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Review

IMMOBILIZED LIPASES AS PRACTICAL CATALYSTS

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Attractive features of lipase systems include versatility, substrate selectivity, regioselectivity, enantioselectivity and catalysis at ambient temperatures and pressures. To fully exploit the technical and economical advantages of lipases, it is recommended to use them in an immobilized form to reduce the cost and the poor stability of the free lipase. This paper summarizes various methods of lipases immobilization including covalent attachment to or adsorption on solid supports, encapsulation and entrapment within the membrane and in polymeric matrices. The effects of immobilization conditions on lipase properties and stability of biocatalysts are considered. Applications of immobilized lipases in the feasible reaction system as well as probable future trends in lipase catalyzed process are discussed.

KEYWORDS: Lipases; immobilization; adsorption; covalent immobilization; entrapment; hydrogel beads; membrane reactor

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are serine hydrolases of considerable physiological significance and industrial potential that can catalyze numerous reactions such as hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis (1-2). The broad substrate specificity makes lipases usable in a wide range of applications and their market is still growing (3). Lipases are used in the dairy and food industries, in the production flavor and aroma components, in oleochemical industry and in medical application (4-7). In addition, they are used in the leather and detergent industry, in the production of surfactants (8) and for analytical purposes. One of the most promising fields of lipases application is in the production of optically active compounds for the agrochemical and pharmaceutical industries (9, 10).

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Considering the high catalytic power and exceptional stereo-, regio- and chemoselectivity of lipases, the small number of their commercial applications in the industry is surprising. The reason is that the lipase reaction systems are too complex, usually consisting of two immiscible phases: an aqueous phase with dissolved enzyme and an organic phase with dissolved substrate (5). In other words, unlike other hydrolases that work in aqueous phase, lipases are activated only when adsorbed to an oil/water interface. The application of lipase has been deterred in these reaction systems by the high cost of enzymes, contamination of products with residual protein, slow reaction rate, and lack of an ideal emulsion reactor system to cater the complex interfacial heterogeneous hydrolysis. Most of the above problems could be overcome using immobilized lipases. Immobilization of lipases on, mainly, water insoluble carriers, helps in their economic reuse and in the development of continuous bioprocesses. Immobilization also facilitates the separation of products, enhances lipase properties such as thermostability and activity in non-aqueous media, and provides more flexibility with enzyme/substrate contact by using various reactor configurations. A lot of research work has therefore been performed in order to optimize immobilization techniques and procedures in view of the development an efficient lipase immobilized system (11, 12).

The design of an efficient lipase immobilized system is a rather difficult task. The selection criteria for immobilization technique and carrier are largely dependent on the particular lipase type, the type of reaction system (aqueous, organic solvent or two-phase system), the process conditions (pH, temperature and pressure) and the goal of immobilization. Furthermore, these will then determine desirable bioreactor design (batch, stirred-tank, membrane reactor, column and plug-flow).

This review deals with the general aspects of lipase immobilization focusing on lipase catalyzed hydrolytic and synthetic reactions. There are various methods of immobilization of lipases on many different types of supports. Each immobilization method has its advantages and disadvantages since immobilization is generally accompanied by changes in enzymatic activity, optimum pH, temperature and stability. Experimental investigations have produced unexpected results, such as a significant reduction or even an increase in lipase activity compared with soluble enzyme. The reasons for these observations will be discussed in this article, together with recent advances in the development of an efficient lipase immobilization system that would enable us to circumvent such peculiarities and achieve the desired benefits.

IMMOBILIZATION OF LIPASE

Techniques for immobilization have been broadly classified into four categories, namely adsorption, covalent binding, entrapment and microencapsulation. A combination of two or more of these techniques has also been investigated. It should be emphasized that in terms of economy of a process, both the activity and the operational stability of the biocatalysts are important. The yield of enzyme activity following immobilization does not only depend on losses caused by the immobilization procedure but can be further reduced by mass-transfer effects. However, improved stability under process conditions can compensate for such drawbacks, resulting in an overall benefit. Altogether, these parameters are a measure of productivity of an immobilized lipase system.

ADSORPTION

Immobilization by adsorption is the easiest and least expensive technique to prepare solid-support biocatalysts. It is based on the physical adsorption or ionic binding, or both, of the lipase to the surface of the support. The weak linkages established between enzyme and support (mainly van der Waals, hydrogen bonds and hydrophobic interactions) have little effect on catalytic activity (13). Regeneration of the immobilized biocatalyst is often possible. However, because the bonds are so weak, the enzyme can easily be desorbed from the carrier. Adsorption should not be used if enzyme cannot be tolerated in the product.

Immobilization of lipases by non-covalent adsorption has been shown to be very useful in non-aqueous systems, in which desorption can be neglected owing to the low solubility of lipases in organic solvents (14). For this reason, and due to simplicity of adsorption procedure, the use of adsorbed lipases is widespread for catalysis in water-immiscible solvents on an industrial scale (12).

The various adsorption protocols used with lipases have been extensively reviewed by Malcata et al. (12). A great number of synthetic or natural carriers with different shapes/sizes, porous/non-porous structures, different aquaphilicities and binding capacity have been used for lipase immobilization. Among these supports that have been studied are alumina, silica, celite, ceramics, metal oxides, porous glass, sepharose, sephadex, cellulose, zeolites, polyethylene, polypropylene, polystyrene, nylons polyacrylates, and others. Most of these materials are commercially available and much information is known about their operational characteristics (15).

The adsorption of lipases onto carrier materials depends on factors such as pH, ionic strength, isoelectric point of the lipase, surface and protein properties, as well as the history dependence of lipase-adsorption kinetics. Most supports usually bind from 2 to 50 mg protein per gram of support. While some supports are claimed to bind as high as 170 mg protein per gram of support, such high binding capacity may result in steric interference problems and loss of enzyme activity (16).

In general, the maximum adsorption is observed at pHs close to the isoelectric point of the lipase. In addition, porous particulate supports are superior to nonporous supports for immobilization of lipases due to their greater surface area. However, porous supports must have an internal morphology that allows not only the lipase binding but also an easy accessibility to substrate molecules in order to minimize diffusional limitation. It appears that pore sizes best suited for lipase adsorption are at least 100 nm in diameter (17). Smaller pore sizes can result in diminished availability of lipase molecules within the pores and in restricted diffusing substrate molecules. Such limitations lead to a lowered efficiency. The affinity of a lipase for an adsorbent generally increases with the hydrophobicity of the surface, and lipases desorb more easily from hydrophilic than from hydrophobic surfaces (18). Finally, an efficient adsorption process also dependents on the enzyme concentration. The most lipase adsorption isotherms display a well-defined “box” shape characterized by a sharp and steep slope at the lower concentration range, and a “plateau” at the high range. The adsorbed amount is usually up to the equivalent of a close packed monolayer, indicating the absence of multilayer adsorption. Although lipase adsorption systems were mostly described by the Langmuir isotherm (12), a deeper insight showed that the lipases distribution on the support surface could be best described by the Freund-

lich (19, 20) or even the Redlich-Peterson models (21). Namely, the Langmuir isotherm reflects irreversible adsorption and is based on the assumption of a structurally homogeneous adsorbent, where all adsorption sites are identical and energetically equivalent, which is rarely fulfilled (13). Nevertheless, many authors have used Langmuir model to correlate experimental data in order to compare different lipase/adsorbent systems. Hence, two parameters are sufficient to provide an accurate description of the affinity of a lipase for the support: the dissociation constant and a constant that provides a measure of monolayer coverage of the surface. Experimental values of the dissociation constant for the adsorption process and the surface area associated with each molecule for the different lipase/adsorbent systems are in the range of 10^{-8} and 10^{-6} M and 4,000 and 6,000 A^2, respectively (12).

Adsorption process could be described by two basic steps: diffusion of the enzyme from the bulk of the solution to the surface of the support and binding of the enzyme at adsorption sites on the carrier surface. The rate of lipase binding to the surface is much higher than the rate of diffusion and therefore the adsorption process is often diffusion controlled. However, in the case of some non-porous supports such as zeolite type Y, adsorption process is controlled by surface kinetics (19, 22).

The first goal of any immobilization procedure is to obtain a high yield of immobilized enzyme activity. There is no rule to predict the activity and stability of an immobilized enzyme upon adsorption. The activity of adsorbed lipase can vary from nil to fairly high values. In some cases the relative activity of adsorbed lipase even exceed that of the soluble enzyme (23). The nature of carrier (e.g. pore size, hydrophilic/hydrophobic balance, surface chemistry) strongly dictates the catalytic properties of the lipase (e.g. activity, selectivity and stability). The level of observed enzyme activity of such preparations is also dependent on the binding capacity of the support and on the diffusional and steric restrictions of the immobilized enzyme. It appears that the hydrophilic/hydrophobic balance of the support material is very important factor since the support material affects the water content in the microenvironment of the enzymes and the partitioning of substrates and/or products in the reaction mixture. Lipase activity is generally higher with hydrophobic supports.

Adsorption on to a hydrophilic carrier is a very simple procedure for lipase immobilization, for it requires just the mixing of the lipase solution with the carrier. Furthermore, in many cases, instead of previous molecular adsorption of lipases on the supports, the enzyme is forced to precipitate on the support by vacuum drying or by addition of chilling acetone to the enzyme and support mixture (4). All these methods are very easy to carry out and very useful to handle lipases in organic media. However, as noted, immobilization of lipases on hydrophilic supports, e.g., celite, duolite, cellulose, silica gel, kieselguhr, clay, nylon, sepharose, sephadex, alumina, and porous glass, often leads to structural deformations, to inability to structure/activity relationships and to reduced catalytic activity (3-30%) (12).

Most lipases display a large increase in activity when adsorbed on hydrophobic supports. This characteristic has been shown to be associated with conformational changes in the enzyme upon adsorption, creating an open, substrate-accessible active site. Thus, lipases recognize hydrophobic surfaces similar to those of their natural substrates and they undergo interfacial activation during immobilization. Special emphasis is paid to the selective adsorption of lipases on tailor-made strongly hydrophobic support surfaces (24).

Palomo et al. investigated several highly hydrophobic supports materials (e.g. octyl-agarose, octadecyl-Sepabeads) for lipases immobilization and found that the activity and enantioselectivity of such derivatives were much higher than that of free lipase (23). For example, esterase activity of lipase from *Humicola lanuginosa* increased up to 20-fold after adsorption on octyl-agarose while the lipase from *Pseudomonas fluorescens* showed a much higher enantioselectivity towards the hydrolysis of ethyl-α hydroxy phenyl butyrate after adsorption on the same support (25). Additionally, this immobilization method provides highly selective adsorption of pure lipases at low ionic strength from crude protein extracts. The binding forces between adsorbed enzyme and support are generally weak, but if hydrophobic interactions are present, the conjugates may exhibit a sufficient stability even in aqueous systems. These results imply that the proposed immobilization technique provide a promising solution for the applications of lipase for enantioselective esterification and hydrolysis in high water-activity systems on the industrial scale.

COVALENT IMMOBILIZATION

Covalent binding of lipases to different inorganic and organic carriers is more advantageous than other methods since diffusional restrictions to substrate or products are decreased considerably. In addition, covalent immobilization offers the greatest advantages by increasing the stability of the enzyme and preventing it from leaking into solution. There are numerous inorganic and organic supports available for lipase immobilization, including porous alumina, metallic oxides, stainless steel, and controlled pore glass (CPG), cellulose, starch, chitin, Sepharose and synthetic polymers. These supports, however, do not possess reactive groups for direct coupling of enzyme but rather hydroxy, amino, amide, and carboxy groups, which have to be activated for immobilization of enzymes. The various methods of activation of these supports are described in the literature (26). The covalent bond is usually formed by active bridge molecules, such as CNBr, and bi- or multifunctional reagents such as glutaraldehyde. The most general methods for activation of supports containing carboxy or amino groups involove the use of carbodiimides and similar reagents. It is very important that these active derivatives enable mild covalent coupling of lipase.

Although inorganic carriers have many advantages over organic polymers such as high mechanical strength, thermal and chemical stability, resistence to organic solvents and microbial attack, easy handling, and easy regenerability by a simple pyrolysis process, their application for covalent attachment is more limited as they are activated by only a few techniques. One of the most popular derivatization methods of inorganic carriers is the silanization method, which involves the use of trialkoxy silane derivatives containing an organic functional group. These organic groups are further modified to produce activated intermediates, which in turn are reacted with lipases. Besides, the reaction conditions required are relatively complicated and not mild, and the efficiency of the immobilization procedure is often quite low. Thus, *Rhizopus oryzae* lipase covalently attached to χ-aminopropyltriethoxysilane and glutaric aldehyde treated alumina retained only 23% of its initial activity (27). Another derivatization method of inorganic carriers involves the use of trichlorotriazine for activation of hydroxyl groups. Moreno and Sinisterra have described the properties of lipase covalently bound to some trichlorotriazine activated inorga-

nic supports such as alumina, silica and controlled pore glass (28). According to these workers, lipase immobilized on silica and alumina exhibited greater activity and stability than their immobilized counterpart on controlled pore glasses. This difference could be explained by the morphological characteristics of the support materials used for immobilization. The main disadvantage of porous carriers is that most of the surface available for protein immobilization is internal and owing to the diffusion limitations, the immobilized enzyme within the deep part of the pore structure might be inefficient. Lipase from *Candida cylindracea* immobilized on trichlorotriazine activated silica retained rather high activity of 69% and stability while lipase from *Candida antarctica* immobilized under the same conditions retained 31% activity (29). It appeared that the immobilization method is highly specific and should be optimized for a particular lipase-carrier system.

Polymer supports based on polysaccharide are very popular because they contain hydroxyl groups that can be activated directly by introduction of an electrophilic group, reactive toward enzyme, into the support, but they tend to swell and have poor stability against physical, chemical, thermal, and microbial degradation. Lipases have often been immobilized with epoxy or tresyl activated polysaccharide (29). Despite the rather long spacer arm of the epoxy-activated sepharose (12 carbons), the activity of bound lipase was poor (49%). Instead, lipase from *Candida antarctica* immobilized on tresyl activated sepharose, where the enzyme is closely attached to the matrix, showed higher activity (72%). Steric hindrance by the matrix cannot explain the epoxide immobilized lipase inactivation. Additionally, the immobilization conditions are not harsher than those employed for the immobilization of lipase to tresyl-sepharose. A possible explanation for the loss of lipase activity is the potential for oxirane reactive group to link protein through the hydroxyl, amino, and thiol groups of their amino acids, then an interaction between the oxirane group of the epoxy-activated sepharose and hydroxyl group of *serine 105* of the catalytic triad could be involved to some extent during the immobilization. Thus, it is very important that the immobilization of lipase by covalent attachment to a support should involve only functional groups of the lipase that are not essential for its catalytic action.

In general, the conditions required for the covalent attachment of a lipase to an insoluble carrier are such that some loss of activity is inevitable. However, since lipases are glycoproteins, some of these problems can be obviated by covalent bonding through the carbohydrate moiety that is not essential for enzyme activity. In general, functional aldehyde group can be introduced in a glycoprotein by oxidizing the carbohydrate moiety by periodate oxidation without significantly affecting the enzyme activity. The enzyme could then be covalently linked to a support containing an alkyl amine group through the Schiff base reaction. Hence, Arroyo et al. have immobilized lipase from *C. cylindracea* to tosyl activated supports (agarose and corn cob) by enzyme coupling via hydroxylic groups of the sugar component and the biocatalyst with high activity and stability was obtained (30).

Recently, different authors have focused their attention on studying lipase immobilization on Eudragit S-100, an anionic copolymer of methacrylic acid and methyl methacrylate (31). This polymer is characterized by a reversible solubility undergoing fast changes in its microstructure, by modifying reaction conditions such as pH, temperature or salt concentration. Thus, the catalytic reaction can be performed in a homogenous single phase, free from solid-liquid mass transfer problem and pore diffusion limitations with respect to substrate or product. Rodrigues et al. assessed covalent immobilization of *Chromobacterium viscosum* lipase on Eudragit S-100, by activating the polymer with carbo-

diimide and reported that this derivative can be effectively used for the esterification of different alcohols and fatty acids in both organic and two-phase system (31).

The physical structure and chemical composition of the support can also influence the microenvironment of the immobilized lipases and consequently their properties. The plot of activity vs. pH of an immobilized lipase can be broader (32), narrower or identical to that of native enzyme (33). Upon immobilization, the pH optimum for reactions catalyzed by lipases is often shifted to more alkaline values. Such shift in the optimum pH may be as much as 2 to 3 pH units and is caused by the charge of a water-insoluble carrier or chemical modification of the enzyme. It appeared that covalent immobilization is as any other random treatment to increase, decrease, or have no effect on enzyme stability. However, if one uses immobilization to realize a rational stabilization strategy, then it should indeed produce more stable enzyme preparations. Therefore, the lipase from *Candida cylindracea* covalently immobilized on nylon support was quite stable and retained about one third of the initial activity after repeated experiments in the course of 72 days (34). In addition, covalent immobilization onto geometrically well-defined supports enhances the flow characteristics through packed-bed columns.

Entrapment and microencapsulation

Lipase immobilization by entrapment is based on low porosity of matrix which at the same time retains enzyme within the carrier and provides substrate/or products diffusion. Numerous natural and synthetic organic carriers have been investigated as matrix materials. Natural polymers used as carrier materials for lipase immobilization such as alginate, cross-linked with linear chains of Ca^{2+} ions (35, 36), gelatin, carrageenan and agarose (37), chitin and chitosan (38), have the advantages of being nontoxic, biocompatible and biodegradable. However, diffusional problems and enzyme leakage are very evident in these gel entrapped systems.

The existence of diffusional limitations reduces the activity of immobilized lipases and therefore should be minimized. This can be achieved by decreasing the size of immobilized particles, enhancing the stirring or flow rate, increasing the porosity and optimizing the lipase distribution in the beads. Diameter is therefore one of the most important parameters of hydrogel beads when used as biocatalyst carriers. Alginate beads are produced by the so-called dropping method, which consists of droplet formation by pumping of alginate lipase solution through a needle and further solidification of the falling droplets in a hardening solution via counter-ion exchange. Most studies have been conducted with large alginate beads (2-3 mm) produced by droplet formation from a syringe under gravity or under coaxial air flow. Low lipase activities for these have been reported by several researches (36). Acceleration of the dropleting process and significant decrease of the droplet size may be realized by applying electrical potential to polymer solutions passing through the needle (35). This method, called electrostatic extrusion, provided production of small diameter lipase-alginate beads and easy control of bead size by simply varying the applied potential. Our research group improved the production of small diameter lipase-alginate beads by electrostatic extrusion technique and achieved highly efficient biocatalyst (75% of activity with respect to the free enzyme) for the hydrolysis of palm oil in a microaqueous system (35).

Entrapment efficiency and enzyme stability are also critical parameters to be considered in the preparation of hydrogel beads containing a lipase. Low entrapment efficiencies of 43-50% have been reported by several researches (36), and attempts are being made to optimize enzyme loading and minimize the leakage of the enzyme in the reaction medium, while still maintaining or improving the enzyme activity. The pH of the gelling medium and the concentration of the counter-ion were found to have significant effects on the lipase entrapment efficiency of the alginate beads. It appears that more lipase was entrapped with an increase in the calcium concentration until the concentration was above 180 mM (39). Further increase in the concentration of crosslinking agent had no effect on the lipase entrapment. In addition, a remarkable increase in the entrapment efficiency was observed with a decrease in the pH to 5.0, slightly below the isoelectric point of the lipase. This result can be explained by the fact that at pH values below the isoelectric point, lipase is positively charged and an effective ionic attraction of encapsulated enzyme with negatively charged alginate may be achieved. The entrapment efficiency was maximized at almost 100% at calcium concentration of 180 mM and at pH 5.0, probably due to an optimal ratio of cationic and anionic interaction sites under these conditions, leading to the most rapid formation of crosslinked hydrogel system, which consequently prevented further lipase leaking out from the beads into the counter-ion solution during bead formation (35, 39). In addition, the alginate beads have been surface coated with polycations such as poly-L-lysin or chitosan to prevent enzyme leakage and to improve stability of the biocatalysts (40).

A number of synthetic polymers have also been investigated such as the photo-crosslinkable resins, polyurethane prepolymers, and acrylic polymers like polyacrylamide. Lipases are mixed with prepolymers, and the mixture is subjected to radical or photochemical polymerization. For example, lipases can be entrapped into a polyacrylamide gel when they are mixed with acryl-amide, and then N,N`-methylene bisacrylamide and potassium persulfate are added. Urethane prepolymers and other materials have been used for photochemical polymerization (41). With due attention to the degree of cross linking and the nature of the gelling process, *i.e.* minimising the concentration of free radicals, gel entrapment can be applied to efficient lipases immobilization. Photo-crosslinkable resins based on poly(ethylene glycol) or poly(propylene glycol) were mostly used as matrix materials for lipase immobilization (41). The effects of difference prepolymers chain length, surface area and hydrophobicity of the support on the activity and stability of the immobilized lipase were investigated. Among tested samples, lipase immobilized in poly (ethylene glycol) resin prepolymer, ETN-4000 gave the highest activities and stability in all the parameters investigated. It appeared that entrapped lipases exhibit better activities and stabilities when the trapping resin was hydrophobic. Hydrophilic supports, however, lower the solubility of hydrophobic compounds in the vicinity of the enzyme, thereby causing apparent K_m values to increase and the enzyme's catalytic efficiency to decrease.

Alternatively, lipases may be entrapped within semi-permeable membrane or microcapsules that are impermeable to enzyme but permeable to low molecular weight substrates and products. Typical examples include entrapment of lipases in microcapsules produced by interfacial polimerization, liquid drying or phase separation (42) and in liposomes (43). The advantage of this type of immobilization is that each enzyme is in much closer contact with the surrounding solution than are those entrapped in the interior of gels. But

the drawbacks are the possibility of enzyme incorporation in the membrane wall and restriction of the substrate to low molecular weight substances.

LIPASE CATALYZED REACTIONS IN A MEMBRANE REACTOR

Lipase-catalyzed reactions, which take place at the oil-water interface, are commonly carried out in emulsion reactors. The practical use of lipase in emulsion reactors raises technical and economic difficulties such as lipase inactivation by intensive stirring of emulsion mixture and contamination of the products with surfactants (5). On the other hand, a two-phase membrane reactor with lipase immobilized in the membrane might be a suitable solution. The use of membrane reactors offers several advantages including high specific surface area, simultaneous reaction and separation of substrate and/or products in a single unit, reuse of the enzyme, and continuous operation of the process. In addition, the immobilization of enzymes onto membrane offers a number of advantages over beaded supports, when operated in continuous systems such as low pressure drop, short residence time and high operational stability with a low external and internal diffusional resistences (44).

Organic synthetic membranes, either in hollow fibers or in flat configuration, have been extensively used as lipase carriers for a number of applications. Several authors have studied lipase immobilization and fat and oil hydrolysis in hollow fiber reactors. Hoq et al. presented a hydrophobic hollow-fiber membrane system for fat hydrolysis, where the lipase was immobilized at the water-glycerol side of the membrane (45). Several groups of authors used this hydrophobic reactor system for fat and oil hydrolysis and ester synthesis (46-48). On the other hand, Pronk et al. presented a hydrophilic hollow-fiber membrane system for the hydrolysis of soybean oil, where the lipase was immobilized at the oil side of the membrane (49). In recent years, many reports have been published about lipase catalysis in this hydrophilic membrane system (50-52). Both membrane systems have advantages and it is not yet determined which membrane system should be preferred for industrial application. The advantage of the hydrophilic membrane system could be reduced enzyme desorption because of its insolubility in the organic phase. In addition, the catalytic activities per membrane area were all of the same order of magnitude and independent of membrane type or thickness, but catalytic activities presented per amount of immobilized enzyme are much higher for hydrophilic membranes (53).

For design and operation of lipase membrane reactor systems, proper rate equations and kinetic parameters are necessary. In most studies performed in hollow fiber reactor systems with immobilized lipase, reactor operating regimes and reaction kinetics were not thoroughly investigated and the Michaelis-Menten approach was commonly used (49, 51). However, the reaction kinetics is more complex than the commonly assumed one-substrate one-product irreversible reaction (54). Operating regime affected reactor performance in respect of lipase desorption and reaction rates and should be optimized for a particular reaction system. In order a reactor to function at its optimal performance, it should work in a reaction-limited regime rather than in a diffusion-limited regime. Fat and oil hydrolysis, enantioselective ester synthesis and hydrolysis seem to be the most promising industrial applications of such systems. It was shown that lipase from *Candida rugosa* immobilized on Cuprophane hollow fibers was suitable for oil hydrolysis and attractive

for further exploration in long-term applications. Under optimal flow conditions immobilized lipase was stable for 137 hours of operation (52, 55).

CONCLUSION

Immobilized lipases technology offers favorable solutions for many potential biotechnological applications. Immobilization often stabilizes structure of the enzymes, thereby allowing their applications even under harsh environmental conditions of pH, temperature and organic solvents, and thus enable their uses in nonaqueous media. In the search for novel materials and immobilization procedures it is essential to carry out fundamental work on the physicochemical factors affecting the activity and stability of immobilized lipases. This information is essential to further reactor development and industrial scaleup. Several attractive concepts for lipase immobilization and bioreactor designs for lipase catalyzed process have been developed over the last decade. Mostly used systems are based on lipase adsorbed or covalently attached to solid supports, or entrapped in gel matrices in packed and fluidized bed reactors. Membrane reactor with lipase immobilized in the pore of membranes is another promising approach to many lipase catalyzed processes. This system may help in the future to integrate the bioprocess with downstream processing with an effort to increase the productivity while minimizing product recovery cost. Immobilized lipase technology may also be useful in nonaqueous media, not only in terms of stabilization of the biocatalysts but also in the development of continuous bioreactors. The use of immobilized lipases would be especially useful for specific applications in the pharmaceutical, oleochemical and food industry.

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ИМОБИЛИСАНЕ ЛИПАЗЕ КАО КАТАЛИЗАТОРИ ОД ПРАКТИЧНОГ ЗНАЧАЈА

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Липазе су ензими од великог потенцијалног практичног значаја због особине да селективно катализују велики број реакција под благим условима и без примене кофактора. Савремени приступ примени ових ензима у индустријским условима представqа примена имобилисаних ензима, чиме се повећава њихова стабилност и омогућује примена у континуалним системима. У раду је дат преглед различитих метода и носача за имобилизацију липаза укључујући ковалентну имобилизацију, адсорпцију, микроинкапсулацију и заробљавање ензима унутар полимерне матрице и у поре мембране, при чему је указано на предности и недостатке сваког од имобилисаних система. Дискутовани су утицаји услова имобилизације и природе носача на особине и стабилност биокатализатора. Такође су разматране могућности примене имобилисаних липаза у реакцијама од практичног значаја, као и развој нових имобилисаних система.

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