Original Article

Stabilization of enzymes for biotechnological applications

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Abstract

The biotechnological applications use stable and eco-friendly biocatalysts, with good operational properties, reusable and easily separable. An efficient method of improving the stability of enzymes as biocatalysts may be the immobilization. The enzymes immobilization research objectives are obtaining stable and versatile insoluble enzyme preparations with high enzymatic activity, capable of catalyzing the reactions of their natural and unnatural substrates. If active and stable water – insoluble enzymes, immobilized enzymes having appropriate substrate specificity can be obtained, it becomes possible to use enzymes conveniently in the same way as ordinary solid catalysts in synthetic biochemical reactions. In addition, since enzymes can catalyse specific reactions under mild conditions (normal temperature and pressure), application of immobilized enzymes in large scale bioconversion processes can reduce energy requirements. For some specific applications simple immobilization methods can be used. To increase the catalytic efficiency of immobilized enzyme preparations combinations of these methods can be handled to maximize the advantages presented by them. The physico-chemical properties of the supports used for enzymes immobilization influence the enzymatic activity and the immobilization yields of the immobilized preparations. The aimed of this work was to find efficient methods to stabilize enzymatic preparations with a good balance between costs associated and biocatalytic efficiency.

Keywords: enzymes, stabilization methods, biotechnological applications.

1. INTRODUCTION

Enzymes are biocatalysts created by nature, sustainable, biocompatible, biodegradable, and synthesized in renewable resources [1]. They can be separated from the natural environment and used in vitro. In green chemistry enzymes are preferred and are increasingly used to obtain biologically active compounds.

Industrial processes are conducted more and more efficiently in enzymatic catalysis using purified enzymes. They work as selective, specific, and efficient biocatalysts in very mild conditions, in aqueous solutions or in organic medium. Mild reaction conditions reduce the risk of degradation of temperature-sensitive compounds, as well as energy consumption or corrosion processes.

The use of biocatalysts in industrial applications is limited because most enzymes are unstable. A method of increasing enzyme stability is their immobilization. The immobilization of enzymes, besides the fact that it leads to an increase in their stability, also ensures an increase in the economic efficiency of industrial processes through the possibility of their reuse. Also, the reactions catalyzed by immobilized enzymes are easier to control, and the bioreactors in which they take place have a simplified constructive model [2].

The immobilization of enzymes can be done, mainly, by binding methods or entrapment on/in solid, insoluble in water, carrier (support); these methods immobilize the enzyme molecules and make them insoluble in aqueous media.

Immobilized biocatalysts have a number of advantages: (a) free or immobilized, biocatalysts are sustainable, nature-friendly catalysts; (b) immobilized biocatalysts used in the food sector reduce the loss of protein residues from enzyme molecules in food products; this aspect is very important if we consider that more and more people are sensitive and can accuse allergies to non-hydrolyzed or partially hydrolyzed protein fragments; (c) the possibility of reusing immobilized biocatalysts reduces process costs; (d) technologies designed for immobilized biocatalysts can be controlled and operated continuously; (e)

separation of products and biocatalysts is easy; (f) immobilization can favorably modify the properties of biocatalysts (activity, stability, operational properties) [3-5].

2. Methods used for enzymes immobilization

The immobilization methods consist in binding or blocking the enzymes to/in a carrier with a high molecular weight, insoluble in water. Thus, the enzymes are separated from the substrate or inhibitor molecules, while the exchange of substances to and from the enzymes is allowed, through the structure of the carrier. Enzyme immobilization can be achieved by various methods. Mainly, they can be linked to the carrier, adsorbed on its surface or physically embedded inside the porous matrix (Figure 1) [3, 4].

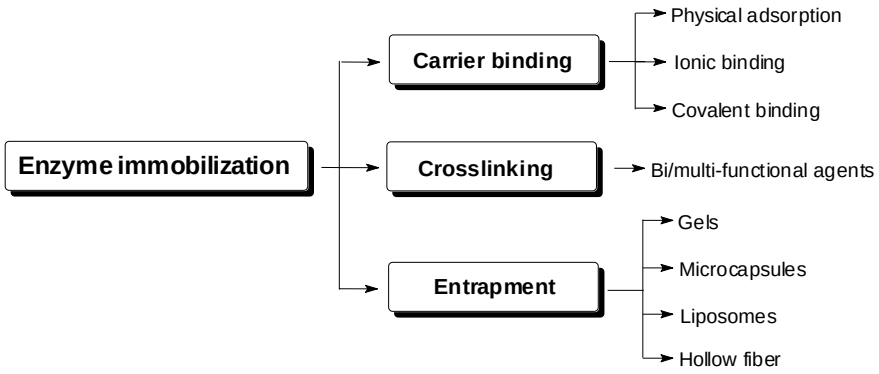

Figure 1. Enzymes immobilization methods

The immobilization of biocatalysts is done in order to increase the performance of enzymes, reflected by improved enzyme activities, stereoselectivities or stability, to allow the recovery and recirculation of biocatalysts and to make them more suitable for continuous processes [6].

2.1. Carrier binding methods

Carrier binding methods consists of attaching the biocatalyst to a water-insoluble support by physical adsorption, ionic or covalent binding [7].

Through the physical adsorption, the enzyme adheres to the surface of a carrier. Adsorption is achieved through physical interactions (hydrogen bonds, Van der Waals forces, hydrophobic interactions, etc.) between the enzyme and the surface of the solid carrier. Considering that no chemical reactions take place, the conformational changes of the enzyme during immobilization are minimal or even non-existent. For a maximum retention of the enzyme activity, a precise control of the environmental variables (pH, temperature, solvent, ionic strength, enzyme and carrier concentration) is essential.

The enzymes can be physically adsorbed with very good results on inorganic carrier (silica, alumina, activated carbon, clay, glass, diatomaceous earth, hydroxyapatite etc.), organic (collagen, glass, diatomaceous earth, various polysaccharides etc.) or organic-inorganic hybrids (mixed silica gels etc.) [3, 8].

The method of immobilization by ionic binding is based on ionic bonds between the enzyme protein and the solid support containing ion exchange residues. The binding forces are ion-ion interactions, stronger than in the case of physical adsorption. Practically, this method is based on both ionic and Van der Waals bonds.

As in the case of immobilization by physical adsorption, considering the nature of the bonds between the enzyme and the support, the conformational changes of the enzyme are very small, obtaining enzyme preparations with high activity.

Ion exchangers are used as enzyme carriers. Most frequently, organic supports with ion exchange residues are used or inorganic supports, especially silicon compounds, with similar ion exchange residues [9, 10].

Immobilization by covalent binding on activated polymers is a widely used method, although it is often difficult to obtain an immobilized enzyme in this way. A water-insoluble support can be covalently bound to the enzyme through the reactive groups of amino acid residues that are not involved in the enzyme's active site or substrate binding site. The covalent bond is stable and there is no possibility for the enzyme to detach from the support, if the concentration of the substrate and the ionic strength change during the reaction. The advantage of this method consists in the wide variety of supports that allow great flexibility in obtaining various types of biocatalysts with specific physical and chemical properties [4, 5].

Covalent bonding is achieved by the reaction of some groups on the support with functional groups on the polypeptide chain of the enzyme (hydroxyl, amino, carboxyl groups). Only a few supports contain reactive groups capable of reacting directly with the enzyme. The most used supports do not contain these reactive groups, but contain HO, NH2, CONH2 and COOH that can be activated for immobilization through various reactions (peptide bond formation, arylation or alkylation reactions, Schiff base formation etc.) [3-5].

2.2. Cross-linking immobilization method relies on the formation of chemical bonds, similar to those formed in the case of covalent binding immobilization, but does not use water-insoluble supports. Enzyme immobilization is achieved by the formation of three-dimensional intermolecular networks between enzyme molecules and a series of bi- or multifunctional reagents. The most used crosslinking agent is glutaraldehyde. The bonds established between the enzyme and glutaraldehyde is irreversible and stable at extreme pH and temperature values [4, 8].

2.3. Entrapment of a biocatalyst can be achieved by inclusion inside the network of a polymer with a high degree of crosslinking.

An important characteristic of these methods is that the biocatalyst is not linked to the polymer matrix network. Thus, steric problems do not appear, as in the case of covalent binding; enzymatic deactivation is much less than in the case of binding methods. In some cases, even intensification of enzyme activity can be observed.

The first gel entrapment techniques were developed using polyacrylamide gel; the enzymes such as trypsin, chymotrypsin, papain, α-amylase, etc. were immobilized in the pores of the gel. Enzymes can be immobilized not only in synthetic polymers but also in natural gels such as some important proteins (collagen, gelatin) and polysaccharides (agar, calcium alginate, κ-carrageenan, chitosan). The method has the advantage that the biocatalyst has no structural changes, being at the same time protected by proteinases [3-5, 7-10].

A large number of methods for enzyme immobilization are known, but there is no a technique that can be considered an ideal universal method. Biocatalyst immobilization methods and technology are continuously developing, as well as the enzymes used in numerous industrial applications, to obtain the most efficient immobilized biocatalysts, with good residual activity and suitable operational stability, with reduced costs of biocatalytic processes.

The success of immobilization is measured calculating three parameters: the immobilization yield, the efficiency of the immobilization and the enzymatic activity found after the immobilization [1].

The immobilization yield represents the percentage of immobilized enzyme activity out of the total enzyme activity of the free solution subjected to immobilization.

The immobilization efficiency is calculated as the ratio of the observed enzyme activity to the immobilized activity. The immobilization efficiency takes into account the possibility of enzyme inactivation during the immobilization process, or becomes inaccessible to the substrate after immobilization.

The recovered enzyme activity is calculated as the percentage of total activity assayed from the total initial activity subjected to immobilization.

3. Carriers used for enzymes immobilization

The success in obtaining efficient and stable immobilized enzymes is the interaction between the enzyme and the carrier. The carriers can be classified according to their organic or inorganic composition and based on their morphology as porous matrices and non-porous matrices (Figures 2 and 3) [3-10].

The inorganic carriers, compared to the organic ones, have a greater stability, but a major disadvantage is the abrasion that can occur in stirred bioreactors. The organic natural carriers have a good biocompatibility with the protein enzymes. The synthetic organic carriers can be synthetized with tailored pores according to the size of the enzyme molecules. The major disadvantage of the organic carriers is the diffusion limitation imposed by the polimeric matrix [11].

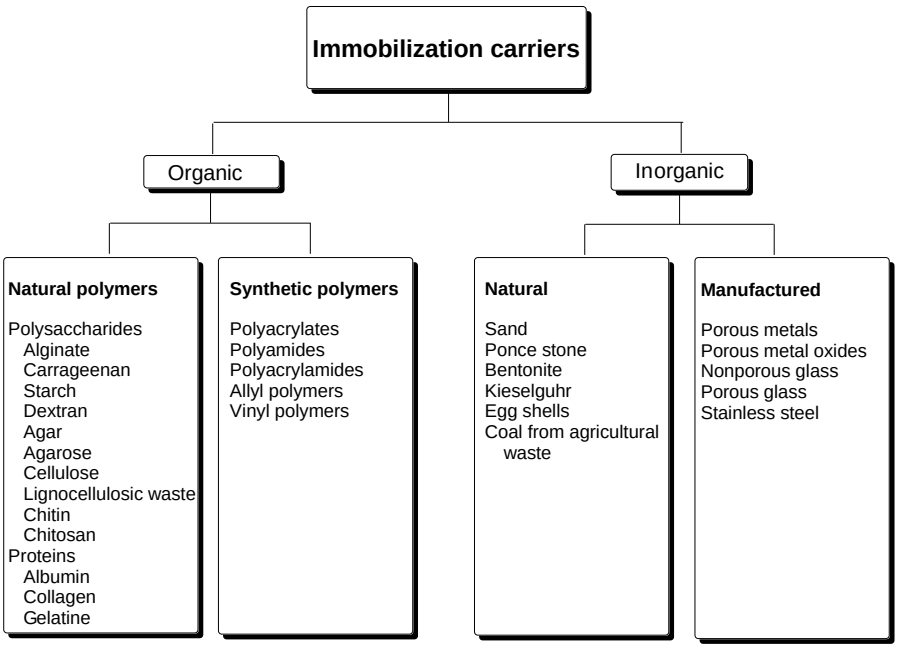

Figure 2. Chemical classification of the carriers

The morphology of the carriers influences the immobilization quality. The characteristics of the carrier that determine the result of the immobilization are: (a) the surface available for the catalyst (internal or external), (b) the loading capacity of the carrier with the catalyst (pore size), (c) the diffusion limitations imposed by the structure of the carrier towards soluble compounds (substrate or product), (d) the way in which the structure the carrier is protected from external environmental factors and allows the use of immobilized preparations in industrial reactors [3-5, 10]. The most important advantages and disadvantages of carriers are presented in Figure 3.

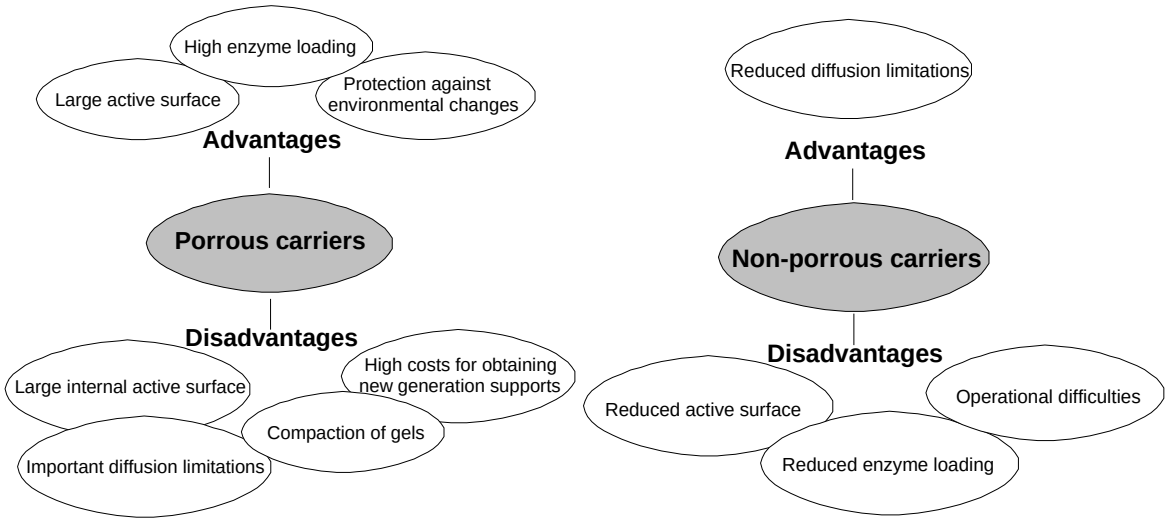

Figure 3. The advantages and disadvantages of the porous and nonporous carriers

The selection of the most suitable immobilization method and the right immobilization carrier is essential in the design of biocatalytic processes considering that their efficiency depends by the reaction conditions and the functional properties of the enzyme. Thus, the carrier used for the immobilization of biocatalysts used in the agro-food industry must meet certain characteristics [12]: (a) purity required by the food industry, (b) complete inertness to avoid the detachment of residues that can affect the quality of the food product, (c) chemical, biological, thermal and mechanical stability, (d) resistance to enzymatic attack, solvents or frictional forces during the process.

4. Immobilized enzymes in biotechnological applications

The enzymes have been an essential component in numerous human activities since ancient times. Applications of enzymes, in free or immobilized form, are in obtaining food and alcoholic beverages, even before their structure or function was known [10].

The new biotechnologies have enabled the fast development of more specific enzymes and have also enabled amazing improvements in enzyme process performance. This has made biocatalysis to step from a niche technology to a mass process, widely expanded, finding relevant applications for different chemical transformations [6].

Today, enzymes are used in many biotechnological applications to produce food and feed, food and feed additives, pharmaceuticals, fine chemicals, agrochemicals, detergents, etc. To increase the enzymes performance and reduce the technologies costs, the development of enzyme stabilization technologies is considered important.

The first use of immobilized enzymes was in the kinetic resolution process of the racemic mixture of D,L-amino acids with immobilized Aspergillus oryzae aminoacylase. Other major applications of immobilized enzymes are the industrial production of sugars, amino acids, and pharmaceuticals [12-15]. The industrial production of high-fructose syrup with glucose isomeraze, semy-synthetic penicillins with penicillin acylase, acrylamide with nitrile hydratase are other important technologies that use enzymes in immobilized form [12, 15].

At the beginning of their development, industrial processes mainly used hydrolases, these being much studied enzymes; in addition, hydrolases do not require a cofactor. Today, many other microbial enzymes with significant industrial impact are known, most of them being oxidoreductases, isomerases, lyases.

Oxidoreductases

Green chemistry aims to find selective catalysts in the reduction and oxidation reactions of key substrates for obtaining intermediates for fine chemicals. The oxidoreductases have been studied a lot in recent years and they have proven to be very efficient in the bioconversion of natural and unnatural substrates. The microbial oxidases and dehydrogenases and their coenzymes are very specific biocatalysts for obtaining in a synthetic route optically active compounds acting as intermediates for pharmaceuticals or other fine chemical compounds [16].

The alcohol dehydrogenase from Lactobacillus kefir was immobilized in PVA beds. The immobilized enzyme presented an improved thermal and operational stability in the enantioselective reduction reaction of prochiral ketones to the corresponding (R)-secondary alcohols in n-hexane as solvent. The NADP co-factor was immobilised together with the enzyme and their regeneration was done within the PVA gel matrix. The products were obtained with high enantiomeric excess. The entrapped enzyme had a good stability in the organic solvent [15].

The most efficient biosensors are obtained using enzymes and are based on their biocatalytic activity. Several sensors have been obtained for the detection of different pollutants, such as organophosphorus pesticides. Interesting results were obtained in the biodetection of nitrates using a nitrate reductase immobilized in sol-gel. The characteristic changes in the UV/VIS spectrum in the presence of nitrate reductase allow the detection of nitrates in concentrations of the order of μmol/l. This fact is important because nitrate-based fertilizers are increasingly used in agriculture and their detection is essential for human health and environmental protection [17].

Important research has been carried out to obtain sensitive, selective, and cheap glucose sensors. Many glucose sensors, especially those used in vivo, are based on measuring the rate of the glucose oxidation reaction, a reaction catalyzed by glucose oxidase (GOD). Effective immobilization of GOD is the essential step in obtaining an efficient biosensor. Xiaohong et al. [18] obtained a biosensor for glucose by coimmobilizing the enzyme with bovine serum albumin in a sol-gel matrix. Yu and coworkers use titanium-based sol-gel materials for enzyme immobilization [19]. Their further studies use titanium-based sol-gel films to immobilize GOD under neutral conditions and obtain efficient biosensors. GOD is entrapped with a regular distribution and a higher biological activity than the enzyme in solution. The titanium-based sol-gel films containing trapped hemoglobin were also used to obtain an amperometric biosensor for hydrogen peroxide, which is an important mediator in food analysis, pharmaceutical and clinical, industrial and environmental analysis [20, 21].

The phenol is an important contaminant of the environment (soil, surface water), and the monitoring of the phenol level has become an important topic. Many spectrophotometric and chromatographic techniques have been used to monitor phenol, but electrochemical techniques, especially amperometric biosensors, are considered promising methods due to the advantages offered (good selectivity, low cost, automation etc.) [22, 23]. Liu et al [24] obtained a sol-gel material based on aluminum oxide for the immobilization of tyrosinase in order to obtain biosensors for phenol. Yu and colleagues, studying titanium-based sol-gel materials, succeeded in immobilizing tyrosinase and obtaining a high-performance biosensor for phenol [25].

Mushroom polyphenol oxidase (PPO) immobilized sodium alginate, sodium alginate-PVA and sodium alginate-PVA-Ag nanoparticles were used for the determination of p-cresol degradation. From all the PPO immobilized in sodium alginate-PVA-Ag nanoparticles has achieved the highest degradation rates and higher than the free PPO. It was reused for more than 12 cycles, without losing any degradation capacity, showed high tolerance to temperatures or pH changes compared to the free PPO. The improved characteristics of the PPO immobilized in sodium alginate-PVA-Ag nanoparticles recommend it to be used in the bioremediation of environment polluted with p-cresol or other phenolic compounds [26].

The bitter gourd peroxidase was entrapped in calcium alginate–starch hybrid matrix. The enzyme activity was higher at the surface of the gel. The internal carbohydrate moieties made the immobilized enzyme more stable to denaturants like urea [27].

To be used for the treatment of textile bleaching effluents, in the harsh conditions required by the process (temperatures above 60°C, pH 9 and above), catalase must be used in immobilized form. Alumina-based supports are indicated in the scientific literature as a suitable support for immobilization in industrial applications. The immobilized product has good mechanical stability under working conditions and can be reused with very good results [2].

Catalase was immobilized on nanoporous silica spheres (pore size of up to 40 nm), coated with three layers of poly-dimethyldiallylammonium chloride and 21 nm silica nanoparticles. The activity of immobilized enzyme was 75 times that of catalase immobilized on mesoporous silica spheres [15].

Catalase was also immobilized in hybrid matrix Fe3+-collagen. The collagen, a natural polymer, made possible retaining a high amount of activity even after 26 cycles of reuse of the immobilized enzyme [27].

Hydrolases

Classic industrial processes use hydrolases as biocatalysts in the production of food and feed, powder and liquid detergents, fabric and leather products, intermediates for the pharmaceutical and agrochemical industries and more [10].

Proteases represent the largest category of industrial enzymes, regardless of their animal, vegetable or microbial origin [13-16].

Amino acids can be obtained from natural sources such as proteins through chemical or enzymatic hydrolysis. The enzymatic hydrolysis of proteins under controlled conditions is a process that takes place at a constant pH value, in the neutral or slightly alkaline range, a value maintained by continuous addition of alkali. The method of controlled enzymatic hydrolysis is applicable to all types of proteins, but sometimes the results obtained experimentally are particular for each type of protein and enzyme.

In the limited hydrolysis of proteins and the determination of the sequence of amino acids in polypeptides, proteolytic enzymes are used in a free state, but also immobilized by binding to a solid carrier. For this purpose, were used proteases immobilized by covalent binding to copolymers (trypsin, chymotrypsin, aminopeptidase, papin), or porous glass (pronase of Streptomyces griseus), also physically adsorbed on calcium phosphate gel (leucine aminopeptidase) etc. [3].

Peterson and collaborators obtained an enzymatic microreactor with a volume of 470 nL, by immobilizing trypsin on a porous monolithic polymer, located in silica capillaries. This microreactor is used in the digestion of proteins in a very short time, less than a minute, the resulting peptides being analyzed by mass spectrometry [28]. Similar research using immobilized trypsin on porous glass beads was carried out by Bonneil and collaborators [29].

Amino acids can be obtained from their functional derivatives such as esters or amino acid amides, through enzymatic hydrolysis. In organic medium the hydrolysis of amino acid esters with subtilisin occurs with much better reaction yields than in an aqueous environment [13-15].

D-aryl-glycines (side chains of semi-synthetic penicillins and cephalosporins) were obtined by stereospecific hydrolysis with immobilized subtilisin in a biphasic water-organic solvent system [30]. The methyl ester of N-acetyl-DL-phenylglycine, slightly soluble in water, is dissolved in the organic phase (eg methylisobutyl ketone). The L-form is hydrolyzed in the aqueous phase by the immobilized enzyme. The D form of the substrate remains unhydrolyzed in the organic phase.

Obtaining enantiomerically pure L-amino acids through the resolution of N-acetyl-DL-amino acids in the presence of aminoacylase is the first industrialized process by which tons of product have been obtained so far. Initially, the resolution of N-acetyl-DL-amino acids with aminoacylase was carried out with soluble enzyme, then in the Tanabe Seiyaku process (Japan) a column packed with Aspergillus oryzae aminoacylase immobilized by ionic bonding on DEAE-Sephadex was used, in a continuous operating process [31].

The synthesis of peptides, a reaction catalyzed by protease, requires stable biocatalysts under specific conditions, and the immobilization of specific enzymes represents an efficient reaction strategy. Immobilization by cross-linking (CLEC) is a method of obtaining robust, insoluble and stable biocatalysts, with high specific activity, by cross-linking with a bifunctional agent such as glutaraldehyde. Subtilisin thus immobilized is used with high efficiency in organic synthesis in continuous and repeated batch operating systems. Enzyme aggregates immobilized by cross-linking and embedded in hydrophilic hydrogels are successfully used in the synthesis of β-lactam antibiotics in organic medium [1, 15, 16].

The esterification of Z-dipeptides with papain in ethanol containing 2% phosphate buffer solution (pH 9) was achieved with yields of up to 50%. Papain immobilized by entrapment using Amberlite XAD-8 was used in the esterification of Z-alanine (Z-Ala-Ala-OH and Z-Val-Ala-OH) under the same conditions. The esterification yields obtained with immobilized papain were

higher than those obtained for free papain. After 24 hours of reaction, for both reactions, the ester yield was 1.5 times higher when immobilized papain was used [32].

An interesting example of enzyme technology was developed to obtain the neuroactive dipeptide kyotorphin with a role in pain regulation. The reaction was catalyzed by chymotrypsin immobilized and bound to a membrane. Another pain regulator, a pentapeptide, enkephalin, was obtained with a 30% yield using a proteinase immobilized on Celite. Also, di, tri and tetra bioactive peptides were obtained with yields between 67-74%, in biocatalysis processes with chymopapain and substilisin deposited on Celite [16].

Eutectic solvents can be used with very good results in numerous biocatalytic processes, being alternatives to organic solvents and ionic liquids. Based on existing studies, it can be said that eutectic solvents have low toxicity and can be considered biodegradable solvents, suitable for use in green chemical technology. Zhao et al investigated the activity of subtilisin and α-chymotrypsin immobilized in chitosan, in the transesterification reaction conducted in eutectic solvent based on chloride or choline acetate/glycerol [33, 34]. The biocatalytic efficiency of the two proteases in eutectic solvent medium with a 4% water content was superior to that measured in the reaction conducted in t-butanol. Enzymatic synthesis of peptides with α-chymotrypsin proceeded with very good productivities in eutectic solvent based on choline chloride and glycerol/urea/xylitol, 10-30% water [35]. The synthesis of N-(benzoylcarbonyl)-alanyl-glutamine with papain immobilized on a magnetic support proceeded in 71.5% yield in choline chloride/urea (1:2) [36].

Lipases are important catalysts in organic chemical synthesis, involved in the hydrolysis of carboxylic acid derivatives in aqueous medium. For lipase immobilization by binding methods numerous carriers were used (e.g. different types of glass and silica etc.), the most ones being the biopolymers, ion exchange resins, Celite [37, 16].

Reetz and collaborators showed that lipase from Pseudomonas cepacia significantly increases its activity following immobilization in a sol-gel matrix obtained from TMOS, methyltrimethoxysilane (MTMS), as well as from other precursors. The relative stability of the entrapped lipase depends on the alkyl groups of the precursors and on the additives used for immobilization [38-40].

CaLB lipase was immobilized by absorption on silica granules. In an aqueous medium the physical bonds between the enzymes molecules and the silica granules are broken and the enzymes are released. Lipase immobilized by this method is suitable for use in the composition of solid detergents, as it can be easily released in the washing environment. Lipase - silica granules composites, due to carrier structure, is more appropriate to be used in organic medium [15].

Lipase from Rhizopus oryzae immobilized onto ion exchange resin Amberlite IRC 50 proved a good long-term stability, the carrier having a high adsorption capacity [16].

Candida antarctica lipase B was immobilized by covalent binding to different supports, like alumina and silica. Immobilization by covalent bonding has the advantage of ensuring a significant increase in the stability of the immobilized preparation in biocatlysis in aqueous or organic medium, while the most important disadvantage is the possibility of important loss of enzyme activity if the covalent bonds are formed with amino acids from the catalytic site [41, 37, 16].

The immobilized lipase was used for obtaining pharmaceuticals. The immobilization method influences the biotransformation catalyzed by lipases. The lipase from Serratia marcescens immobilized in spongy matrix was used for industrial synthesis in a membrane bioreactor of the chiral intermediate in the reaction of production of Diltiazem. Lipase immobilized in the cross-linked enzyme crystals form (CLECs) has been used for production of pharmaceuticals ibuprofen and naproxen. CLECs have been also used for production an important organic compound methanol and were tested in biodiesel technology [16].

Amylases are hydrolases that catalyse the cleaving of α - 1,4- and α-1,6-glucosidic bonds in starch. Amylases are widely used as enzymatic components in the structure of detergents. A microbial amylase from Aspergillus niger was immobilized onto zirconia-coated alkylamine glass beads by glutaraldehyde coupling. The washing power of the detergent containing immobilized amylase was improved compared to that of the detergent without immobilized amylase [2].

Numerous supports, such as charcoal, CNBr-activated Sepharose, alkyl substituted Sepharose, DEAE cellulose and other large surface area matrices were successfully used for amylase immobilization. The high porosity and the high capacity of adsorption of these carriers made the immobilized enzyme preparations have a very good residual catalytic activity and functionality at extreme values of pH, temperature and high salt concentrations [27].

Cellulases are also hydrolases, but they catalyze the breaking of bonds in cellulose. Three types of cellulases are known, endo- and exoglucanases and beta-glucosidases. This cellulolytic system with synergistic action is used in the textile industry. In order to obtain superior quality textiles, cellulases capable of functioning and being stable under controlled conditions are needed. Dinçer and Telefoncu immobilized an acidic cellulase on chitosan beads coated with maleic anhydride-modified polyvinyl alcohol. The immobilized enzymes had improved stability in the neutral pH range [42].

Lyases - aldolases

Aldolases are enzymes that catalyze C-C bond formation reactions. Considering that C-C bond formation reactions are very important reactions in chemical synthesis, aldolases are used in obtaining products for fine chemistry and pharmaceutical industry [43].

Many different enzymes were immobilized by different techniques, using numerous carriers, and were used in industrial applications. However, the information related to the immobilization of aldolases is very little. The microbial aldolases FucA and 2-deoxy-D-ribose 5-phosphate aldolase (DERA) from E. coli and SHMT from Streptococcus thermophilus were immobilized by covalent binding to glyoxyl – agarose. The immobilized enzyme had a reduced enzymatic activity due to the inactivation process. The remaining enzyme activity was only 10-20%, although the immobilization yield was 80-90%. The immobilized preparation had much better residual activity in the presence of Co2+. The type of the metal and the metal-chelate support influences the immobilization result [16].

5. Conclusions

Enzymes represent specific biocatalysts for the development of new ways of obtaining valuable chiral intermediates. The need for enantiopure compounds with biological activity is increasing, as well as the development of industrial processes that use enzymes as very specific catalysts. Immobilization is a way of stabilizing enzymes and ensuring their functioning under the conditions required by specific industrial processes. The reuse of immobilized biocatalysts increases the economic efficiency of industrial processes. New enzymes and new reaction strategies are required by the continuously developing biotechnological industry. Future research must be focused on new biotechnological approaches that could be built on obtaining immobilized multi-enzymatic biocatalysts with high-performance for the production of biologically active compounds with industrial biotechnological applications.

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