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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

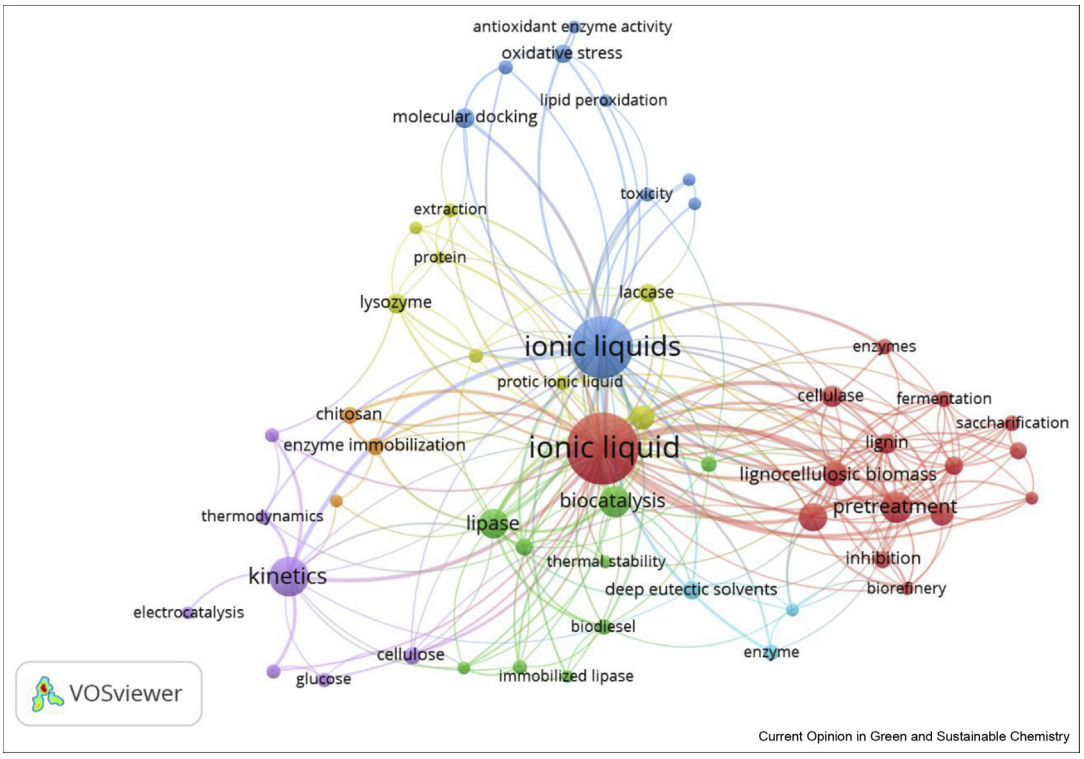
There is growing interest in using ionic liquids (ILs) for enzymatic reactions, including polymerization and other syntheses, wherein certain organic solvents are incompatible. Not all ILs are environmentally friendly. However, in the past 2 years, emphasis has moved from first-generation to second- 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,

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.

hexafluorophosphates, and tetrafluoroborate. Researchers have used such ILs for both physical and chemical 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

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

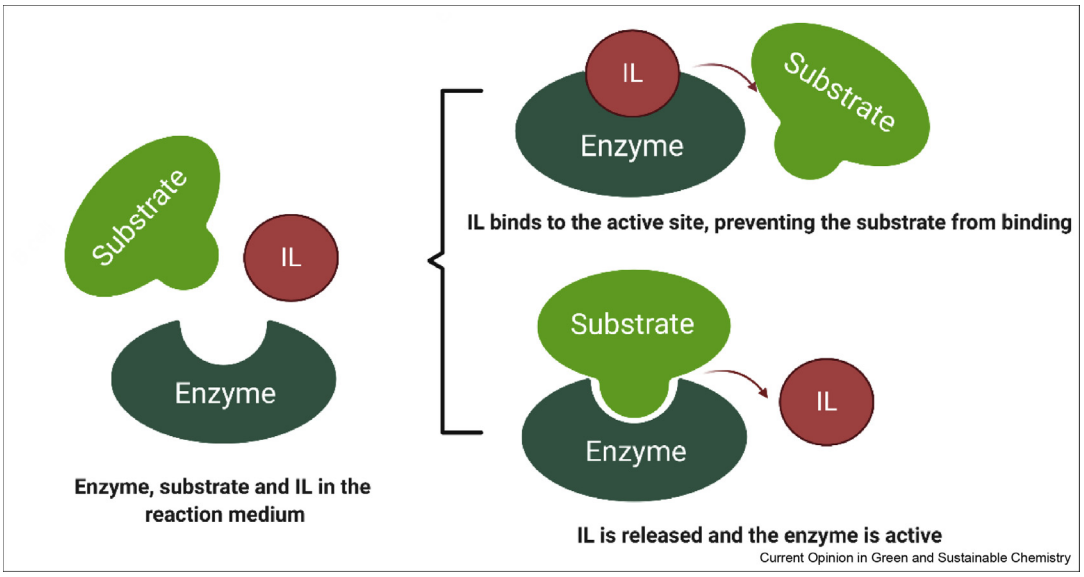


Illustration of reversible, competitive inhibition of enzymes in ILs. Created by Biorender.com web application (license ID: D67A6E35-0001). IL, ionic liquid.

lipozyme (*Rhizomucor miehei* lipase; RML), proteinase K (from *Tritirachium album*), immobilized Eupergit C, and many others.

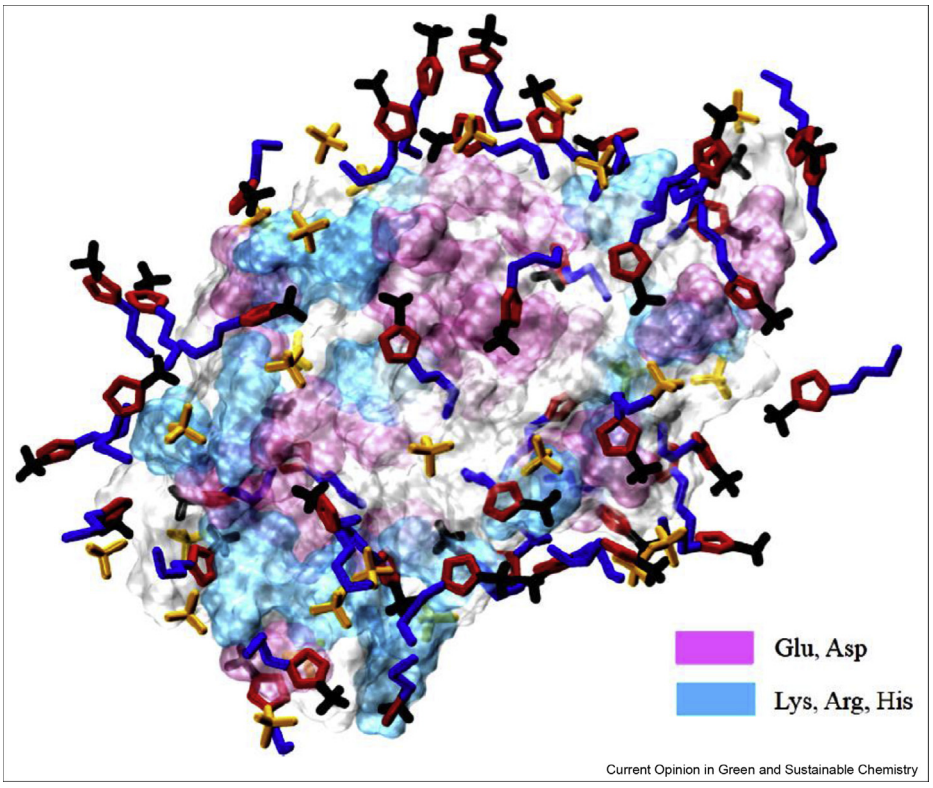
Several inorganic materials can be used as a support. Among those materials, Scherer et al. [41] explored mobile crystalline material—type mesoporous silicas from the M41S family synthesized using 1-hexadecyl-3-methylimidazolium chloride, $[C_{16}mim]Cl$. Based on their properties, such as uniformity 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 $[CH_3OCH_2CH_2\text{-}PBu_3][Tf_2N]$ 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

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.

PPL, which showed a 382% improvement in the activity 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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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Elgharbawy AAM, Moniruzzaman M, Goto M: **Recent advances of enzymatic reactions in ionic liquids: Part II**. *Biochem Eng J* 2020, **154**:107426. https://doi.org/10.1016/j.bej.2019.107426.

2. Schindl A, Hagen ML, Muzammal S, Gunasekera HAD, Croft AK: **Proteins in ionic liquids: reactions, applications, and futures**. *Front Chem* 2019, **7**:1–31.

3. Nasirpour N, Mohammadpourfard M, Heris SZ: **Ionic liquids: promising compounds for sustainable chemical processes and applications**. *Chem Eng Res Des* 2020, **160**:264–300.

4. Zappi D, Gabriele S, Gontrani L, Dini D, Sadun C, Marini F, Antonelli ML: **Biologically friendly room temperature ionic liquids and nanomaterials for the development of innovative enzymatic biosensors: Part II**. *Talanta* 2019, **194**:26–31.

5. Singh SK, Savoy AW: **Ionic liquids synthesis and applications: an overview**. *J Mol Liq* 2020, **297**:112038. https://doi.org/10.1016/j.molliq.2019.112038.

6. Sivapragasam M, Moniruzzaman M, Goto M: **An overview on the toxicological properties of ionic liquids toward microorganisms**. *Biotechnol J* 2020, **15**:1900073. https://onlinelibrary.wiley.com/doi/abs/10.1002/biot.201900073.

7. Umeda K, Kobayashi K, Minato T, Yamada H: **Atomic-scale three-dimensional local solvation structures of ionic liquids**. *J Phys Chem Lett* 2020, **11**:1343–1348.

8. Almeida HFD, Neves MC, Trindade T, Marrucho IM, Freire MG: **Supported ionic liquids as efficient materials to remove non-steroidal anti-inflammatory drugs from aqueous media**. *Chem Eng J* 2020, **381**:122616. https://doi.org/10.1016/j.cej.2019.122616.

9. Priyanka VP, Gardas RL: **Mono- and di- cationic ionic liquids based aqueous biphasic systems for the extraction of diclofenac sodium**. *Sep Purif Technol* 2020, **234**:116048. https://doi.org/10.1016/j.seppur.2019.116048.

10. Moshikur RM, Chowdhury MR, Wakabayashi R, Tahara Y, Moniruzzaman M, Goto M: **Ionic liquids with methotrexate moieties as a potential anticancer prodrug: synthesis, characterization and solubility evaluation**. *J Mol Liq* 2019, **278**:226–233.

11. Bisht M, Jha I, Venkatesu P: **Does choline-based amino acid ionic liquid behave as a biocompatible solvent for stem bromelain structure?** *Process Biochem* 2018, **74**:77–85.

12. Barbosa MS, Freire CCC, Souza RL, Cabrera-Padilla RY, Pereira MM, Freire MG, Lima ÁS, Soares CMF: **Effects of phosphonium-based ionic liquids on the lipase activity evaluated by experimental results and molecular docking**. *Biotechnol Prog* 2019, **35**:1–10.

13. Yang W, Zhou M, Yan L, Ju X, Li L: **Diversity of *Paenibacillus sp.* LLZ1 cellulase and its improved enzyme activity and stability in the ionic liquid 1-Ethyl-3-methylimidazolium diethyl phosphate**. *BioResources* 2019, **14**:3132–3145.

14. da Silva VG, de Castro RJS: **Biocatalytic action of proteases in ionic liquids: improvements on their enzymatic activity, thermal stability and kinetic parameters**. *Int J Biol Macromol* 2018, **114**:124–129.

15. Gomes FO, Maia LB, Delerue-Matos C, Moura I, Moura JJG, Morais S: **Third-generation electrochemical biosensor based on nitric oxide reductase immobilized in a multiwalled carbon nanotubes/1-n-butyl-3-methylimidazolium tetrafluoroborate nanocomposite for nitric oxide detection**. *Sensors Actuators, B Chem* 2019, **285**:445–452.

16. Calderón C, Contreras R, Campodónico R: **Surfactant-mediated enzymatic superactivity in water/ionic liquid mixtures, evaluated on a model hydrolytic reaction catalyzed by α-chymotrypsin**. *J Mol Liq* 2019, **283**:522–531.

17. Saha D, Mukherjee A: **Effect of water and ionic liquids on biomolecules**. *Biophys Rev* 2018, **10**:795–808.

18. Mai NL, Koo Y-M: **Enzymatic reactions in ionic liquids**. In *Emerging areas in bioengineering*. Edited by N Chang H, Ed, Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA; 2018:35–65.

19. Nascimento PAM, Pereira JFB, de Carvalho Santos-Ebinuma V: **Insights into the effect of imidazolium-based ionic liquids on chemical structure and hydrolytic activity of microbial lipase**. *Bioprocess Biosyst Eng* 2019. http://doi:10.1007/s00449-019-02121-w.

20. * Geniselli V, Janser R, De Castro S: **Biocatalytic action of proteases in ionic liquids : improvements on their enzymatic activity , thermal stability and kinetic parameters**. *Int J Biol Macromol* 2018, **114**:124–129.
In line with enzymatic reactions in ionic liquids, commercial proteases activity rises by 150% in contrast with the control samples. The most positive effects observed in this study included proteases with improved activity and stability properties and a higher affinity for the substrate.

21. Ben Hmad I, Gargouri A: **Ionic liquid-tolerant cellulase system of Stachybotrys microspora exploited in the in situ saccharification of lignocellulosic biomass**. *J Mol Liq* 2020, **310**:113167. https://doi.org/10.1016/j.molliq.2020.113167.

22. Fernandez FJH: **Keys for the use of ionic liquids as reaction media in enzyme-catalyzed processes**. *J Bioprocess Biotech* 2018, **8**:9–11.

23. Zhang Y, Zhen B, Li H, Feng Y: **Basic ionic liquid as catalyst and surfactant: green synthesis of quinazolinone in aqueous media**. *RSC Adv* 2018, **8**:36769–36774.

24. He W-S, Li L-L, Huang Q-J, Yin J, Cao X-C: **Highly efficient synthesis of phytosterol linolenate in the presence of Bronsted acidic ionic liquid**. *Food Chem* 2018, **263**:1–7.

25. Lozano P, Alvarez E, Nieto S, Villa R, Bernal JM, Donaire A: **Biocatalytic synthesis of panthenyl monoacyl esters in ionic liquids and deep eutectic solvents**. *Green Chem* 2019, **21**:3353–3361.

26. Carvalho NB, Vidal BT, Barbosa AS, Pereira MM, Mattedi S, Freitas LDS, Lima ÁS, Soares CMF: **Lipase immobilization on silica xerogel treated with protic ionic liquid and its application in biodiesel production from different oils**. *Int J Mol Sci* 2018, **19**:1829. https://doi.org/10.3390/ijms19071829.

27. Wang F, He S, Zhu C, Rabausch U, Streit W, Wang J: **The combine use of continuous-flow microchannel reactor and ionic liquid cosolvent for efficient biocatalysis of unpurified recombinant enzyme**. *J Chem Technol Biotechnol* 2018. https://doi.org/10.1002/jctb.5621.

28. Zhang Y, Di X, Wang W, Song M, Yu Q, Wang Z, Yuan Z, Chen X, Xu H, Guo Y: **Kinetic study of lipase-catalyzed esterification of furoic acid to methyl-2-furoate**. *Biochem Eng J* 2020, **161**: 107587. https://doi.org/10.1016/j.bej.2020.107587.

29. Fan Y, Cai D, Wang X, Yang L: **Ionic liquids: efficient media for the lipase-catalyzed Michael addition**. *Molecules* 2018, **23**: 2154. https://doi.org/10.3390/molecules23092154.

30. Rajapriya G, Morya VK, Mai NL, Koo YM: **Aspergillus Niger whole-cell catalyzed synthesis of caffeic acid phenethyl ester in ionic liquids**. *Enzyme Microb Technol* 2018, **111**:67–73.

31. Sikora A, Chałupka J, Marszałł MP: **The use of ion liquids as a trojan horse strategy in enzyme-catalyzed biotransformation of (R,S)-atenolol**. *Catalysts* 2020, **10**:1–11.

32. Villalobos MC, Gonçalves AG, Noseda MD, Mitchell DA, Krieger N: **A novel enzymatic method for the synthesis of methyl 6-O-acetyl-α-D-glucopyranoside using a fermented solid containing lipases produced by Burkholderia contaminans LTEB11**. *Process Biochem* 2018, **73**:86–93.

33. Park S, Doan TTN, Koo YM, Oh KK, Lee SH: **Ionic liquids as cosolvents for the lipase-catalyzed kinetic resolution of ketoprofen**. *Mol Catal* 2018, **459**:113–118.

34. Amin R, Khorshidi A, Shojaei AF, Rezaei S, Faramarzi MA: **Immobilization of laccase on modified Fe3O4@SiO2@Kit-6 magnetite nanoparticles for enhanced delignification of olive pomace bio-waste**. *Int J Biol Macromol* 2018, **114**:106–113.

35. Tonova K: **Long-term preservation of α-amylase activity in highly concentrated aqueous solutions of imidazolium ionic liquid**. *Green Process Synth* 2018, **7**:106–113.

36. Li J, Huang WC, Gao L, Sun J, Liu Z, Mao X: **Efficient enzymatic hydrolysis of ionic liquid pretreated chitin and its dissolution mechanism**. *Carbohydr Polym* 2019, **211**:329–335.

37. * Elgharbawy AA, Alam MZ, Moniruzzaman M, Kabbashi NA, Jamal P: **Chemical and structural changes of pretreated empty fruit bunch (EFB) in ionic liquid-cellulase compatible system for fermentability to bioethanol**. *3 Biotech* 2018, **8**:236. https://doi.org/10.1007/s13205-018-1253-8.

Pretreatment of biomass was conducted in an integrated system of ionic liquid (choline acetate) and locally-produced cellulase. The process yielded 87.3% of the theoretical glucose yield, where the majority of the cellulose was converted to glucose after 48 h. The same vessel was used for bioethanol production where the IL did not suppress the growth of the yeast.

38. Fan Y, Wang X, Li J, Zhang L, Yang L, Gao P, Zhou Z: **Kinetic study of the inhibition of ionic liquids on the trypsin activity**. *J Mol Liq* 2018, **252**:392–398.

39. Janati-Fard F, Housaindokht MR, Monhemi H, Esmaeili AA, Nakhaei Pour A: **The influence of two imidazolium-based ionic liquids on the structure and activity of glucose oxidase: experimental and theoretical studies**. *Int J Biol Macromol* 2018, **114**:656–665.

40. Wang B, Zhang C, He Q, Qin H, Liang G, Liu W: **Efficient resolution of (R,S)-1-(1-naphthyl)ethylamine by *Candida antarctica* lipase B in ionic liquids**. *Mol Catal* 2018, **448**:116–121.

41. Scherer GCRS, Nyari NLD, Hillesheim EL, Paulazzi AR, Da Silva BA, Zeni J, Mignoni ML: ***Pseudomonas fluorescens* AK lipase immobilization on MCM-48-type mesoporous support in the presence of ionic liquid**. *Ind Biotechnol* 2018, **14**:222–229.

42. ** Suo H, Xu L, Xu C, Chen H, Yu D, Gao Z, Huang H, Hu Y, Xu L, Xu C, *et al.*: **Enhancement of catalytic performance of porcine pancreatic lipase immobilized on functional ionic liquid modified Fe_3O_4-chitosan nanocomposites**. *Int J Biol Macromol* 2018, **119**:624–632.

Magnetic chitosan nanocomposites modified with functional IL were efficaciously prepared and applied as a novel support for the immobilization of lipase. The enzyme retained its activity for ten cycles and can be easily recovered by applying a magnetic field. This study showed the emerging application of nanotechnology and enzyme-catalyzed reactions.

43. Fan Y, Wang X, Zhang L, Li J, Yang L, Gao P, Zhou Z: **Lipase-catalyzed synthesis of biodiesel in a hydroxyl-functionalized ionic liquid**. *Chem Eng Res Des* 2018, **132**:199–207.

44. Barbosa MS, Santos AJ, Carvalho NB, Figueiredo RT, Pereira MM, Lima ÁS, Freire MG, Cabrera-Padilla RY, Soares CMF: **Enhanced activity of immobilized lipase by phosphonium-based ionic liquids used in the support preparation and immobilization process**. *ACS Sustain Chem Eng* 2019, **7**:15648–15659.

45. Qiu X, Qin J, Xu M, Kang L, Hu Y: **Organic-inorganic nanocomposites fabricated via functional ionic liquid as the bridging agent for laccase immobilization and its application in 2,4-dichlorophenol removal**. *Colloids Surf B Biointerfaces* 2019, **179**:260–269.

46. * Zhao H, Kanpadee N, Jindarat C: **Ether-functionalized ionic liquids for nonaqueous biocatalysis: effect of different cation cores**. *Process Biochem* 2019, **81**:104–112.

This is a unique study using ether-functionalized ILs in enzyme reactions due to their low viscosity in two reactions: (1) the transesterification of ethyl sorbate with 1-propanol (2) the ring-opening polymerization (ROP) of ε-caprolactone. Both reactions were conducted successfully at moderate temperatures. This study demonstrates the possibilities of advancements in ILs applications by introducing new ILs with modified properties.

47. Qiu X, Wang Y, Xue Y, Li W, Hu Y: **Laccase immobilized on magnetic nanoparticles modified by amino-functionalized ionic liquid via dialdehyde starch for phenolic compounds biodegradation**. *Chem Eng J* 2019, **391**:123456. https://doi.org/10.1016/j.cej.2019.123564.

48. Xie W, Wang H: **Immobilized polymeric sulfonated ionic liquid on core-shell structured Fe_3O_4/SiO_2 composites: a magnetically recyclable catalyst for simultaneous transesterification and esterifications of low-cost oils to biodiesel**. *Renew Energy* 2020, **145**:1709–1719.

49. Suo H, Gao Z, Xu L, Xu C, Yu D, Xiang X, Huang H, Hu Y: **Synthesis of functional ionic liquid modified magnetic chitosan nanoparticles for porcine pancreatic lipase immobilization**. *Mater Sci Eng C* 2019, **96**:356–364.

50. Suo H, Xu L, Xue Y, Qiu X, Huang H, Hu Y: **Ionic liquids-modified cellulose coated magnetic nanoparticles for enzyme immobilization: improvement of catalytic performance**. *Carbohydr Polym* 2020, **234**:115914. https://doi.org/10.1016/j.carbpol.2020.115914.

51. Bui-Le L, Clarke CJ, Bröhl A, Brogan APS, Arpino JAJ, Polizzi KM, Hallett JP: **Revealing the complexity of ionic liquid–protein interactions through a multi-technique investigation**. *Commun Chem* 2020, **3**:1–9.

52. Kadotani S, Nokami T, Itoh T: **Enhanced activity and modified substrate-favoritism of *Burkholderia cepacia* lipase by the treatment with a pyridinium alkyl-PEG sulfate ionic liquid**. *Tetrahedron* 2019, **75**:441–447.

53. ** Grabner B, Nazario MA, Gundersen MT, Loïs S, Fantini S, Bartsch S, Woodley JM: **Room-temperature solid phase ionic liquid (RTSPIL) coated ω -transaminases: development and application in organic solvents**. *Mol Catal* 2018, **452**:11–19.

The enzyme, ω-transaminases, was coated with room-temperature solid-phase ILs (RTSPILs) with there different techniques; (1) precipitation coating, (2) melt coating, and (3) co-lyophilization. Interestingly, not only the enzyme has enhanced activity and recyclability, but the cofactor essential for transaminase activity was protected from degradation due to the coating.

54. ** Zhao H, Harter GA, Martin CJ: **"water-like" dual-functionalized ionic liquids for enzyme activation**. *ACS Omega* 2019, **4**: 15234–15239.

A unique combination of the water-like structure of IL designed using ether and *tert*-alcohol groups enables very high synthetic activity of lipase (CALB). This study is the first report in mimicking water structure to enhance enzyme activity.

55. Ghanta KP, Pal T, Mondal S, Bandyopadhyay S: **Microscopic understanding of the effect of ionic liquid on protein from molecular simulation studies**. *J Phys Chem B* 2020, **124**:3909–3921.