Biodiesel can be produced from vegetable oils, animal

Table 5: Survey of the immobilized peroxidases, laccases and polyphenol oxidases used in bioremediation

Enzyme and source	Immobilization support	Substrate	Reference
Lipase from *C. rugosa*	Polypropylene membrane	Dimethylphthalate	Mita *et al*., 2010
Laccase from *T. versicolor*	Silica	Reactive dyes	Peralta-Zamora *et al*., 2003
Polyphenol oxidase	Chitosan coated polysulphone membrane	Industrial phenolic effluent	Edwards *et al*., 1999
Polyphenol oxidase from *S. tuberosum*	Celite 545	Textile and non-textile dyes	Khan and Husain, 2007a
Peroxidase from *M. charantia*	Con A-sephadex	Textile dyes	Akhtar *et al*., 2005a, b
Laccase from *T. versicolor*	Silica modified with imidaxole	Textile reactive dyes	Peralta-Zamora *et al*., 2003
Laccase from *M. thermophila*	Epoxy activated carriers	Synthetic reactive dyes	Kunamneni *et al*., 2008
Fangal laccase	Porous glass beads	Anthraquinone and indigoid dyes	Champagne and Ramsay, 2010
Dye decolorizing	Silica based mesocellular foam	Anthraquinone dyes	Shakeri and Shoda, 2008
Laccase	Alginate-gelatin beads	Reactive red B-3BF	Wang *et al*., 2008
Polyphenol oxidase from tuberosum and *S. melongena*	Celite-545	Textile and non-textile dyes	Khan and Husain, 2007a

fats, microalgal oils and waste products of vegetable oil refinery or animal rendering and used frying oils. Immobilized enzymes could be employed in the biodiesel production with the aim of reducing the production cost by reusing the enzyme (Jegannathan *et al*., 2008). Literature about the biodiesel production by immobilized enzymes shows that most of the researchers have used lipases from different sources for biodiesel production (Table 4). Different immobilization supports like ceramics, kaolinites, silica and zeolites have been used for lipase immobilization (Yagiz *et al*., 2007) (Table 5). Costs of chemical biodiesel production have still been lower than those of the enzymatic processes, however if the pollution of the natural environment is also taken into consideration, these costs are comparable (Canakci and Gerpen, 2003).

USE OF IMMOBILIZED ENZYMES FOR BIOREMEDIATION

There are >100,000 commercially available dyes with over 7×10^5 ton of dyestuff produced annually worldwide and used extensively in textile, dyeing and printing industry (Akhtar *et al*., 2005a). It is estimated that about 10-15% of the dyes are lost in industrial effluents. The discharge of wastewater that contains high concentration of reactive dyes is a well-known problem.

Anaerobic transformation of azo dyes begins with the reductive fission of the azo-linkage, resulting in the formation and accumulation of colorless aromatic amines which can be highly toxic and carcinogenic (Akhtar *et al*., 2005b; Khan and Husain, 2007a). Recent studies indicate that an enzymatic approach has attracted much interest in the removal of phenolic pollutants from aqueous solutions as an alternative strategy to the conventional chemical as well as microbial treatments that pose some serious limitations (Khan and Husain, 2007b).

Conventional physical and chemical methods of dye decolorization/degradation are actually outdated due to some unresolved problems. Biodegradation appears a promising technology but unfortunately the analysis of contaminated soil and water has been shown that these toxic pollutants persist even in the presence of microorganisms. Often the environment of the microorganisms is not optimal for rapid degradation. Recent studies indicate that an enzymatic approach has attracted much interest in the removal of phenolic pollutants from aqueous solution as an alternative strategy to the conventional chemical as well as microbial treatments that pose some serious limitations (Chen and Lin, 2007). Recently, peroxidases from bitter gourd (*M. charantia*) immobilized on some cheaper supports have been found highly effective in decolorizing reactive textile dyes compared to its soluble counterpart as the immobilized enzyme looses only 50% activity even after 10 cycles of usage (Akhtar *et al*., 2005b). Furthermore, laccases from a number of enzymes have been immobilized on a number of supports for the decolorization/degradation of various textile and non-textile dyes and phenolic compounds. Table 5 lists some recent research about the use of peroxidase, laccases, polyphenol oxidases in different immobilized forms for dye decolorization/degradation and phenolic compounds removal.

CONCLUSION

Recent advances in the design of immobilization supporting materials with tailorable pore size and surface functionality has enabled more precise control of immobilization of enzymes. New simulations of the surface characteristics of the target enzymes can be used to aid in the design of appropriate support materials. As the structure and mechanism of action of enzymes becomes available more controlled immobilization methods will be generated. The development of cheaper and disposable array biosensors, bioreactors and biochips for the simultaneous detection of clinically important metabolites and rapid screening of diseases has attracted much attention during the recent past. We believe that the use of more and more immobilized enzymes in clinical, biotechnological, pharmacological and other industrial fields has great promise among future technologies.

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