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Review Article

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Enzymes immobilized on polymeric supports as potential catalysts

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ABSTRACT

Enzymes immobilized on polymeric supports are being extensively used for a number of chemical reactions and syntheses especially esterification, trans-esterification and enantioselective syntheses. The use of immobilized enzymes have several advantages including low cost product formation, reusability, rapid termination of reactions, ease of separation and many more. Immobilized enzymes have been reported to show enhanced thermal and chemical stability as compared to the free enzymes. The product formed is not only pure but yield is also higher as compared to free enzymes. Many reports regarding the use of immobilized enzymes as biocatalyst in esterification, trans-esterification reactions, and enantioselective hydrolysis are available in literature. The use of immobilized enzymes in place of hazardous chemicals (generally acids and bases) in chemical reactions/syntheses is an important step in the direction of green synthetic tools and processes. Some of the remarkable advantages those make the use of immobilized enzymes environmental friendly and in accordance with the basic principles of 'Green Chemistry' includes: use of simple benign operating conditions against the usual use of strong acids and bases, no separation of water is required as most of the polymeric supports/hydrogel are good water sorbent, no solvent and auxiliary chemical are required and high yields with high purity.

Keywords: enzyme; immobiliztion; lipase; activity; catalyst

INTRODUCTION

In order to minimize the use of hazardous chemicals, need to increase the interaction between polymer science and biotechnology is being realized. This interaction has manifested the immobilization of enzymes especially lipase on polymeric supports and use of these immobilized enzymes in many industrial applications and syntheses. Immobilization of enzymes not only enhances their thermal, chemical, mechanical and conformational stability but also efficiency of such enzymes are more than the free enzymes. Some enzymes can catalyse reactions both in aqueous and nonaqueous solvents whereas there are certain enzymes those can catalyze reactions in nonaqueous solvents only and these reactions are difficult or even impossible to carry out in water. In the recent past, many research groups have reported the use of immobilized enzymes for the biosynthesis of molecules in organic solvents [2-4]. It has been observed that the behavior of enzymes is different in organic phase from that in the aqueous phase. Further, most of the proteins are sparingly soluble in organic solvents, and hense it becomes necessary to immobilize enzyme onto a suitable porous matrix or support. The matrix or support is mostly polymeric in nature and provides an increased interfacial surface area, easy separation of catalyst, and reuse of immobilized enzyme. As compared to free enzyme, the immobilized enzyme is associated with many advantages like enhanced thermal [5] and chemical stability, ease of handling, easy recovery, and reuse relative to nonimmobilized forms [6–8].

No doubt, immobilized enzymes have above mentioned advantages over free enzymes but at the same time, it should be kept in mind that the immobilized enzymes are less active than free enzymes [9,10]. In view of the foregone discussion, enzymes/lipases immobilization requires specific polymeric supports for higher activity of immobilized enzymes/lipases, especially under harsher conditions such as adverse pHs, high ionic concentrations and higher temperatures.

Several factors are responsible to affect the extent of the immobilization and activity of immobilized enzymes. These factors include the nature of the polymeric support and many environmental factors such as time of immobilization, the pH of the medium, temperature, and nature of the reaction medium. Keeping in view all these factors, designing a suitable support for enzyme immobilization requires stringent and specific protocol. As most of the enzymes exhibit catalytic properties both in aqueous and non aqueous solvents and this behaviour is highly sensitive, a combination of hydrophilic and hydrophobic monomers in a hydrogel/polymeric support is desirable for better results. [11]

Proper selection of polymeric supports/hydrogels has great role in tailoring and designing them as support for enzyme immobilization. Such enzymes have broader spectrum of applications and can be used in adverse pH and strongly ionic solutions [12,13]. Activity of immobilized enzymes/lipases is also affected by the nature of the reaction medium both from kinetic and thermodynamic points of view [14,15]. It has been established that a support of moderate hydrophilicity not only provides higher conformational stability to enzymes/lipases but also increases the surface area of hydrogels [10]. It has been reported that lipase acts as an hydrolase in an aqueous medium [16] whereas it acts as an esterase in hydrophobic organic solvents [17]. After a close look at the literature available, it has been observed that the major concerns in the area of use of immobilized lipase is its low activity as compared to free enzymes. At the same time most of the immobilized lipases do not act as biocatalysts in aqueous medium but are very good catalysts in the organic solvents. These limitations can be improved by designing suitable supports for lipase immobilization. The present review aims at highlighting the recent advances in the immobilization of enzymes especially lipases on polymeric supports and their uses as catalysts in organic syntheses.

Some example of lipase immobilization and their subsequent use in organic syntheses

In literature there are numerous reports on the use of immobilized lipases as biocatalyst in esterification, trans-esterification reactions, and enantioselective syntheses.

Chauhan *et al*. reported the use of tailored polymeric supports for lipase immobilization. N-aminoethylacrylamide and N-aminoethylmethacrylamide were crosslinked with N,N-methylene bisacrylamide followed by reacting them separately with acrylic acid and methacrylic acid. The hydrogels thus formed consisting of both amide and carboxylic functional groups were characterized by nitrogen analysis, SEM, FTIR and by swelling in water as a function of time, temperature, pH, and in solutions of sodium dodecyl sulfate and cetyl trimethyl ammonium bromide. These hydrogels were found to be environmentally sensitive and in the presence of surfactants, they show micellization and swell to the maximum at the critical micellar concentration (CMC) of the surfactants. Lipase was immobilized on these hydrogels and the one exhibiting the maximum activity was further used as biocatalyst to explore nonconventional green routes for the synthesis of some known and novel vinyl monomers. Yields obtained have been high and the immobilized lipase showed remarkable reusability [18].

Eras *et al*. reported the conversion of chlorotrimethylsilane to chlorohydrin esters by using lipase immobilized onto poly(acrylic acid) hydrogels [19]. Polymeric supports based on Poly(AAc) have been used for the immobilization of lysozyme [20] and trypsin [21]. The effect of the number of carbon atoms in acids and alcohols on the extent of reaction in esterification catalyzed by Rhizomucor miehei lipase has been reported. Lipase catalysed reactions involving acids containing C-2 to C-5 and alcohols containing C-1 to C-8 were carried out [22]. Beier *et al.* reported lipase catalyzed trans-esterification reactions in homogenous perfluorocarbon and hydrocarbon solvents. The catalysis not only gave remarkable yield but also enabled approximately 95% direct enantiomeric partitioning of the products by liquid–liquid separation [23]. Immobilized lipase obtained from Candida species has been used in the synthesis of 2-ethylhexyl palmitate with esterification degree of 91% [24]. Ethyl esters of short chain fatty acids were synthesized by using whole cell lipase from Rhizopus chinensis in nonaqueous medium [25].

Enantioselective trans-esterification of esters of 2-bromo-tolylacetic by using immobilized lipase has been reported [26]. The fatty acid chain length has been reported to affects regioselectivity and initial specific reaction rate of the lipase catalyzed esterification of disaccharides. It has been found that initial specific reaction rate increases with the

decreasing chain length of the acyl donor [27]. Immobilized lipases have been used in the syntheses of oleoyl ester of L-ascorbic acid by using series of solvents (ethanol, THF, butanol, t-amyl alcohol, hexanol, octanol, pyridine and hexane) Activity of lipases and yield of the reactions were found maximum in tertiary amyl alcohol [28]. Kanwar *et al*. reported the immobilization of a purified alkaline thermotolerant bacterial lipase of Bacillus coagulans MTCC 6375 onto crosslinked poly(N-Aminoethylacrylamide-co-Acrylic acid) hydrogel at pH 8.5 and at temperature 55^{o}C. The immobilized lipase was used for the synthesis of ethyl propionate by using ethanol and propionic acid in a ratio of 300 : 100 mM in n-nonane along with 10 mg of hydrogel-bound lipase resulting in 52% conversion. Addition of molecular sieves (3 A°, 0.7 g/reaction volume) further enhanced the conversion rate to 82.4% [29].

Immobilization of lipase on polymeric supports based on acrylamide and methacrylates has been reported. The polymeric supports were prepared by crosslinking copolymerization using acrylamide and three different methacrylates viz. methyl methacrylate, dodecyl methacrylate and octadecyl methacrylate with ethylene glycol methacrylate and N,N-methylenebisacrylamide as crosslinker. Lipase immobilization on selected hydrogels was studied as a function of the concentration of the methacrylate used in the feed and the nature of the crosslinker. The activity of the hydrogel series that showed the highest activity of the immobilized lipase was investigated further as a function of the methacrylate concentration, pH, and temperature. Activity of the immobilized lipase in a few organic solvents were studied. From these studies, it was observed that the activity of the immobilized lipase was more than that of the free lipase. Further, the activity was affected by the structural attributes of the polymeric supports and by the nature of the solvent [30].

A large number of factors are responsible to influence the activity of immobilized enzymes and the yield of the products formed. Thus, before using an immobilized enzyme, it becomes necessary to study the effect of these factors to get the desired activity and yields. Nature of solvent plays an important role in immobilized enzyme catalysed reactions, especially for esterification and trans-esterification reactions. [31-38]. In certain cases the enzyme may be inactive in dehydrated systems [39] In some other cases, production of acidic or basic species in the reaction system or direct addition of exchangers, such as salt hydrates [40] or zeolites [41,42] can also alter the performance of enzyme in organic solvents.

CONCLUSION

From the foregone discussion, it can be concluded that enzymes especially lipases obtained from different sources can be immobilized on polymeric supports of varied compositions. Immobilization not only show enhanced conformational, thermal and chemical stability as compared to the free enzymes but also provide alternative green and clean routes for many esterification, trans-esterification, hydrolysis and enantioselective syntheses. Other than this, use of immobilized enzymes as biocatylysts have several advantages viz. reusability, low cost product formation, rapid termination of reactions, ease of separation and higher yield. Most important aspect associated with the use of immobilized enzymes is its environmental friendly nature. Use of immobilized enzymes avoids the use of hazardous chemicals (generally acids and bases) in chemical reactions/syntheses which is an important step in the direction of green synthetic tools and processes. There are many advantages associated with the use of immobilized enzymes those make the use of immobilized enzymes in accordance with the basic principles of 'Green Chemistry'. These advantages include use of simple benign operating conditions against the usual use of strong acids and bases, no separation of water is required as most of the polymeric supports/hydrogel are good water sorbent, no solvent and auxiliary chemical are required and high yields with high purity.

REFERENCES

[1] AM Klibanov. *Nature*, **2001**, 409, 241-246.
[2] M Noel; D Combes. *J Biotechnol*, **2003**, 102, 23-32.
[3] M Gargouri, P Drouet; M D Legoy. *J Biotechnol*, **2002**, 92, 259-266.
[4] E Castillo; F Pezzotti; A Navarro; A Lopez-Munguia. *J Biotechnol*, **2003**, 102, 251-259.
[5] A Zaks; AM Klibanov. *Science*, 1984, 224, 1249.
[6] SS Kanwar; M Srivastva; IA Ghazi; SS Chimni; RK Kaushal; GK Joshi. *Acta Microbiol Immunol Hugarica*, **2004,** 51, 57-73.
[7] SS Kanwar; HK Verma; S Pathak; Y.Kumar; ML Verma; GS Chauhan. *Acta Microbiol Immunol Hungarica*, **2006**, 53, 195-207.
[8] FX Malcata; HR Reyes; HS Garcia; CG Hill; CH Admunson. *J Am Oil Chem Soc*, **1990**, 67, 890-910.

[9] JF Shaw; R Chang; FF Wang; YJ Wang. *Biotechnol Bioeng,* **1990**, 35, 132-137.
[10] RB Liebernnan; DF Ollis. *Biotechnol Bioeng,* **1975,** 17, 1201-1219.
[11] BD Ratner; J Millerm. *J Polym Sci Part A-1: Polym Chem,* **1972,** 10, 2475-2484.
[12] PAD Filippo; MB Fadda; A. Rescigno; A Rinaldi,; ESD Teulada. *Eur Polym J,* **1990,** 26, 545-547.
[13] K Mosbach. *FEBBS Lett (Suppl) Immobilized Enzymes,* **1976**, 62, 80-95.
[14] F Borzeix; F Monot; JP Vandecasteele. *Enzyme Microb Technol*, **1992**, 14, 791-797.
[15] F Yang; A Russell. *J Biotechnol Bioeng,* **1995**, 47, 60-70.
[16] FH Mattson; RA Volpenheim. *J Lipid Res*, **1969,** 10, 271-276.
[17] A Zaks; A Klibanov. *Proc Natl Acad Sci USA,* **1985**, 82, 3192-3196.
[18] GS Chauhan; SS Kanwar; R Kumar; Y Kumar, S Chauhan. *Journal of Applied Polymer Science*, **2008**, 108, 3200–3209.
[19] J Eras; JJ Mendez; M Balcells; R.Canela. *J Org Chem.*, **2002**, 67, 8631-8634.
[20] T Hirotsu; C Tagaki. *Thin Solid Films*, **2004**, 457, 20-25.
[21] H.Ahmad; MAJ Miah; MS Pervin; MM Rahman. *J Colloid Polym Sci*, **2003**, 281, 897-902.
[22] S Divakar. *Ind J Chem B Org Chem Incl Med Chem,* **2002**, 41,1919-1922.
[23] P Beier; D.O Hagan. *Chem Commun,* **2002**, 16, 1680-1681.
[24] X He; B Chen; TW Tan. *J Mol Catal B Enzymol*, **2002**, 18, 333-339.
[25] Y Xu; D Wang; X Mu; GA Zhao; K Zhang. *J Mol Catal B Enzymol,* **2002**, 18, 29-35.
[26] D Guieysse; C Salagnad; P Monsan; M Remaund-Simeon. *Tetrahedron: Asymmetry*, **2003,** 14, 317-323.
[27] NR Pedersena; R Wimmera,; J Emmersena; P Degnb; LH Pedersen. *Carbohydr Res*, **2002**, 337, 1179-1184.
[28] Q Song; D Wei. *J Mol Catal B Enzymol*, **2002**, 18, 261-266.
[29] SS Kanwar; RK Kaushal; A Aggarwal; S Chauhan; SS Chimni; GS Chauhan. *Journal of Applied Polymer Science*, **2007**, 105, 1437-1443.
[30] GS Chauhan; S Chauhan; Y Kumar, US Thakur; SS Kanwar; R Kaushal. *Journal of Applied Polymer Science*, **2007**, 105, 3006–3016.
[31] SS Kanwar; HK Verma; S Pathak, Y Kumar; M. L Verma; GS Chauhan. *Acta Microbiol Immunol Hungarica*, **2006,** 53, 195-207.
[32] SS Kanwar; M Srivastva; IA Ghazi; SS Chimni; RK Kaushal; GK Joshi. Acta *Microbiol Immunol Hugarica*, **2004**, 51, 57-73.
[33] SS Kanwar; GS Chauhan; SS Chimni; Y Kumar; GS Rawat, RK Kaushal, *J Appl Polym Sci*, **2006,** 100, 1420-1426.
[34] JL Boyer; B Gilot; R Guirand. *In Recent Progress en Geniedes Procedes, Nouvelles Applications de La methodologie de Genie des Procedes; Storck, A.; Grevillot, G, Eds.; Lavoisier Technique et Documentation: Paris, France*, **1987**, p 7.
[35] B Cambou; AM Klibanov. *J Am Chem Soc*, **1984**, 106, 2687-2694.
[36] T Nishio; T Chicano; M Kamimura. *Agric Biol Chem*, **1988**, 52, 1203-1208.
[37] SS Kanwar; RK Kaushal, ML Verma; Y Kumar; G. S Chauhan; R Gupta; SS Chimni. *Indian J Microbiol*, **2005**, 45, 187-193.
[38] A Zaks; AM Klibanov. *J Biol Chem*, **1988**, 263, 3194-3201.
[39] M Goldberg; D Thomas; MD Legoy. *Eur J Biochem*, **1990**, 190, 603-609.
[40] N Fontes; N Harper; PJ Halling; S Barreiros. *Biotechnol Bioeng*, **2003**, 82, 802-808.
[41] N Fontes; J Patridge; PJ Halling; S Barreiros. *Biotechnol Bioeng*, **2002**, 77, 296-305.
[42] N Harper; S Barreiros. *Biotechnol Prog*, **2002,** 18, 1451-1454.