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Review Article

The Application of Ionic Liquids in Enzyme Immobilization and Enzyme Modification

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Abstract

Owing to the unique chemical and physical properties, ionic liquids have captivated us for decades. Many research works revealed that ionic liquids met some new kinds of demands for enzyme technology. In this paper, we reviewed the use of ionic liquids as carrier/support to immobilize enzyme or as functional regents to modify support materials and enzyme. Meanwhile, the effects of ionic liquids on enzyme were summarized and discussed. At last, the problems in the present studies were pointed out and the further development was also put forward.

Keywords: Ionic liquids; Enzyme immobilization; Enzymatic; Carrier/support; Reaction medium

Introduction

Ionic Liquids (ILs) are a class of salts that are liquid at or close to room temperature. They are generally composed of organic cations and inorganic anions. The increasing interest in ILs is derived from their special properties, such as high thermal stability and chemical stability, low vapor pressure, high density, widely tunable polarity and hydrophobicity, high electric conductivity, and the solubility of organic compounds [1-3]. Studies spanning over the last decade have greatly promoted the use of ionic liquids in organic synthesis [4,5], extraction [6-10], basic life science [11,12], and enzyme immobilization/catalysis [5,13-15].

The potential application of ionic liquids in biocatalysis was proposed in 2000 [16] and grown rapidly in the recent decade [1-3]. So far, many ILs have been used in enzymatic technology to perform a variety of biocatalysis [14,17]. Compared with conventional reagents, ILs will make enzyme with higher catalytic activity, higher enantioselectivity, and better stability. Although most of the enzymes in ionic liquids have many advantages, a few enzymes still showed a relative lower activity in ionic liquids than that in organic solvents [18].

Recently, the usage of ionic liquids as enzyme immobilization agents exploits a new frontier. It is a new approach to develop an efficient biocatalyst by using ionic liquids to modify carriers or as carriers to immobilize enzymes, which shows an enhanced activity, thermostability and enantioselectivity. To date, a variety of techniques for IL modifications have been used in enzyme immobilization [19]. Here, we will review the effect of ionic liquids on enzyme from the use of ILs as carrier/support to immobilize enzyme or as functional regents to modify support materials and enzyme.

Ionic Liquids Used as Carrier

As well known, the property of carrier material may have a key influence on the performance of immobilized enzyme. Recently, Room Temperature Solid-Phase Ionic Liquids (RTSPIL) has emerged as alternative carrier materials for enzyme immobilization (Table 1). These RTSPILs could be used to immobilized enzyme *via* coat/entrapment [20-22], or physical adsorption [23,24], or covalent bond. Among these methods, physical coat/entrapment into a matrix is relatively simple and inexpensive, and causes a relatively small perturbation to the native enzyme structure and function. The pioneering study of Ionic Liquid-Coated Enzyme (ILCE) was first reported by Lee and Kim [21]. In their study, lipase from *Pseudomonas cepacia* was firstly mixed with [PPmim][PF_6] which was liquid above 53°C, then the mixture was cooled down to room temperature until solidification. As a result, the obtained ILCE showed a better enantioselectivity and stability than native PCL in the transesterification reactions. Considering that the enzyme activity has no obvious enhancement, they further introduced a new idea, i.e., the enzyme was coated by RTSPILs during lyophilization in aqueous medium [22]. The obtained ILs-coated enzyme showed a noticeable enhancement in its catalytic activity. Moreover, an improved enantioselectivity was also observed for the immobilized enzyme compared with the free one. Recently, Abdul Rahman et al. [25] have synthesized two kinds of RTSPIL ionic liquids, including tetraethylammonium L-histidinate and tetraethylammonium L-asparaginate, whose melting point is below 60°C. And these ILs was considered to be a suitable choice for coating material. Furthermore, they employed these two ionic liquids to coat *Candida rugosa* lipase, and then used these coated lipases as biocatalysts to catalysize the esterification of oleyl alcohol with fatty acids in hexane [26]. These coated lipases showed higher catalytic activity than the non-coated lipase.

Besides, ionic liquid polymer materials can also be used as carriers to immobilize enzyme *via* encapsulation. One such approach is that Horseradish Peroxidase (HRP) was encapsulated in Polymerized Ionic Liquid Microparticles (pIL-MP) [20], which were prepared by polymerization of 1-vinyl-3-ethylimidazolium bromide ([Veim][Br])

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Table 1: The performance of enzyme immobilized in ILs or polymerization of ILs.

RTSPIL carriers	Enzyme	Immobilization technology	Enzyme performance	References
polymerization of [Veim] [Br]	Horseradish Peroxidase (HRP)	encapsulation	The activity of HRP was enhanced by 2-fold.	[20]
[PPMIM]-[PF_6] ([PPMIM] = 1-(3'-phenylpropyl)-3-methylimidazolium)	Lipase from *Pseudomonas cepacia*	coat	The enantioselectivity was enhanced without losing the activity.	[21]
1-dodecyl-3-methylimidazolium ([C12MIM])	*Burkholderia cepacia* lipase	co- lyophilized	The co-lyophilized enzyme was 660-fold more active compared to its RTSPIL-free counterpart.	[22]
1-dodecyl-2,3-dimethyl-imidazolium ([C12DMIM])	*Burkholderia cepacia* lipase (BCL)	coat	The activity of the coated-lipase was enhanced by 6.3 times.	[23]
[MOPMIM]-[PF_6] ([MOPMIM] = 1-(3'-methacryloyloxypropyl)-3-methylimidazolium)	*Burkholderia cepacia* lipase (BCL)	coat	The enantioselectivity of the coated-lipase was improved significantly.	[24]
tetraethylammonium l-histidinate [N2222][his]	lipase from *Candida rugosa*	coat	The coated-lipase activity was enhanced up to 0.5 fold than that of non-coated lipase.	[26]
(1-vinyl-3-ethylimidazolium bis(trifluoromethyl-sulfonyl) amide) ([veim][Tf2N])	*Candida rugosa* lipase	micro- encapsulation	Lipase encapsulated within IL remained its active and exhibited excellent stability.	[27]

in the presence of the crosslinker N,N'-methylenebis(acrylamide) in a concentrated water-in-oil (W/O) emulsion. pIL-MP encapsulating HRP chemically-modified with comb-shaped polyethylene glycol (PM13-HRP) exhibited excellent activity for guaiacol oxidation in aqueous solution. The PM13-HRP in pIL-MP showed more than 2-fold higher activity than that of the enzyme encapsulated in a polyacrylamide microparticle. Moreover, pIL-MP could be easily recovered from the reaction mixture by centrifugation, which made it possible to recycle the biocatalyst for repeated oxidation reactions. More recently, *Candida rugosa* lipase was microencapsulated into W/IL microemulsions, and then incorporated into the poly ionic liquid ([veim][Tf_2N]) *via* free radical initiated polymerization [27]. The performance of such lipase encapsulated in IL polymer materials was evaluated *via* enzymatic hydrolysis of *p*-Nitrophenyl Butyrate (*p*-PNB) as a model reaction. As a result, the immobilized lipase exhibited excellent active and stability in aqueous solutions. After 50 days incubation, the immobilized lipase still retained 95% of its initial activity, while the native lipase only retained less than 50% of its activity after 1 h incubation. More importantly, the immobilized lipase could be recovered from the reaction mixture simply by centrifugation and the immobilized lipase retained most of its activity after five reaction cycles.

Hydrophobic Room Temperature Solid-Phase Ionic Liquids (RTSPIL) are solid under room temperature or close to room temperature, which thus can be used as carrier to immobilize enzyme *via* physical adsorption. The previous studies on enzyme immobilization *via* physical adsorption, such as enzyme immobilization on ceramic, diatomite or polymer, have been frequently performed in nonionic environment. Considering the specific effect of ILs' charge on enzyme molecule, we can expect that enzyme immobilized on RTSPIL would be more efficient than that immobilized on the traditional carriers. Therefore, the hydrophobic RTSPILs are promising supporting materials in enzyme immobilization. Interestingly, the enzyme immobilized on ionic liquids performed excellent stability and catalytic activity in organic solvents or solvent-free systems compared with the free enzyme. As an illustration, Gamba and co-workers [28] employed *Pseudomonas cepacia* lipase adsorption-immobilized on 1-*n*-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ionic liquid to catalyze synthesis of biodiesel. The IL-immobilized lipase was found to be an excellent biocatalyst and the reaction conversion was up to 96% in the presence of 70% MeOH. Moreover, the synergistic effect also improved the reusability of enzyme. The recovered IL-enzyme biocatalyst could be re-used at least four times without loss of catalytic activity and selectivity. Lee [23] investigated the application of ten RTSPILs as carrier for the *Burkholderia cepacia* lipase (BCL) immobilization *via* physical adsorption. These ten RTSPILs were composed of various imidazolium (or pyridinium) cation derivatives and a PF_6 anion, which provided the hydrophobic character to immobilize enzyme in a buffer solution. The obtained BCL/RTSPILs were used for the transesterification of *sec*-phenethyl alcohol with vinyl acetate in toluene. Compared with native BCL, BCL/RTSPILs often exhibited a remarkable enhancement in catalytic activity. A better stability and enantioselectivity was also observed after 5 times reuse. Although the reason why ionic liquids improve the stability and activity of enzyme remains unclear, we speculate that the ionic environment provided by ILs is responsible for the same.

Ionic Liquids Covalent Modification of Carriers

So far, the development of promising carriers for enzyme immobilization was flooded. Particularly, many researchers expect that the introduction of ionic liquids in modifying the traditional carriers can make a good influence on enzyme performance. Recently, Hu et al. [29] used mesoporous silica SBA-15 modified by imidazole based ionic liquids with various functional groups as novel carrier (IL-SBA) for *Burkholderia Cepacia* Lipase (BCL) immobilization and studied the performance of the immobilized lipase (BCL-IL-SBA). The BCL-IL-SBA showed a higher thermal stability, reusability, storage stability and stability in organic solvent than BCL directly immobilized on SBA-15 (BCL-SBA). More recently, they further employed a task-specific amino acid ionic liquids 1-methyl-3-(3-(trimethoxysilyl) propyl) imidazoleium lysinate to modify SBA-15 [30], and then grafted the *Porcine Pancreas* Lipase (PPL) on the novel carrier surface *via* ionic binding and cross linking. The results indicate that this new developed mesoporous material, SBA-15 modified with amino acid ionic liquid (AA-SBA), was a high effective carrier for enzyme immobilization and the immobilization efficiency of PPL could reach as high as 98%. Compared with the PPL immobilized on SBA *via* cross-linking (PPL-cro-SBA), the lipase immobilized on AA-SBA *via* ionic-binding and cross-linking methods (PPL-AA-SBA) showed dramatically higher thermal stability and reusability. As another illustrative example, Jiang et al. [31] have immobilized lipase from *Candida rugosa* (CRL) on the Fe_3O_4 magnetic nanoparticles modified with ionic liquids (MNP-IL) and the immobilized lipase showed higher catalytic activity, thermal stability than the native lipase in the

solvent-free esterification reactions. The enhancement of operational stability was also observed, e.g. it remained 92% of its initial activity after 5 cycles and 60% after 8 cycles, while no activity was detected after 6 cycles for the native counterpart. Subsequently, their group further prepared a series of IL-modified magnetic nanoparticle-grafted lipase and preferentially located them at the oil-water interface to study the lipase-catalyzed ester hydrolysis [32]. In this case, ionic liquids on the carrier surface could control the oil-water interfacial characteristics as well as the behavior of the immobilized lipase. The enzymatic activity increased as increasing the hydrophilicity of ionic liquids. Moreover, the ionic liquids environment favored the accumulation of substrate at the ionic liquids interlayer on the carrier surface. Also, the Magnetic Silica Nanoparticles (MSNPs) were also modified by functionalized ionic liquids through forming chemical bonds between the ethoxy groups of ILs and the silanol groups on MSNPs [33]. Then, Penicillin G Acylase (PGA) was successfully immobilized onto the magnetic nanoparticles modified with ionic liquids (MNP-IL) *via* physical adsorption. The immobilized PGA exhibit an excellent reusability and the activity of immobilized PGA was 75% remained after 5 cycles and 62% even after 10 cycles, while it's hardly impossible for native PGA.

Although the reasons why the ionic liquid incorporated into carrier can affect the enzyme immobilized on carrier is still not well understood, some possible reasons have already been suggested. On the one hand, it has been suggested that ILs as salts may stabilize enzyme by protecting the hydration layer surrounding the enzyme. On the other hand, the IL-modified carrier may activate the catalytic activity of enzyme by tuning its conformation structure. According to the previous report [34], the presence of [Emim][NTf_2] on carrier surface clearly stabilized lipase PS from *Burkholderia cepacia* against inactivation and preserved enantioselectivity. MALDITOF mass spectrometric analysis has also shown that the cationic part of an IL can bind with the lipase protein [35]. In addition, when enzyme is immobilized on IL-modified carrier, a thin IL layer is established between the enzyme and reaction medium, which is proved to be enough in retaining similar characteristics of enzyme with enzyme in a bulk IL solvent. The thin ionic liquid layer may protect the enzyme protein and enhance its flexibility [36]. Thus, it is promising to construct new materials based on IL modification for enzyme immobilization.

Enzyme Immobilization Using Ionic Liquids as Additives

Another effective way is that ionic liquids are used as additives to modify the carriers during enzyme immobilization. In the process of sol-gel immobilization of enzyme, there is always some shrinkage of gel during condensation and drying process, which may cause partial denaturation of enzymes [37]. To overcome these drawbacks, Polyvinyl Alcohol (PVA), amino acids, polyols, crown ethers, sugars and surfactants have been usually used as additives to modify the supports [38]. These additives can increase thermal stability and activity of immobilized proteins by altering hydration of protein and reducing shrinkage *via* a 'pore filling' effect. As an alternative additive, ILs can protect the shrinking of gel structure by pore filling and have great potential for enhancing the activity, selectivity and stability of enzyme. Recently, Lee et al. [37] have used ILs as additives to prevent the inactivation of enzymes from the released alcohol and shrinking of gel during the sol-gel process. The *Candida rugosa* lipases immobilized by using ILs in sol-gel process showed higher activity and stability than lipase immobilized without ILs. Subsequently, they further investigated the influence of various factors, including ionic liquids structure, ionic liquids content and types of precursor in the sol-gel process, on the activity and stability of immobilized lipase [39]. They found that the highest hydrolytic activity of immobilized lipase was obtained when the hydrophilic ionic liquid, [C_2mim][BF_4], was used as an additive, while the highest stability of immobilized lipase was obtained by using hydrophobic ionic liquid, [C_{16}mim][Tf_2N]. Therefore, they used the binary mixtures of these ionic liquids as additives and obtained the optimal immobilized lipase, which showed both high activity and stability. After that, Zarcula et al. [40] demonstrated once again that the utilization of 1-octyl-3-methyl-imidazolium tetrafluoroborate ([Omim][BF_4]) as immobilization additive for OcTMOS/TMOS silane sol–gel entrapping *Pseudomonas fluorescens* lipase led to a 2-fold increase in the total activity and 1.56-fold increase in the recovery yield of total activity in the acylation of racemic 2-heptanol by vinyl acetate in hexane. In order to identify how the presence of ILs could modify the morphology of sol-gel preparates and to have a possible confirmation of the partial incorporation of the IL in the matrix, they investigated the detailed structure of the OcTMOS/TMOS silane sol-gel immobilized lipase with ILs as additives *via* scanning electron microscope (SEM). The SEM image of the lipase immobilized in OcTMOS/TMOS silane sol-gel without IL additive shows an irregular porous structure, with spherical nano particles, while the SEM image of the lipase immobilized in OcTMOS/TMOS silane sol-gel with [Omim]BF_4 as additive shows a more amorphous structure. More recently, the use of protic ionic liquids as additives has been reported for the immobilization of lipase from *B. cepacia* in hybrid sol-gel matrices [41]. And the total activity recovery yield was increased by 35 times when employing the hydrophobic protic ionic liquid N-methylmonoethanolamine pentanoate (C_5-PIL) as additive. In summary, it is very effective to enhance the activity and stability of the immobilized enzyme by using ionic liquids as additives to modify supports. It is considered that ILs in the sol-gel process can act as a template during gelation and behave as a stabilizer to protect the enzyme from the inactivation.

Enzyme Immobilization Using Ionic Liquids as Solvent/Media

ILs can also be used as solvents to dissolve some polymer molecules which are insoluble in traditional solvents during the process of enzyme immobilization *via* encapsulation. As an illustration, cellulose is well known to be one of the most abundant renewable biopolymer and it has excellent thermal and mechanical properties and biocompatibility. Thus cellulose, especially cellulose gel, is a promising material for enzyme immobilization. However, the development of cellulose gel has been hampered by the difficulty of dissolving cellulose because cellulose is hardly insoluble in traditional solvents. To overcome this problem, ILs can be used as solvents to dissolve cellulose. Recently, by dissolving the cellulose into the biocompatible ionic liquid [Emim][Ac], Kim and co-workers [42] had prepared cellulose hydrogel and had reported for the first time the encapsulation of *Candida rugosa* lipase in a series of cellulose-biopolymer (cellulose-carrageenan, cellulose-chitosan, cellulose-

agarose, and cellulose-agar) composite hydrogel beads. The result suggested that the usage of ionic liquid [Emim][Ac] as solvent for cellulose dissolution has improved the immobilization yield. Moreover, lipase entrapped in the cellulose-biopolymer composite hydrogels showed excellent activity and protein loading amount. More recently, ionic liquids were used as solvent once again to prepare novel Nanoporous Magnetic Cellulose-Chitosan Composite Microspheres (NMCM) by sol-gel transition method [43]. Then the composite microspheres were activated by glutaradehyde to immobilize laccase. The immobilized laccase showed higher pH, thermal and operational stabilities than the native laccase. Moreover, the laccase immobilized on NMCM displayed a remarkable reusability and activity.

Sometimes excellent results can be obtained when using ionic liquids as reaction media during enzyme immobilization. For instance, the immobilization of enzymes onto carbon nanotubes has been normally performed in aqueous media such as buffer. However, the inherent insolubility of carbon nanotubes in buffer, resulting from the intrinsic van der Waals forces of SWNTs [44], often makes the immobilization inefficient. This limitation might be overcome by performing the immobilization in room-temperature ionic liquids, in which carbon nanotubes became better soluble or dispersable compared to aqueous media [45]. The results showed that the immobilization efficiency in ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]) was much higher than that in aqueous buffer. The resulting immobilized lipase displayed high activity in the transesterification of 1-phenylethyl alcohol in the presence of vinyl acetate in toluene.

Enzyme Modification with Ionic Liquids

Chemical modification is an important approach to alter protein function by introducing non-natural fragments into proteins [46]. This specific modification can expand the efficiency of native biocatalysts, also may improve enzyme properties. Several modifiers such as Z-proline and phthalic anhydride that improve the catalytic performance of enzyme have been reported in recent years [47,48]. In particular, ionic liquids are potential modifiers thanks to the presence of enzymes with excellent activity and stability. Recently, Bekhouche et al. [49,50] reported that IL covalently bound to Formate Dehydrogenase (FDH) could improved its stability and activity in ILs, and found that a higher modified degree from a more chaotropic cation contributed to significantly improved activity and stability. In their study, the enzyme was chemically modified by cholinium, hydroxyethyl-methylimidazolium and hydroxypropyl-methylimidazolium cations usually present in ionic liquids [49]. After modification, the FDH activity present 0.06 μmol min−1 mg−1 in 70% [MMIm][Me_2PO_4] (v:v) (30–45% of their activity in aqueous buffer) while the native enzyme is inactive at this ionic liquid concentration, proving the stabilizing effect of ionic liquids on enzymes. More recently, a series of functional ionic liquids was covalently bound on *Porcine Pancreatic* Lipase (PPL) *via* carbonyldiimidazole [51]. Then the modified enzyme was employed to catalyze hydrolysis of *p*-Nitrophenyl Palmitate (pNPP) and racemic 1-phenethyl acetate. It was demonstrated that hydrolytic activity was enhanced by ILs with chaotropic cations and kosmotropic anions compared with the free enzyme. Moreover, modifications by ILs bearing kosmotropic cations and chaotropic anions have contributed to lipase thermostability and enantioselectivity. In order to further verify the above observations, they further investigated the performance of IL-modified lipase in water-miscible 1,3-dimethylimidazolium methylsulfate ([MMIm][$MeSO_4$]) aqueous [52]. The results showed that the free enzyme lost 18% of its initial activity in 0.4 [MMIm][$MeSO_4$], whereas the activities of the modified enzyme increased by 2 times. Moreover, the modified enzyme exhibited higher thermostability in [MMIm][$MeSO_4$] aqueous compared with the native enzyme at high temperature.

Conclusion

Ionic liquids have captivated us for quite a long time owing to their remarkable properties. In summary, this review has summarized that ionic liquids serve as carriers and modification agents of support or enzyme for lipase immobilization. Ionic liquids have displayed great potential in the biocatalysis field since they can help to maintain or even enhance the enzymatic activity, stability, enantioselectivity and reusability due to the unique physical and chemical properties of ILs. We believe that the combination of enzyme with ionic liquids will provide a powerful tool for the development of enzyme technology and stimulate the use of them in much more potential applications. In particular, more and more new ILs recently have been synthesized, but are not applied to biocatalysis. It is thus necessary to investigate the effect of these new ILs on enzymes and explore their potentials. Moreover, it will be extremely valuable to enzymatic processes if the enzyme-compatible ILs can dissolve substrates that are not soluble in traditional organic solvents.

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References

1. Zhao H. Methods for stabilizing and activating enzymes in ionic liquids-a review. J Chem Technol Biotechnol. 2010; 85: 891-907.
2. Hernandez-Fernandez FJ, de los Rios AP, Lozano-Blanco LJ, Godinez C. Biocatalytic ester synthesis in ionic liquid media. J Chem Technol Biotech. 2010; 85: 1423-1435.
3. Zhang ZC. Catalytic transformation of carbohydrates and lignin in ionic liquids. WIREs Energy Environ. 2013; 2: 655-672.
4. Pârvulescu VI, Hardacre C . Catalysis in ionic liquids. Chem Rev. 2007; 107: 2615-2665.
5. Greaves TL, Drummond CJ. Protic ionic liquids: properties and applications. Chem Rev. 2008; 108: 206-237.
6. Cardiano P, Lazzara G, Manickam S, Mineo P, Milioto S, Lo Schiavo S. POSS--tetraalkylammonium salts: a new class of ionic liquids. Eur J Inorg Chem. 2012; 2012: 5668-5676.
7. Guo L, Chen J, Shen L, Zhang JP, Zhang DL, Deng YF. Highly selective extraction and separation of rare earths (iii) using bifunctional ionic liquid extractant. ACS Sustainable Chemistry & Engineering. 2014; 2: 1968-1975.
8. Bai L, Nie Y, Li Y, Dong HF, Zhang XP. Protic ionic liquids extract asphaltenes from direct coal liquefaction residue at room temperature. Fuel Process Technol. 2013; 108: 94-100.
9. Wang L, Sun HW, Zhang GH, Sun SJ, Fu XM. Application of ionic liquids for the extraction and passive sampling of endocrine-disrupting chemicals from sediments. J Soils Sediment. 2013; 13: 450-459.
10. Stojanovic A, Keppler BK. Ionic liquids as extracting agents for heavy metals. Sep Sci Technol. 2012; 47: 189-203.

11. Li X, Ma J, Lei W, Li J, Zhang Y, Li Y. Cloning of cytochrome P450 3A137 complementary DNA in silver carp and expression induction by ionic liquid. Chemosphere. 2013; 92: 1238-1244.

12. Zhang T, Gai Q, Qu F, Zhang Y. Ionic liquid-assisted SDS-PAGE to improve human serum protein separation. Electrophoresis. 2011; 32: 2904-2910.

13. Naushad M, Alothman ZA, Khan AB, Ali M. Effect of ionic liquid on activity, stability, and structure of enzymes: a review. Int J Biol Macromol. 2012; 51: 555-560.

14. Lu X, Luo Z, Yu S, Fu X. Lipase-catalyzed synthesis of starch palmitate in mixed ionic liquids. J Agric Food Chem. 2012; 60: 9273-9279.

15. Khare SK, Pandey A, Larroche C. Current perspectives in enzymatic saccharification of lignocellulosic biomass. Biochem Eng J. 2015; 102: 38-44.

16. Cull SG, Holbrey JD, Vargas-Mora V, Seddon KR, Lye GJ. Room-temperature ionic liquids as replacements for organic solvents in multiphase bioprocess operations. Biotechnol Bioeng. 2000; 69: 227-233.

17. Galonde N, Nott K, Debuigne A, Deleu M, Jerome C, Paquot M, et al. Use of ionic liquids for biocatalytic synthesis of sugar derivatives. J Chem Technol Biotech. 2012; 87: 451-471.

18. Abe Y, Kude K, Hayase S, Kawatsura M, Tsunashima K, Itoh T. Design of phosphonium ionic liquids for lipase-catalyzed transesterification. J Mol Catal B: Enzym. 2008; 51: 81-85.

19. Moniruzzaman M, Kamiya N, Goto M. Activation and stabilization of enzymes in ionic liquids. Org Biomol Chem. 2010; 8: 2887-2899.

20. Nakashima K, Kamiya N, Koda D, Maruyama T, Goto M. Enzyme encapsulation in microparticles composed of polymerized ionic liquids for highly active and reusable biocatalysts. Org Biomol Chem. 2009; 7: 2353-2358.

21. Lee JK, Kim MJ. Ionic liquid-coated enzyme for biocatalysis in organic solvent. J Org Chem. 2002; 67: 6845-6847.

22. Lee JK, Kim MJ. Ionic liquid co-lyophilized enzyme for biocatalysis in organic solvent: Remarkably enhanced activity and enantioselectivity. J Mol Catal B: Enzym. 2011; 68: 275-278.

23. Lee JK. Immobilization of enzyme on hydrophobic room temperature solid-phase ionic liquids and its use for biocatalytic transesterification in organic solvent: remarkably enhanced activity of lipase. Bull Korean Chem Soc. 2012; 33: 3458-3460.

24. Lee JK, Kim MJ. Room temperature solid-phase ionic liquid-immobilized enzyme for biocatalysis in organic solvent: markedly enhanced enantioselectivity. Bull Korean Chem Soc. 2011; 32: 3149-3151.

25. Abdul Rahman MB, Jumbri K, Basri M, Abdulmalek E, Sirat K, Salleh AB. Synthesis and physico-chemical properties of new tetraethylammonium-based amino acid chiral ionic liquids. Molecules. 2010; 15: 2388-2397.

26. Abdul Rahman MB, Jumbri K, Hanafiah NAMA, Abdulmalek E, Tejo BA, Basri M, et al. Enzymatic esterification of fatty acid esters by tetraethylammonium amino acid ionic liquids-coated Candida rugosa lipase. J Mol Catal B: Enzym. 2012; 79: 61-65.

27. Moniruzzaman M, Ino K, Kamiya N, Goto M. Lipase incorporated ionic liquid polymers as active, stable and reusable biocatalysts. Org Biomol Chem. 2012; 10: 7707-7713.

28. Gamba M, Lapis AAM, Dupont J. Supported ionic liquid enzymatic catalysis for the production of biodiesel. Adv Synth Catal. 2008; 350: 160-164.

29. Hu Y, Tang SS, Jiang L, Zou B, Yang J, Huang H. Immobilization of Burkholderia cepacia lipase on functionalized ionic liquids modified mesoporous silica SBA-15. Process Biochem. 2012; 47: 2291-2299.

30. Zou B, Hu Y, Jiang L, Jia R, Huang H. Mesoporous material sba-15 modified by amino acid ionic liquid to immobilize lipase via ionic bonding and cross-linking method. Ind Eng Chem Res. 2013; 52: 2844-2851.

31. Jiang YY, Guo C, Xia HS, Mahmood I, Liu CZ, Liu HZ. Magnetic nanoparticles supported ionic liquids for lipase immobilization: enzyme activity in catalyzing esterification. J Mol Catal B: Enzym. 2009; 58: 103-109.

32. Jiang YY, Guo C, Gao HS, Xia HS, Mahmood I, Liu CZ, et al. Lipase immobilization on ionic liquid-modified magnetic nanoparticles: ionic liquids controlled esters hydrolysis at oil–water interface. AIChE J. 2012; 58: 1203-1211.

33. Zhou HC, Li W, Shou QH, Gao HS, Xu P, Deng FL, et al. Immobilization of penicillin G acylase on magnetic nanoparticles modified by ionic liquids. Chinese J Chem Eng. 2012; 20: 146-151.

34. Hara P, Mikkola JP, Murzin DY, Kanerva LT. Supported ionic liquids in Burkholderia cepacialipase-catalyzed asymmetric acylation. J Mol Catal B: Enzym. 2010; 67: 129-134.

35. Itoh T, Matsushita Y, Abe Y, Han S, Wada S, Hayase S, et al. Increased enantioselectivity and remarkable acceleration of lipase-catalyzed transesterification by using an imidazolium PEG- alkyl sulfate ionic liquid. Chemistry. 2006; 12: 9228-9237.

36. Cantone S, Hanefeld U, Basso A. Biocatalysis in non-conventional media—ionic liquids, supercritical fluids and the gas phase. Green Chem. 2007; 9: 954-971.

37. Lee SH, Doan TTN, Ha SH, Koo YM. Using ionic liquids to stabilize lipase within sol–gel derived silica. J Mol Catal B: Enzym. 2007; 45: 57-61.

38. Peter F, Poppe L, Kiss C, Szoc-Biro E, Preda G, Zarcula C, et al. Influence of precursors and additives on microbial lipases stabilized by sol-gel entrapment. Biocatal Biotransfor. 2005; 23: 251-260.

39. Lee SH, Doan TTN, Ha SH, Chang WJ, Koo YM. Influence of ionic liquids as additives on sol–gel immobilized lipase. J Mol Catal B: Enzym. 2007; 47: 129-134.

40. Zarcula C, Corici L, Croitoru R, Ursoiu A, Peter F. Preparation and properties of xerogels obtained by ionic liquid incorporation during the immobilization of lipase by the sol–gel method. J Mol Catal B: Enzym. 2010; 65: 79-86.

41. de Souza RL, de Faria ELP, Figueiredo RT, dos Santos Freitas L, Iglesias M, Mattedi S, et al. Protic ionic liquid as additive on lipase immobilization using silica sol–gel. Enzyme Microb Tech. 2013; 52: 141-150.

42. Kim MH, An S, Won K, Kim HJ, Lee SH. Entrapment of enzymes into cellulose–biopolymer composite hydrogel beads using biocompatible ionic liquid. J Mol Catal B: Enzym. 2012; 75: 68-72.

43. Peng S, Meng HC, Zhou L, Chang J. Synthesis of novel magnetic cellulose-chitosan composite microspheres and their application in laccase immobilization. J Nanosci Nanotechnol. 2014; 14: 7010-7014.

44. Fukushima T, Kosaka A, Ishimura Y, Yamamoto T, Takigawa T, Ishii N, et al. Molecular ordering of organic molten salts triggered by single-walled carbon nanotubes. Science. 2003; 300: 2072-2074.

45. Lee HK, Lee JK, Kim MJ, Lee CJ. Immobilization of lipase on single walled carbon nanotubes in ionic liquid. Bull Korean Chem Soc. 2010; 31: 650-652.

46. Díaz-Rodríguez A, Davis BG. Chemical modification in the creation of novel biocatalysts. Curr Opin Chem Biol. 2011; 15: 211-219.

47. Evran S, Telefoncu A. Modification of porcine pancreatic lipase with Z-proline. Prep Biochem Biotechnol. 2005; 35: 191-201.

48. Xiong YH, Su JH, Liu XP. Study on the property of pocine Pancreas lipase modified with phthalic anhydride. J South China Agric Univ. 2011; 32: 122-124.

49. Bekhouche M, Doumeche B, Blum LJ. Chemical modifications by ionic liquid-inspired cations improve the activity and the stability of formate dehydrogenase in [MMIm][Me_2PO_4]. J Mol Catal B: Enzym. 2010; 65: 73-78.

50. Bekhouche M, Blum LJ, Doumeche B. Ionic liquid-inspired cations covalently bound to formate dehydrogenase improve its stability and activity in ionic liquids. ChemCatChem. 2011; 3: 875-882.

51. Jia R, Hu Y, Liu L, Jiang L, Zou B, Huang H. Enhancing catalytic performance of porcine pancreatic lipase by covalent modification using functional ionic liquids. ACS Catal. 2013; 3: 1976-1983.

52. Jia R, Hu Y, Huang H. Improving the catalytic performance of porcine pancreatic lipase in the presence of [MMIm][$MeSO_4$] with the modification of functional ionic liquids. Process Biochem. 2014; 49: 668-672.