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Facilitating enzymatic reactions by using ionic liquids: A mini review

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Over the past two decades, ionic liquids (ILs) have been widely used for enzymatic conversions of substrates — especially substrates that are insoluble in common organic solvents and water — resulting in high conversion rates, high selectivity, and improved enzyme stability, wherein the ILs are recoverable and recyclable. Compared with performance in first-generation ILs, researchers recently considerably improved the technological utility of enzymes in second- and third-generation ILs composed of enzyme-benign cations and anions. Use of upgraded ILs with enzymes offers further improved activity and stability compared with research studies in the past decade, rendering IL-assisted biocatalytic processes more environmentally and economically attractive. This short review briefly presents recent developments of enzymatic reactions in ILs. The review covers approaches for and modifications of enzymes and ILs within the past 2 years for improved enzymes performance in ILs.

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Introduction

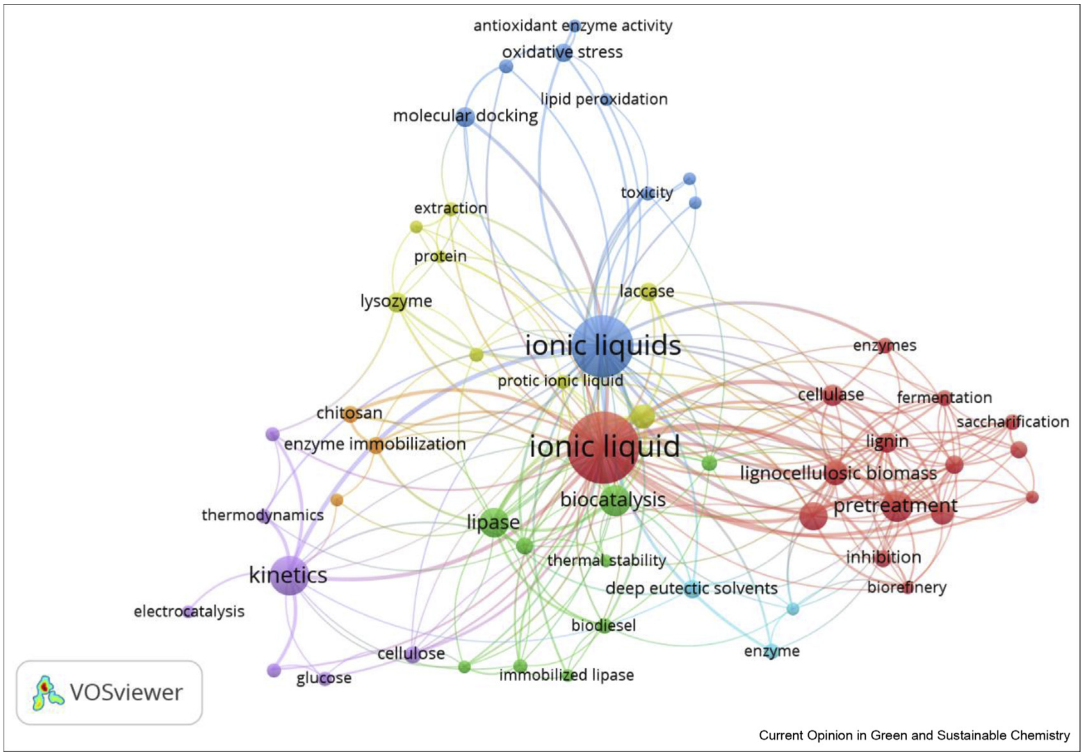
There is growing interest in using ionic liquids (ILs) for enzymatic reactions, including polymerization and other syntheses, wherein certain organic solvents are incompatible. Not all ILs are environmentally friendly. However, in the past 2 years, emphasis has moved from first-generation to second- and third-generation ILs, which are more compatible with enzymes [1,2] and can be derived from environmentally friendly and relatively inexpensive renewable sources [3,4]. A Scopus search of the literature from 2018 to 2020 generated many keywords related to ILs and enzymes, such as enzyme activity, enzyme immobilization, thermostability, biomass, pretreatment, and biofuel production. Figure 1 shows a corresponding network visualization created using VOSviewer.

The aim of this review is to highlight recent developments in IL-assisted enzymatic reactions, particularly those that are challenging to perform in water or organic solvents. In enzymatic reactions, ILs can be used in various ways. In this short review, we give special consideration to enzyme-catalyzed reaction innovations in ILs such as hydrolysis for biofuel production, transesterification, esterification, delignification of biomass, and new techniques for immobilization and stabilization, all reported within the past 2 years. This period has witnessed progressive applications; 625 articles were published with the keywords 'ionic liquid' and 'enzyme' from 2018 to the date of this review.

Second- and Third-generation ILs

Second-generation ILs are compatible with substances such as ion composites, energetic substances, and lubricants [5–7]. ILs' tailorable physicochemical properties facilitate development of new, useful materials. Third-generation ILs with biological activity can be active pharmaceutical ingredients [8–10]. Over the last few years, extensive reports of biocatalysis in second-generation ILs have demonstrated that a number of enzymes display outstanding selectivity and stability [11–14]. Enzymes have also maintained excellent operating and thermal stability in ILs. In other words, researchers have developed groundbreaking ILs with enhanced green efficiency. Recently, third-generation ILs have begun to emerge with the construction of abundant biodegradable and nontoxic ions, including naturally occurring sugars, amino acids, alkalis, and carboxylic acids [15]. Alkylpyridinium, dialkylimidazolium, phosphonium, and ammonium are extensively used cations. The commonly used anions are halides, hexafluorophosphates, and tetrafluoroborate. Researchers have used such ILs for both physical and chemical

Figure 1



Network visualization of keywords from the Scopus database (2018–2020) for the search terms ‘ionic liquids’ and ‘enzyme,’ as of July 20, 2020.

applications. Because third-generation ILs are pertinent to environmental and biological applications, they are of much interest [8–10].

Enzyme stabilization in ILs

Enzymes are biocatalysts that contribute considerably to current advances in industry, supporting various processes. As a consequence of their adaptability, some enzymes exhibit greater activities in ILs than without ILs. Enzymes are extensively used in industrial and research biocatalysis (in the pharmaceutical, waste management, food, and energy sectors). Furthermore, enzymes offer advantageous ecofriendly features compared with chemical catalysis [12]. Many studies have focused on the dynamic and structural characteristics of enzymes and other proteins dissolved in IL media [12,16,17]. Reactions catalyzed by enzymes can be performed in an IL-based monophasic or biphasic system consisting of ILs/supercritical CO_2, ILs/molecular solvents, or ILs/water. Researchers have used ILs as additives or reaction media for whole-cell processes, solvents, or cosolvents in such systems [18]. For instance, *Aspergillus niger* lipase showed enhanced activity in ILs with a short cation alkyl side chain length, namely, 1-butylimidazolium chloride, [bmim]Cl, and 1-hexyl-3-methylimidazolium chloride, [hmim]Cl [19]. Researchers have tested commercial proteases (Neutrase 0.8 L, Flavourzyme 500 L, and Alcalase 2.4 L) with the ILs choline chloride, tetramethylammonium bromide, and [emim]Br and observed a 20%, 15%, and 150% increase in protease activity, respectively, with respect to control samples [20]. IL-tolerant cellulose, obtained from the halophile *Stachybotrys microspora*, exhibited enhanced activity of 115.5% and 114.5% in the presence of 5 v/v% 1-ethyl-3-methylimidazolium diethyl phosphate, [emim]DEP, and 1-allyl-3-methylimidazolium chloride, [amim]Cl, respectively [21]. In the following sections, we will elaborate further on the reactions catalyzed by enzymes in ILs.

Current developments in enzymatic reactions in ILs

ILs can play a similar role as an organic solvent in influencing enzyme function as follows: (1) the IL replaces the water surrounding the enzyme; (2) when entering the microaqueous phase, the IL interacts with the enzyme by modifying the conformation, dynamics, or active site; and (3) the IL interacts with the products and substrates, by reacting with or changing their partitioning between the nonaqueous and aqueous phases [22]. Lipases are common components of IL–enzyme systems. Such reactions include surfactant synthesis [23], food and medicinal applications [24], ester synthesis [25], biodiesel preparation [26], transesterification [27], and interesterification. We therefore summarize recent studies of lipase-catalyzed reactions, followed by other enzymes (Table 1).

Fan et al [38] reported that the inactivation induced by ILs is consistent with reversible, competitive inhibition after investigating the kinetics of trypsin inhibition by various imidazolium- and ammonium-based ILs. After removing the IL, the enzyme can recover its activity (Figure 2).

Janati-Fard et al [39] studied the conformational stability and enzymatic activity of glucose oxidase in two imidazolium-based ILs: [bmim]Br and [hmim]Br. They found that hexyl derivatives have a more stabilizing effect than other imidazolium derivatives. Wang et al. [40] used *Candida antarctica* lipase B (CALB) for the resolution of (*R*,*S*)-1-(1-naphthyl)ethylamine [(*R*,*S*)-NEA] in [hmim]Tf_2N. They reported that the conversion of (*R*,*S*)-NEA and enantiomer excess of (*R*)-*n*-octyl acyl-NEA was 49.3% and 99.2%, respectively, under optimal conditions. Moreover, circular dichroism experiments showed that in [hmim]Tf_2N, CALB has a stable secondary structure and increased β-sheet content compared with no IL. A general comparison of enzymes in ILs with lipases in ILs is unavailable. Nevertheless, there has been a rapid increase in new studies such as catalase, chitinase, and oxidase. Furthermore, IL enzyme−catalyzed reactions are clearly an influential research topic.

Functionalization, immobilization, and modification of enzymes with ILs

Most enzymes can be deactivated in ILs, particularly hydrophilic enzymes. This limitation can be overcome by immobilization of the enzyme on a solid support to enhance its biocatalyst properties. One commercially available immobilized enzyme formulation for CALB is Novozyme 435, and it is the most extensively used lipase formulation. Additional examples include lipozyme (*Rhizomucor miehei* lipase; RML), proteinase K

Table 1

Enzymatic reactions in ionic liquids.

Entry	Enzyme	IL	Reaction	Main findings	Reference
1	CALB, TLL RML, CRL, lipases	[bmim]PF_6	Furoic acid → methyl-2-furoate (MF)	Yield of MF: 82.5% at 24 h Reusability: 1 cycle, 20% for 4 more cycles.	[28]
2	*Candida ruosa* lipase, type VII	[C_1C_3OHim] Tf_2N	Lipase-catalyzed Michael addition: hydroxycoumarin (4-HC) → benzylideneacetone (BA), warfarin	Warfarin yield was 83.7% and purity was 99.3%. Reusability: 5 cycles	[29]
3	*Aspergillus niger* EXF 4321	[emim]Tf_2N	2-phenyl ethanol + caffeic acid → caffeic acid esters + water	Yield: 84%. Reusability: 5 cycles	[30]
4	*Candida rugosa* lipase (CRL)	[emim]BF_4	(R,S)-Atenolol → (S)-atenolol acetate	Enantioselectivity (E) = 56.07, enantiomeric excesses of product (ee_p) = 95.23% Reusability: 5 cycles	[31]
5	*Burkholderia contaminans* (DFS3) lipase	[emim]CH_3SO_3	α-D-glucose + vinyl acetate → methyl 6-O-acetyl-α-D-glucopyranoside	Yield was 76%.	[32]
6	*Candida rugosa lipase,* type VII	[bmim]BF_4 [bmim]CH_3SO_4	Isopropanol + ketoprofen ethyl ester → ketoprofen	Yield was 45% Reusability: 3 cycles (90% activity)	[33]
7	*Trametes versicolor* laccase Immobilized on $Fe_3O_4@SiO_2@$KIT-6-NH_2 nanoparticles	[bmim]PF_6	Phenols and lignin degradation	Phenols: degradation 76.5% Lignin degradation: 77.3% Reusability: 11 cycles Enzyme activity: 70% after 21 days	[34]
8	α-amylase *Bacillus licheniformis*, type XII−A	[bmim]Br	Starch → glucose	90% of the activation remained for 2 weeks, and 50% remained for one month.	[35]
9	Chitinase from *Streptomyces albolongus* ATCC 27414	[emim]OAc	IL-chitin → N-acetylglucosamine	76.11%	[36]
10	Cellulase from *Trichoderma reesei*	[Cho]OAc	Hemicellulose and cellulose → glucose	Yield was 0.62 g of sugar/g of biomass. Cellulase was stable for 48 h.	[37]

[bmim]PF_6, 1-butyl- 3-methylimidazolium bishexafluorophosphate; [C_1C_3OHim]Tf_2N, 1-methyl-3-(3-hydroxypropyl) imidazolium bis(trifluoromethylsulfonyl)imid; CH_3SO_3, methanesulfonate; BF_4, tetrafluoroborate; CH_3SO_4, methylsulfate; OAc, acetate; CALB, *Candida antarctica* lipase B; IL, ionic liquid; TLL, *Thermomyces lanuginosus* lipase; RML, *Rhizomucor miehei* lipase; CRL, *Candida rugosa* lipase.

Figure 2

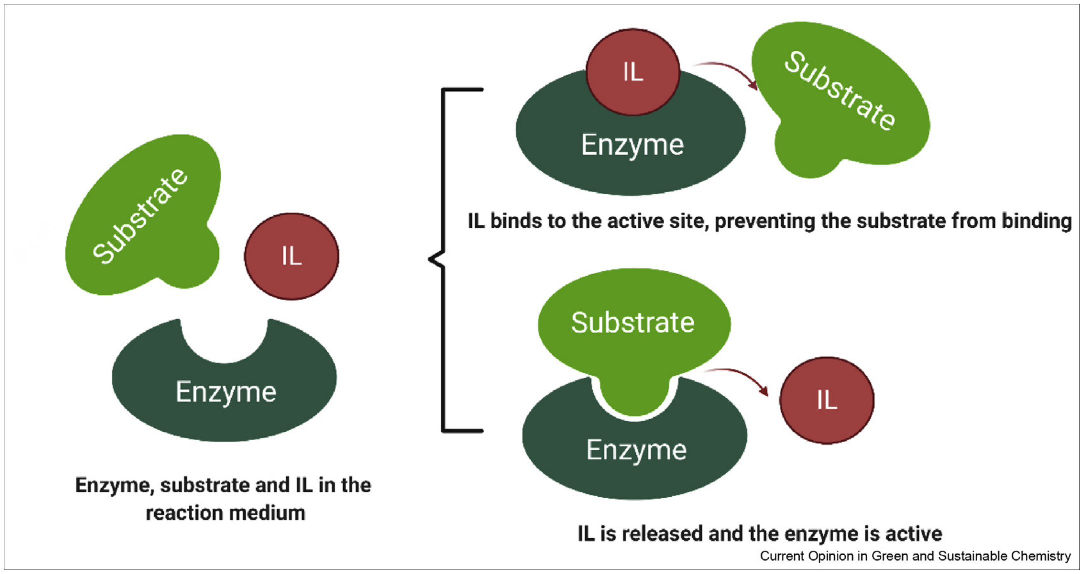


Illustration of reversible, competitive inhibition of enzymes in ILs. Created by Biorender.com web application (license ID: D67A6E35-0001). IL, ionic liquid.

(from *Tritirachium album*), immobilized Eupergit C, and many others.

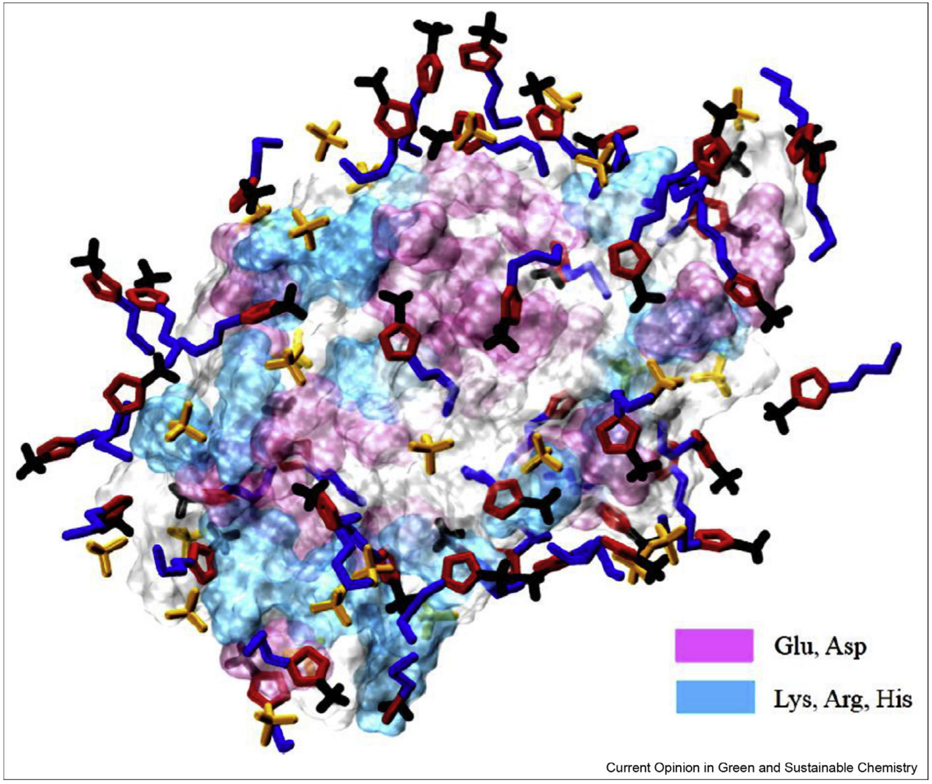
Several inorganic materials can be used as a support. Among those materials, Scherer et al. [41] explored mobile crystalline material–type mesoporous silicas from the M41S family synthesized using 1-hexadecyl-3-methylimidazolium chloride, [C_{16}mim]Cl. Based on their properties, such as uniformity and three-dimensional channel arrangement, mesoporous materials synthesized using ILs have potential as environmentally friendly materials for chemical processes. Immobilization of AK lipase presented a 66% esterification yield (ethyl and geranyl oleates) and an activity of 578 U/g in four cycles. Immobilization of porcine pancreatic lipase (PPL) was similarly performed on magnetic chitosan nanocomposites, modified by an imidazolium-based IL, wherein 91.5% of the PPL initial activity was maintained even after 10 cycles [42].

Fan et al. [43] used a hydroxyl-functionalized IL [C_1C_3OHPyr][Tf_2N] as the reaction medium to improve biodiesel yield to 85%. Barbosa et al. [44] investigated a phosphonium-based IL, [$P_{666(14)}$][Tf_2N], on the activity of immobilized *Burkholderia cepacia* lipase by two approaches: (i) IL–silica support and (ii) immobilization using ILs. On combining both approaches, the relative activity increased up to 231%, and the immobilization yield was 98%. Moreover, the biocatalyst material was recycled 26× while maintaining 50% of the activity. Researchers have integrated organic–inorganic nanoparticles, such as mesoporous silica SBA-15 and chitosan, using carboxyl-functionalized IL as a bridging agent (SBA–CIL–CS). The nanoparticles immobilized laccase (*Aspergillus oryzae*) through physical adsorption, wherein the enzyme retained 75.3% of its initial activity and reusability for five cycles with a 58.8% removal rate for 2,4-dichlorophenol [45].

Researchers have investigated ether-functionalized ILs in the ring-opening polymerization of ε-caprolactone at 70 °C, catalyzed by Novozyme 435. The IL [$CH_3OCH_2CH_2$-PBu_3][Tf_2N] generated the maximum molecular weight (up to 25,400 Da) in the polymerization reaction [46]. Qiu et al. [47] used modified magnetic nanoparticles using amino-functionalized ILs and dialdehyde starch as a cross-linker (Fe_3O_4–NIL–DAS). They achieved an immobilization efficiency of 85.8%; activity retention of 73.7%; removal efficiencies of 86.1%, 93.6%, and 100% for phenol, 4-chlorophenol, and 2,4-dichlorophenol, respectively; a stability higher than 80% after 30 days; and reusability over six cycles with 83% of the activity [47].

Xie and Wang [48] prepared magnetic Fe_3O_4/SiO_2 composite nanoparticles and used polymeric acidic IL (1-vinyl-3-(3-sulfopropyl)imidazolium hydrogen sulfate, [VSim][HSO_4]) for immobilization. The solid catalyst displayed high activity for both esterification of free fatty acids and transesterification of soybean oil. Researchers used ILs to modify magnetic chitosan nanoparticles (CS–Fe_3O_4) and used them to immobilize PPL, which showed a 382% improvement in the activity

Figure 3



Representative snapshot of the surface of α-lactalbumin as obtained from simulations, highlighting the distributions of the IL cations and anions (bmim$^+$ and BF_4^-). Reprinted with permission from Ghanta et al. [55]. Copyright (2020) American Chemical Society. IL, ionic liquid.

and withstood 10 cycles with 84.6% of its activity. Moreover, using a magnetic field, the immobilized enzyme was readily recovered [49]. Suo et al [50] formulated IL-modified magnetic nanoparticles of carboxymethyl cellulose (IL–MCMC) and used them as carriers for enzyme immobilization, whereby the specific activity of immobilized PPL was 1.43-fold higher than that of free lipase. Clearly, the emerging field of nanotechnology has enabled synthesis of more support materials for enzymatic reactions in ILs.

IL-coated enzymes for biocatalysis

ILs may offer protective functions and could act as a support for enzymes [51]. For instance, researchers used the pyridinium salt 1-ethylpyridin-1-ium cetyl-PEG10 sulfate for a *Burkholderia cepacia lipase* coating, whereby an alcohol transesterification proceeded more quickly than a free lipase reaction [52]. Room-temperature solid-phase ILs have been used to coat ω-transaminases with three different techniques: precipitation coating, melt coating, and colyophilization. Grabner et al. [53] found that melt coating and colyophilization increased the activity and recyclability of transaminases. Interestingly, the cofactor essential for transaminase activity (pyridoxal 5′-phosphate) was protected from degradation because of the coating. A unique study enhanced the activity of CALB in a water-like IL (an imidazolium-based IL functionalized with both ether and *tert*-alcohol groups). The water-like IL enabled very high transesterification up to 2- to 4-fold compared with commonly used ILs such as [bmim][Tf_2N] [54].

To understand the coating mechanism, Ghanta et al. [55] investigated α-lactalbumin (protein) in the presence of [bmim][BF_4] ranging from 20 v/v% to 80 v/v%. Calculations revealed that the protein, in general, tends to have reduced conformational fluctuations and is more rigid in the IL. The enhanced protein rigidity is associated with increased fractions of secondary structures, namely, α-helices and β-sheets. In addition, in the presence of the IL, the protein forms an increased number of salt bridges that are stronger than the intermolecular forces in neat aqueous solution [55] (Figure 3). We suggest that researchers extend this concept to enzymes.

Conclusions and prospects

In conclusion, ILs facilitate many enzyme-catalyzed reactions with outstanding yields, enhanced activities, and reusability, which are key factors for industrial applications. Thus, mechanisms and interactions of ILs and enzymes require further investigation to more fully understand activation or inhibition. Moreover, emerging nanotechnology in the field of ILs has expanded researchers' opportunity to tackle additional concerns with regard to stabilization and recyclability that until recently were not feasible. The immobilization technique, support material, and technology are crucial for both effective and sustainable processes. Although high yield of production is usually a main concern in industrial applications, recyclability and recovery of the biocatalyst is a major challenge that researchers are striving to tackle. There is no general rule that describes the enzymatic reactions in ILs; hence, more kinetics and molecular studies are needed. Nevertheless, technologies aimed at sustainable and green processing can overcome these obstacles.

Declaration of competing interest

Nothing declared.

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