Received: 21 September 2011 Revised: 18 December 2011 Accepted: 4 January 2012 Published online in Wiley Online Library: 20 February 2012

(wileyonlinelibrary.com) DOI 10.1002/jctb.3745

Use of ionic liquids for biocatalytic synthesis of sugar derivatives

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Abstract

Sugar-based compounds are widely used in pharmaceuticals, cosmetics, detergents and food. They are mainly produced by chemical methods, but the use of enzymes as 'a greener alternative' to organic synthesis has been investigated for more than 20 years. Due to the low polar substrate solubility in organic solvents compatible with enzymes, research has focused on the application of substitutes for biocatalysis, especially ionic liquids (ILs). After introducing the main properties of ILs and especially their ability to solubilize sugars, this review focuses on one of their applications, the biocatalytic synthesis of carbohydrate derivatives. In this context, they can be used in pure IL systems, in IL/IL systems or in IL/organic solvent systems. Finally, this review provides an update on the environmental fate of ILs. Their exploitation in 'green' processes is still limited due to their low degradability but research is currently under way to design new more 'eco-friendly' ILs.

Keywords: biocatalysis; enzymes; lipases; green chemistry; ionic liquids; carbohydrates

NOTATION

Cations

[Mmim]: 1-methyl-3-methylimidazolium
[Emim]: 1-ethyl-3-methylimidazolium
[Pmim]: 1-propyl-3-methylimidazolium
[Bmim]: 1-butyl-3-methylimidazolium
[Pentmim]: 1-pentyl-3-methylimidazolium
[Hmim]: 1-hexyl-3-methylimidazolium
[Omim]: 1-octyl-3-methylimidazolium
[Bdmim]: 1-butyl-2,3-dimethylimidazolium
[Hepmim]: 1-heptyl-3-methylimidazolium
[sBmim]: 1-sec-butyl-3-methylimidazolium
[MOEmim]: 1-methoxyethyl-3-methylimidazolium
[Glycol-Et-Im]: 1-ethyl-3-glycolimidazolium
[$Me(OEt)_3$-Et-Im]: 1-ethyl-3-(2-(2-methoxyethoxy)ethoxy) ethyl)imidazolium
[$Me(OEt)_3$-Et_3N]: Triethyl (2-(2-methoxyethoxy) ethoxy)ethylammonium
[TMBA]: N-trimethyl-N-butylammonium
[TOMA]: Trioctylmethylammonium
[TEMA]: Triethylmethylammonium
[EtN]: Ethylammonium
[Et_2N]: N-diethylammonium
[PrN]: N-propylammonium
[Me_2N]: N-dimethylammonium
[Bu_3N]: N-tributylammonium
[Bu_4N]: N-tetrabutylammonium
[Bmpyrr]: 1-butyl-3-methylpyrrolidinium
[MOEmpyrr]: 1-methoxyethyl-3-methylpyrrolidinium
[Bmpyr]: 1-butyl-3-methylpyridinium
[Bpyr]: 1-butylpyridinium
[Ppyr]: 1-propylpyridinium
[Ompyr]: 1-octyl-3-methylpyridinium
[Hpyr]: N-hexylpyridinium
[MeOcPyr]: 3-methyl-1-octylpyridinium
[$(C_6)_3C_{14}P$]: Trihexyl(tetradecyl)phosphonium
[CABHEM]: PEG-5-cocomonium methylsulfate

Anions

[BF_4]: Tetrafluoroborate
[PF_6]: Hexafluorophosphate
[TFO]: Trifluorosulfonylimide
[Tf_2N]: Bis(trifluoromethanesulfonyl)imide
[$MeSO_4$]: Methylsulfate
[$EtSO_4$]: Ethylsulfate
[$OctSO_4$]: Octylsulfate
[$MDEGSO_4$]: Diethyleneglycolmonomethylethersulfate
[Br]: Bromide
[Cl]: Chloride
[dca]: Dicyanamide
[TOS]: Tosylate
[lactate]: Lactate
[OAc]: Acetate
[NO_3]: Nitrate
[$EtNO_3$]: Ethyl nitrate

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INTRODUCTION

Many sugar derivatives are useful for a large range of commercial applications in pharmaceuticals, cosmetics, detergents and food sectors. These compounds are generally produced by organic synthesis. Over the last 20 years, numerous studies have shown the huge potential of enzymes, as 'greener alternative' to organic synthesis, in carbohydrate chemistry.[1] Hydrophilic solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF) and tetrahydrofuran (THF) which are able to solubilize saccharides at high concentrations, generally have a negative impact on the enzymatic activity. On the other hand, solvents that are more compatible with enzyme activity are not effective for the solubilization of sugars and do not allow the obtention of high yields. Therefore, the development of alternatives to organic solvents is required in order to fully develop enzymatic processes for carbohydrate modification. In this context, researchers have manifested a rising interest in ionic liquids (ILs) for biotransformation as attested by the numerous publications available over the last decade.[2–6] This review first gives a short description of ILs and describes their properties, in particular those related to their advantages and disadvantages for biocatalysis. The second part focuses on the enzymatic modifications of carbohydrates and their solubility in ionic liquid systems. Finally, this review provides an update on the environmental fate of ILs, their degradability and the possibility of designing more 'eco-friendly' ILs.

IONIC LIQUIDS: NEW ALTERNATIVE MEDIA FOR BIOCATALYSIS

Generalities

The first IL [EtN][NO_3] was developed 90 years ago but its application in research was not realistic due to its explosive nature.[7] The use of ILs in chemical synthesis began in the 1990s only.[8,9]

ILs are a new class of purely ionic, salt-like materials that are liquid at unusually low temperatures. Their melting point is below 100 °C and some of them even have melting point below 0 °C. Because of this property, they are called 'room temperature ionic liquids' (RTILs). They remain liquid up to their decomposition temperature (300–400 °C).

ILs are composed of a large asymmetrical organic cation and a smaller organic or inorganic anion. Compared with typical inorganic salts, their asymmetry prevents crystallization and therefore drastically decreases the ILs melting point.[10]

The strong ionic interactions within this class of solvents result in negligible vapor pressure, non-flammable, highly thermal, mechanically and electrochemically stable products. Spectroscopic (infrared, NMR) and diffraction (Raman, X-ray) studies, theoretical calculations and simulations (molecular modeling) of ILs confirmed the presence of a hydrogen-bond network and Van der Waals interactions. They are considered 'hydrogen bond polymeric supra-molecules' with polar areas (similar to water) and non-polar areas (similar to organic hydrophobic solvents).[11,12] Consequently, ILs cannot be considered as homogeneous solvents. The ions nature has a large influence on ILs properties. Typical structures of the most common anions and cations composing ILs are shown in Fig. 1.

ILs characteristics can be modulated by a judicious choice of the ions; which is why ILs are considered to be 'tunable solvents'.[4] An IL could be designed for each particular application. The combination of the available anions and cations gives the possibility of designing 10^{18} ILs in theory. Currently, approximately 300 ILs are commercially available and about 1000 ILs have been reported in the literature.[13]

The physico-chemical properties influencing biocatalysis

Viscosity

As a rule, ILs are much more viscous than conventional organic solvents. Their viscosity, comparable with those of oils, varies from 10 to more than 1000 cP at room temperature.[14] This can be a disadvantage for biocatalysis, as it may lead to limitation of the mass transfer. Nevertheless, the viscosity can be minimized by increasing the temperature. Moreover, the choice of anion strongly influences the IL's viscosity. For example, small anions such as dicyanamide (dca^-) give less viscous ILs than fluorinated anions.[15]

Polarity

Solvent polarity has an important impact in biocatalysis. Apolar solvents are unable to dissolve polar substrates but on the other hand, polar solvents such as DMSO or DMF tend to deactivate enzymes. Polarity influences both enzymatic activity and stability. The polarity of ILs depends on the anion size, on the nature of the substituent of the cation and its alkyl chain length. Small cations and anions will lead to highly polar ILs.[15] Solvent polarity can be easily determined by the E^N_T Reichardt's scale.[16] According to this scale, ILs are considered as moderately polar with values close to those found for short chain alcohols like methanol and ethanol, as shown in Table 1.[15,17,18]

Hydrophobicity

Anions and cations have an impact on the hydrophilic/hydrophobic character of an IL. Indeed, the alkyl chain length on the cation as well as the nature of the anion (for example, PF_6^- is more hydrophobic than BF_4^-) can strongly influence this property.[12] Hydrophobicity is quantified by the logP value, which is the logarithm of the partition coefficient of a compound between water and 1-octanol.[12] According to the values of their logP, ILs seem to be very hydrophilic compared with organic solvents (logP = −2.39 for [Bmim][PF_6], −0.33 for acetonitrile and 3.5 for hexane).[16] Depending on the enzymatic reaction, hydrophobic ionic liquids can enhance the enzymatic activity or not; this will be discussed in more detail below with the results reported for the biocatalyzed synthesis of glycosylated compounds in pure ILs.

Stability of ILs

The stability of ILs seems principally dependent on the anion nature. Despite their well known air, moisture and temperature stability, the halogenated BF_4^-, PF_6^- and Cl^- anions may generate, via hydrolysis and thermal decomposition, volatile and toxic compounds such as hydrofluoric acid (HF), trifluorophosphate (POF_3) and hydrochloric acid (HCl), which deactivate most enzymes.[14,16] However, [Tf_2N] and [TFO] based ILs constitute an exception and are the most hydrolytically stable.[2] However, it is advisable to use ILs based on non-halogenated anions such as octylsulfates ($OctSO_4^-$), which are not prone to hydrolysis.[2,4,19]

Enzyme solubility and stability

The hydrophilic ILs based on Cl^-, dca^-, NO_3^-, $EtNO_3^-$ or lactate anions are suitable for solubilizing enzymes more readily than

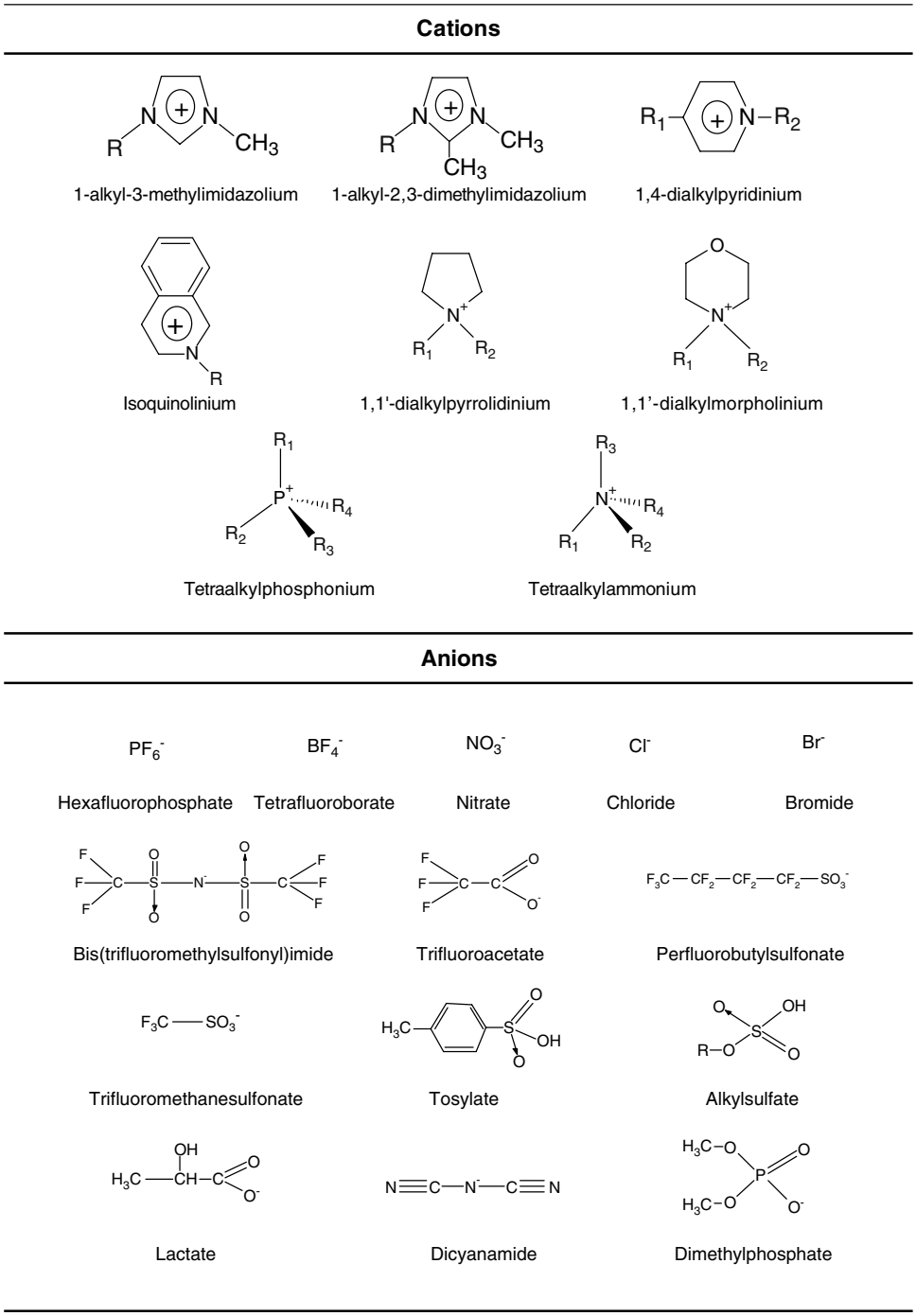

Figure 1. Structure of the major cations and anions used to form ILs.

hydrophobic ILs. Nevertheless, the enzymatic activity can be low since they may cause deactivation of the enzyme, probably due to strong hydrogen interactions with these solvents.[12,20,21]

For example, the conversion rate of the CAL-B-catalyzed transesterification of ethyl butanoate with 1-butanol in [Bmim][BF_4] and [Bmim][PF_6] (78% and 71%, respectively) is comparable with what was obtained in tert-butanol (74%). With ILs containing NO_3^-, $EtSO_4^-$ or lactate anion, a loss of activity is noticed (26% of conversion in [TEMA][$MeSO_4$]) because of their high ability to solubilize the enzyme while the enzyme is dispersed in [Bmim][BF_4], [Bmim][PF_6] and tert-butyl alcohol.[22] Infrared spectroscopy studies showed that the strong interaction by H-bonding of some hydrophilic ILs lead to a conformational change of the free CAL-B and therefore a loss of activity.[22]

Klähn and co-workers[23] recently studied the interaction between the anions (NO_3^-, BF_4^- or PF_6^-) and the cations (imidazolium or guanidinium) of eight ILs and CAL-B by molecular dynamic simulation in combination with an atomistic empirical force field. The anions coordinate directly with polar and charged groups of enzymes through strong H-bonding. Two factors influence the enzyme–anion interaction strength: the anion size and its hydrophobicity. For example [Bmim][NO_3] tends to

Table 1. E^N_T values of various ILs and organic solvents at a given temperature (18). The E^N_T Reichardt's scale is a dimensionless scale based on the position shift of the charge-transfer absorption band of a probe within the visible spectrum in the presence of a solvent. Water ($E^N_T = 1$) and trimethylsilane ($E^N_T = 0$) are used as references[15,17,18]

	Ionic liquid	Temperature (°C)	E^N_T		Ionic liquid	Temperature (°C)	E^N_T
Imidazolium	[Emim][BF_4]	RT	0.710	**Pyridinium**	[Ppyr][BF_4]	RT	0.661
	[Emim][Tf_2N]	RT	0.690		[Bpyr][BF_4]	RT	0.639
	[Bmim][BF_4]	RT	0.680		[Pmpyr][BF_4]	RT	0.670
	[Bmim][PF_6]	RT	0.676		[Bmpyr][BF_4]	RT	0.630
	[Bmim][TFO]	25	0.667		[EtN][NO_3]	RT	0,954
	[Bmim][Tf_2N]	RT	0.645		[PrN][NO_3]	RT	0,923
	[Bmim][Cl]	25	0.614	**Ammonium**	[$(Me)_2N$][Cl]	130	0,914
	[Bmim][NO_3]	RT	0.651		[$(Et)_2N$][NO_3]	RT	1,074
	[Hmim][BF_4]	25	0.707		[$(BU)_3N$][NO_3]	RT	0,803
	[Hmim][PF_6]	25	0.657		[$(Bu)_4N$][Br]	105–130	0,389
	[Hmim][Tf_2N]	RT	0.654		[$(Oc)_4$ MeN][Cl]	125	0,414
	[Hmim][Cl]	25	0.562	**Pyrrolidinium**	[Bmpyrr][Tf_2N]	25	0,544
	[Omim][BF_4]	25	0.670		[MOEmpyrr][NO_3]	RT	0.840
	[Omim][PF_6]	RT	0.636		[MOEmpyrr][Ac]	RT	0,519
	[Omim][Tf_2N]	RT	0.630		[MOEmpyrr][TFO]	RT	0.911

Organic solvent	**Temperature (°C)**	**E^N_T**
THF	25	0.2
Acetone	25	0.35
DMF	25	0.4
DMSO	25	0.45
Ethanol	25	0.65
Methanol	25	0.75
Ethylene glycol	25	0.8

solubilize and denature the enzyme while [Bmim][PF_6] stabilizes the enzyme. Indeed, there are stronger and more interactions between NO_3^- and CAL-B than between PF_6^- and CAL-B because NO_3^- is less hydrophobic and is present in greater numbers in the neighborhood of the enzyme due to its smaller size.[23] Thus, the anion-CAL-B interactions through Coulomb strengths dominate the Van der Waals cation-CAL-B interactions. However, the role of the cation on enzyme–ILs interactions has also to been highlighted despite their minor impact compared to anions. As observed with Bmim cations, the charged region of the protein can interact with the charged ring group of the cation and the butyl group can diffuse easily to the active site to the enzyme preventing substrates to enter and leave the active site of CAL-B.[23]

Recently, some hydrophilic ILs with ether functions have been designed with the aim to dissolve CAL-B in its free form and to improve Novozym's 435® stability.[12,22,24] The improved stability of Novozym 435® in [$Me(OEt)_3$-Et-Im][OAc] compared with tert-BuOH for the transesterification of D-glucose with vinyl laurate has been confirmed by the increase of the conversion rate of D-glucose with decreasing tert-BuOH content (68% with 40% of [$Me(OEt)_3$-Et-Im][OAc] and 60% of tert-BuOH and 85% with 100% of [$Me(OEt)_3$-Et-Im][OAc]).[24]

Advantages and limitations of ILs used as solvent in biocatalysis

ILs offer many advantages for use in biocatalysis compared to conventional organic solvents:[10,25] safe handling as they are non-flammable and non-explosive, low vapor pressure at room temperature and at the temperatures usually used in enzymatic reactions (from 35 to 70 °C), high thermal and chemical stability, solubilization of a large range of compounds.

Their variable water and organic solvent miscibility allows the development of convenient extraction methods. Their characteristics can be easily modified and tuned to a targeted process by adjusting the cation/anion couple. They can be produced by organic chemistry under mild conditions.

Another advantage of using ILs instead of organic solvent in biocatalysis is their capacity to enhance the regio-, stereo-, enantio-selectivity. For example, the regioselective acylation of methyl-6-*O*-trityl-glucosides and galactosides by lipase showed better selectivity in [Bmim][PF_6] and [MOEmin][PF_6] than in THF and chloroform. This improvement of selectivity had been explained by the better structural adaptation of the enzyme in ILs than in organic solvents due to their higher polarity.[26] The same trend was observed for the lipase transesterification of tertiary alcohols by vinyl acetate where the enantioselectivity is improved up to 25 times in ILs compared with organic solvents such as THF and toluene.[27]

Nevertheless, despite their easy synthesis by organic chemistry, the ILs production route can leave traces of contaminants such as halides, water, and inorganic species. Thus their physico-chemical properties can be changed and side reactions can occur.[2] Also, the presence of impurities can cause an alteration of the biocatalyst. For instance, the negative effect of chloride ion impurity in [Omim][Cl] on the activity of lipases had been shown, with a significant decrease of the enzymatic activity with an increase of Cl^- concentration.[28] Their total elimination can be arduous because of their negligible volatility. The effect of ILs on the environment and human health has not yet been fully investigated and despite their potential to be recycled, ILs remain expensive compared with organic solvents, which limits their use.

ENZYMATIC SYNTHESIS OF SUGAR DERIVATIVES IN ILS SYSTEMS

Carbohydrates solubility in ILs

The outcome of the biocatalyzed synthesis of glycosylated compounds (reaction rate, yield, selectivity) depends to a great extent on the substrates solubility in the reaction medium. Saccharides, flavonoïds and nucleosides, the most frequently used substrates to synthesize glycosylated compounds by biocatalysis, are poorly soluble in common organic solvents compatible with enzyme activity and stability. ILs can thus be valuable alternatives for biotransformation of these substrates.

Many investigations are currently underway to find the best ILs to solubilize high concentrations of mono-, di- and polysaccharides and other studies aim to better understand the solubilization mechanism and to highlight the role of the ILs anion and cation.

1-alkyl-3-methylbenzotriazolium based ILs containing nucleophilic anions such as Cl^-, Br^-, dca^-, formate ($HCOO^-$) and acetate (OAc^-) are found to be the best candidates for dissolving monosaccharides such as glucose, fructose and disaccharides like sucrose and lactose as well as β-cyclodextrin.[20,29–33] For example, [Bmim][dca] is able to dissolve β-D-glucose at a much higher concentration (145 g L^{-1}, 25 °C) than [Bmim][BF_4] and [Bmim][PF_6] in which the glucose solubility is less than 0.5 g L^{-1}.[30] These observations demonstrate the large influence of the ILs anion on carbohydrate solubility. The nucleophilic anions are well suited to dissolve carbohydrates as they have a high ability to form a strong H-bond with these substrates.[10,32] However, the anion nucleophilicity leads to the denaturation of enzymes preventing any sugar modification in these medium.[32] To overcome this problem, investigations are being carried out to design new ILs able to solubilize high concentrations of polar substrates without denaturing the enzymes. A suitable strategy must be followed view the large range of ILs potentially available.[34]

Zhao and co-workers[24,31] have designed enzyme-compatible ether-functionalized and glycol-substituted imidazolium and tetraalkylammonium cations coupled with high nucleophilic anions such as acetate and bromide: [$Me(OEt)_3$-Et_3N][OAc], ([Glycol-Et-Im][Br], [Gycol-R_2-N][Br] or [$Me(OEt)_3$-Et-Im][OAc] for examples. In [$Me(OEt)_3$-Et-Im][OAc], the solubility of D-glucose reached 80% wt at 60 °C in and its transesterification with vinyl laurate catalyzed by Novozym 435® gave 85% yield. The anion part of the IL allows high sugar solubility while the cation part stabilizes the enzyme.

Other research teams have developed methods to increase the dissolved sugar concentrations in ILs known to be compatible with enzyme activity. They obtained metastable supersaturated sugar solutions by either the 'conventional' method or the 'water-mediated' method in [Bmim][TFO] and [Bmim][BF_4], known to be the best ILs for enzymatic yields and initial rates.[20,21,35,36] These resulting solutions contain more dissolved solutes than the solubility limit leading to a metastable supersaturated solution of carbohydrates ready for biotransformation.[20] The 'conventional' method is based on the solubilization of carbohydrates in excess by heating at 60 °C and then cooling the solution to 25 °C followed by the removal of the undissolved sugars by centrifugation. The 'water-mediated' method has been introduced as an alternative to the conventional method in order to shorten the solubilization time. Indeed the carbohydrates solubility is much higher in water and as showed in a recent study of simulated glucose solubility in water-mediated [Emim][TFO], water breaks the glucose–glucose interactions, improving the glucose–TFO^- interactions.[37] An aqueous solution of sugar is mixed with the ILs and the water is then removed by vacuum evaporation after dissolution.[20] For instance, supersaturation of [Bmim][TFO] and [Bmim][BF_4] with glucose by the 'water-mediated' method enables concentrations 12 times (363.3 mmol L^{-1}) and 7 times higher (43.4 mmol L^{-1}) in each IL, respectively, compared with classical saturated solutions of glucose in these ILs.[38] This improvement is correlated with an increase in the initial rate as seen for the lipase-catalyzed synthesis of 6-*O*-lauroyl-D-glucose, where the rates are 4.29 and 1.29 µmol min^{-1} g^{-1} in [Bmim][TFO] supersaturated solution and saturated solution, respectively.[38]

Generally, the interest in ILs for the polysaccharides field mainly concerns their extraction and their chemical or enzymatic breakdown.[39,40] Imidazolium based ILs associated with the highly coordinated Cl^-, $MeSO_4^-$ and CH_3COO^- anions, have been shown to be suitable for dissolving cellulose and lignocellulose and hence, may be useful for the conversion of lignocellulosic biomass into new biomaterials, chemical intermediates and biofuels.[41–46] The dissolution mechanism of lignocellulosic biomass investigated by NMR, and computational methods such as the dispersion-corrected density functional theory (DFT-D), coupled with experimental studies show the predominant role of the anions in polysaccharides solubility.[41,44] The interactions between cellulose, lignocellulose and [Bmim][Cl], [Mmim][Cl] or [Mmim][PF_6] studied by molecular modeling supported by NMR are stronger between Cl^- and these polysaccharides than with PF_6^- because the hydrogen-bond network in the carbohydrates is broken in the presence of the former anion.[41] The ILs cation has also a role in the dissolution of lignocellulosic biomass. Indeed the solubility of cellulose decreases with increasing length of the alkyl chain on the imidazolium cations.[47] Cations containing oxygen in their alkyl chain allow additional H-bonds with carbohydrates and thus increase their solubility.[30,48]

Enzyme denaturation prevents any biotransformation of polysaccharides in [Bmim][Cl]. However, Gremos and co-workers successfully esterified cellulose by biocatalysis using [Bmim][Cl] in a pre-treatment step. The IL allows one to obtain an amorphous cellulose by breaking down the numerous intra- and inter-H-bonds between the cellulose chains. This resulting amorphous cellulose is extracted and esterified in a solvent-free system with immobilized esterase from hog liver or immobilized cutinase from *F. solani*. Despite the low degree of esterification with vinyl propionate, vinyl laurate and vinyl sterarate (1.9%, 1.3% and 0.9%, respectively, with cutinase *F. solani*), these results prove the high capacity of ILs for opening new opportunities in enzymatic acylation of polysaccharides for which no suitable organic solvent have been found until now.[42] The interest in esterified polysaccharides for the production of fibers, plastics, films, cosmetics and drugs has become more and more important over the past 10 years.[42,47]

A computational approach, COSMO-RS, has been used to predict the IL solubility of flavonoïds, other interesting substrates for the biocatalyzed synthesis of glycosylated compounds. This has allowed the classification of the ILs tested into three groups from the highest to the lowest solvating power.[34] The most nucleophilic ILs belonging to group I, and group II possess strongly coordinated anions like Cl^- and TFA^- (group I) or sulfate and sulfonate anions (group II). They are able to solubilize the highest concentrations of polyhydroxylated compounds such as esculin, but dramatically reduced or no biocatalysis takes place in these media due to increased interactions between the ILs and the enzymes via H-bonds. In contrast, ILs from group III having low coordinated anions such as BF_4^- and PF_6^- show the highest bioconversion yields.

Type of reaction systems

Pure ILs systems

The first successful use of ILs for the biocatalytic synthesis of glycosylated compounds was reported by Park and Kazlaukas in 2001.[49] The regioselective acylation of D-glucose with vinyl acetate was catalyzed by Novozym 435® in pure IL systems (Table 2). Compared with the results obtained in acetone or THF, the conversion rate as well as the regioselectivity is improved or at least equivalent in all ILs tested.[49] Indeed, the best conversion reached in [MOEmim][BF4] is 99%, equal to that obtained in THF but the proportion of monoacylated compounds in this IL is improved compared with THF (93% and 53%, respectively). The first enzymatic acylations of carbohydrates with long alkyl chain donors were carried out in the most common fluorinated anion based-ILs, [Bmim][BF_4] and [Bmim][PF_6].[50] For the enzymatic synthesis of 6-*O*-lauroyl-D-glucose with vinyl laurate by poly(ethylene glycol)-modified CAL-B (Fig. 2(A)), the hydrophobic [Bmim][PF_6] leads to a slightly better yield (35%) than the hydrophilic [Bmim][BF_4] (30%).[50,51] No reaction took place with fatty acids.[50] However, these first observations are not concordant with other results obtained recently. Indeed, according to the results collected in Table 2, the lipase synthesis of sugar esters in hydrophilic ILs showed better yields than in hydrophobic ones.[20,21] This is confirmed by the enzymatic acylation of konjac glucomannan (KGM), where the best degree of substitution and the best yields are reached with the most hydrophilic ILs.[51] Furthermore, according to the lipase synthesis of 6-*O*-lauroyl-D-glucose with vinyl laurate or lauric acid, the yield follows the decreasing order: [Bmim][TFO] > [Bmim][BF_4] > [Bmim][Tf_2N] > [Bmim][PF_6].[38] This is explained by a lower viscosity and a higher sugar solubility in the hydrophilic ILs leading to better enzymatic activity.[51] The yield for the same glucose ester follows the decreasing ILs order [Bmim][Tf_2N] > [Omim][Tf_2N] showing that a shorter alkyl chain on the cation gives better results.[21] However, in the case of the enzymatic acylation of the nucleoside 1-β-D-arabinofuranosylcytosine (ara-C, Fig. 2(C)), conversion rises with increase of the alkyl chain length attached to the IL's cation (conversion of 17.8% for [Bmim][BF_4] and 60.5% for [Omim][BF_4]). These opposite trends suggest that, depending upon the glycosylated substrate's nature, the enzymatic activity may be influenced by a combination of ILs factors such as nucleophilicity, hydrophobicity, viscosity and impurity.[12]

The nature of the acyl donor can also influence the enzymatic activity in ILs. For example, the lipase-acylation of naringin by vinyl butyrate results in conversion yields of 55.6% in [Bmim][BF_4] and

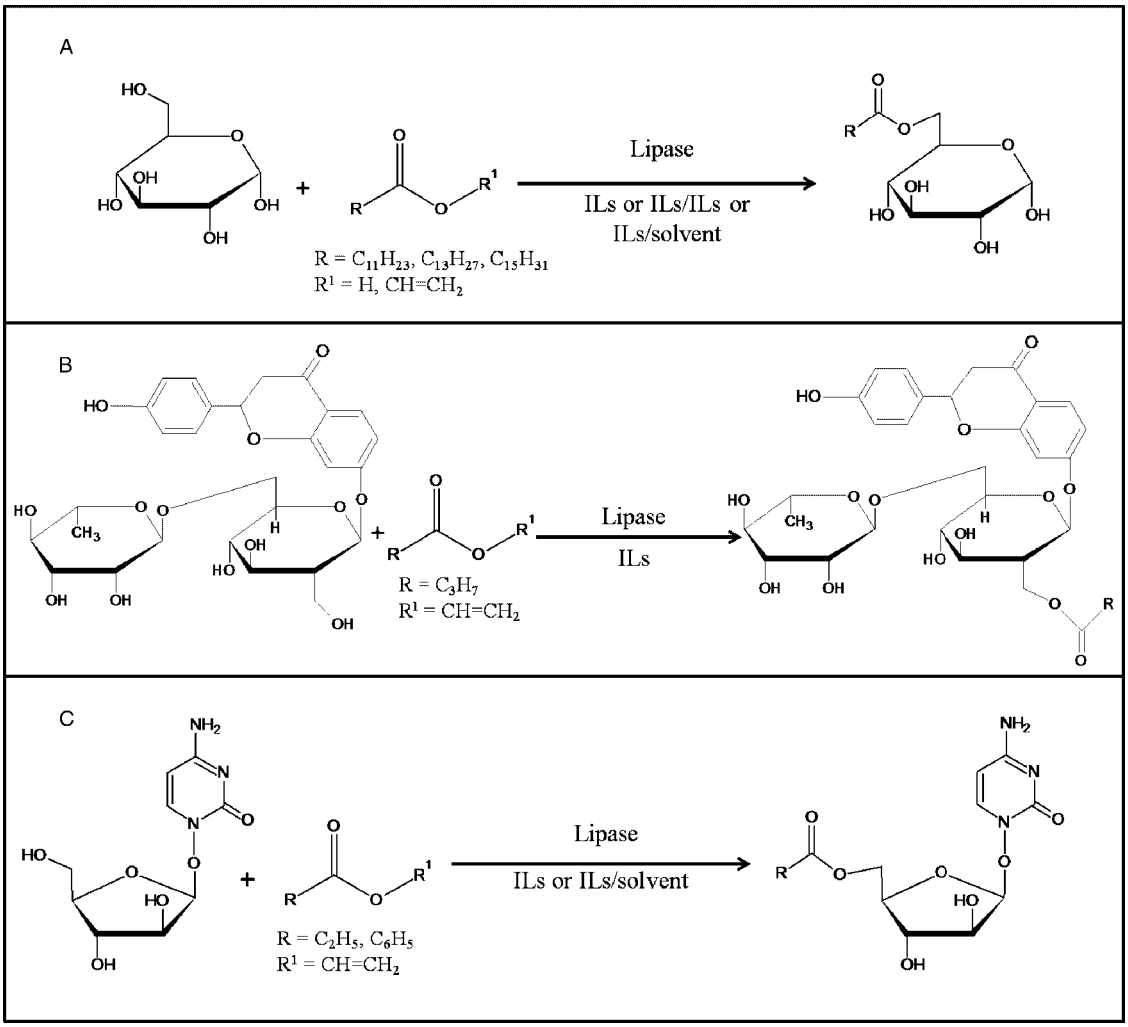

Figure 2. Examples of enzymatic acylation of sugar derivatives. A: Glucose esters synthesis catalyzed by an immobilized CAL-B in pure IL or in IL/IL mixtures or in IL/organic solvent mixtures.[38,50,59] B: Naringin esters synthesis by Novozym 435® in pure IL.[53] C: Arabinofuranosylcytosine esters synthesis by Novozym 435® in pure IL or in IL/organic solvent mixtures.[56]

Table 2. Biocatalysis of sugar derivatives in pure IL systems ($^{\Delta}$: immobilized enzyme)

Biocatalyst		Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate* , yield** (%) or conversion*** (%)	Ref.
Lipases	CAL-B$^{\Delta}$ (*Candida antartica*) immobilized on PEG	Glucose	Vinyl laurate Vinyl myristate	1) [Bmim][BF_4] 2) [Bmim][PF_6]	60 °C	6-*O*-lauroyl-D-Glucose 6-*O*-myristoyl-D-Glucose	1) 30%*** 2) 35%***	50
	Novozym 435®$^{\Delta}$ (CAL-B immobilized on acrylic resin)	Glucose (supersaturated)	Vinyl Laurate	[Emim][MS] [Bmim][TFO]	40 °C, 50 h	6-*O*-lauroyl-D-Glucose	No reaction 96% (24 h)***	38
			Lauric acid	[Bmim][TFO] [Bmim][BF_4] [Emim][TFO]	50 °C, 100 h		91%*** 60%*** 70%***	
	Novozym 435®$^{\Delta}$	Glucose (supersaturated)	Vinyl Laurate	[Bmim][TFO] [Bmim][BF_4] [Bmim][Tf_2N] [Bmim][PF_6]	40 °C, 6 h	6-*O*-lauroyl-D-Glucose	4.21 µmol/min/g* 1.29 µmol/min/g* 0.77 µmol/min/g* 0.76 µmol/min/g*	35
	Novozym 435®$^{\Delta}$	Glucose (supersaturated)	Lauric acid	[Bmim][TFO] [Omim][Tf_2N] [Bmim][Tf_2N]	50 °C, 6 h	6-*O*-lauroyl-D-glucose	3.48 µmol/min/g* 0.72 µmol/min/g* 0.75 µmol/min/g*	21
	Novozym 435®$^{\Delta}$	Fructose (supersaturated)	Palmitic acid	[Bmim][TFO] [Omim][Tf_2N]	60 °C, 6 h	6-*O*-palmitoyl-D-fructose	27.5 µmol/min/g* 4.7 µmol/min/g*	36
	Novozym 435®$^{\Delta}$	Methyl-α-D-glucopyranoside	1) Capric acid	[Bmim][PF_6]	60–80 °C, 1–3 days	1) 6-*O*-caproyl-methyl-α-D-glucopyranoside	1) 19%*** , 2) 31%*** , 3) 65%*** , 4) 70%*** , 5) 50%***	17
			2) Lauric acid	[Bdmim][PF_6]		2) 6-O-lauroyl-methyl-α-D-glucopyranoside	1) 27%*** , 2) 32%*** , 3) 49%*** , 4) 28%*** , 5) 21%***	
			3) Myristic acid	[Pmim][PF_6]		3) 6-O-myristoyl-methyl-α-D-glucopyranoside	1) 25%*** , 2) 23%*** , 3) 51%*** , 4) 65%*** , 5) 11%***	
			4) Palmitic acid	[Hpyr][PF_6]		4) 6-O-palmitoyl-methyl-α-D-glucopyranoside	1) 25%*** , 2) 44%*** , 3) 69%*** , 4) 35%*** , 5) 28%***	

Table 2. (*Continued*)

Biocatalyst		Substrates		Reaction conditions		Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
			5) Oleic acid			5) 6-O-(9Z)-oleyl-methyl-α-D-glucopyranoside		
	CRL (*Candida rugosa*)	Methyl-6-*O*-trityl-β-D-Glucose	Vinyl acetate	1) [Bmim][PF_6]	1) 60 h, 2) 50 h	Methyl-2-*O*-acetlyl-6-O-trityl-β-D-glycosides and	1) 82%**, 2) 76%**	26
		Methyl-6-*O*-trityl-α-D-Glucose		2) [MOEmim][PF_6]	1) & 2) 5 h	Methyl-3-*O*-acetyl-6-*O*-trityl-β-D-glycosides	1) 90%**, 2) 84%**	
		Methyl-6-*O*-trityl-β-D-Galactose			1) 120 h, 2) 96 h		1) 93%**, 2) 95%**	
		Methyl-6-*O*-trityl-α-D-Galactose			1) & 2) 3 h		1) 93%**, 2) 94%**	
	Novozym 435®Δ	Glucose	Vinyl acetate	[Emim][BF_4]	55 °C, 36 h	1) 6-*O*-acyl-D-glucose (α/β), 2) 3,6-*O*-diacyl-D-glucose (α/β)	1) 50% (19/31)***, 2) 0%***	49
				[MOEmim][BF_4]			1) 93% (39/54)***, 2) 6.9% (6.6/0.3)***	
				[Pmim][BF_4]			1) 28% (12/16)***, 2) 0%***	
				[Bmim][BF_4]			1) 69% (31/38)***, 2) 8.7% (4.9/3.8)***	
				[sBmim][BF_4]			1) 79% (35/44)***, 2) 10.8% (6.8/4.0)***	
				[Bmim][PF_6]			1) 11.3% (4.4/6.9)***, 2) 18.1% (9.1/9.0)***	
				[Bpyr][BF_4]			1) 37% (15/22)***, 2) 4.5% (2.1/2.6)***	
				[Ppyr][BF_4]			1) 39% (15/24), 2) 5% (2.2/2.8)	
	Novozym 435®Δ	Levoglucosan	1) Vinyl acetate	[Bmim][BF_4]	55 °C, 96 h	1) & 2) 4-*O*-Acetyl-, 2-*O*-Acetyl-, 2,4-*O*-Diacetyl-, 3,4-*O*-Diacetyl-, 2,3-*O*-Diacetyl-1,6-anhydroglucopyranose	1) 29%**, 2) 29%**, 3) 10%**, 4) 7%**	55

Table 2. (*Continued*)

Biocatalyst	Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
		2) Acetic Acid	[MOEmim][BF_4]			1) 58%**, 2) 31%**, 3) 13%**, 4) 9%**	
		3) Vinyl laurate	[MOEmim][dca]			1) 90%**, 2) 15%**, 3) 19%**, 4) 37%**	
PS-C$^{\Delta}$ (*Pseudomonas cepacea*)		4) Lauric acid	[Bmim][BF_4]			1) 24%**, 2) 15%**, 3) 11%**, 4) 6%**	
			[MOEmim][BF_4]		3)&4) 4-O-Lauryl-1,6-anhydroglucopyranose	1) 56%**, 2) 48%**, 3) 7%**, 4) 5%**	
			[MOEmim][dca]			1) 35%**, 2) 25%**, 3) 4%**, 4) 5%**	
Free PS-C (*Pseudomonas cepacea*)	3,4,6-tri-*O*-acetyl-D-glucal	Decanol	[Bmim][PF_6]	RT, 8 h	4,6-di-*O*-acetyl-D-glucal	84%**	57
			[Bmim][BF_4]	RT, 2 h		13%**	
Novozym 435®$^{\Delta}$	Konjac glucomannan (KGM)	Vinyl acetate	[Emim][BF_4]	50 °C, 48 h, 250 rpm	Acetylated KGM with various substitution degree (SD)	86.9%**	51
			[Bmim][BF_4]			94.3%**	
			[Omim][BF_4]			93.5%**	
			[Bmim][PF_6]			89.2%**	
			[Bmim][Cl]			no reaction	
Novozym 435®$^{\Delta}$	Ascorbic acid	Palmitic acid	[Bmim][BF_4]	60 °C, 24 h	Palmitoyl ascorbate	40%**	90
			[Pentmim][BF_4]			53%**	
Chirazyme® L2$^{\Delta}$ (immobilized and free Cal-B)	Ascorbic acid	Oleic acid	[Bmim][BF_4]	60 °C, 24 h, 300 rpm	Oleyl ascorbate	0.31 µmol/h/mg*	71
			[Emim][TOS]			0.22 µmol/h/mg*	
			[Emim][$OcSO_4$]			0.22 µmol/h/mg*	
			[Bmim][$MDEGSO_4$]			0.12 µmol/h/mg*	
			[Oc3MeN][Tf_2N]			0.14 µmol/h/mg*	
			[CABHEM][$MeSO_4$]			0.12 µmol/h/mg*	
			[Bmim][BF_4] + Salt hydrates			60%**	
Novozym 435®$^{\Delta}$	Rutin	Vinyl butyrate	[Bmim][BF_4]	60 °C, 48 h	Mono- and diester of rutin	51%***	53
Novozym 435®$^{\Delta}$	Naringin		1) [Bmim][BF_4]	60 °C, 96 h	Mono- and diester of naringin	1) 61.6%***, 2) 63%***	

Table 2. (*Continued*)

Biocatalyst		Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
	Lipozyme RMIM$^{\Delta}$ (*Rhizomucor miehei*)			2) $[Bmim][PF_6]$			1) 49.5%***, 2) 50%***	
	Lipozyme TLIM$^{\Delta}$ (*Thermomyces lanuginosus*)						1) 76.9%***, 2) 37.5%***	
	CRL (*Candida rugosa*)						1) Traces, 2) <5 %*** (monoester only)	
	Novozym 435®$^{\Delta}$	Naringin	Vinyl butyrate	1) $[Bmim][BF_4]$	35 °C, 96 h	Mono- and diester of naringin	1) 38.2***, 2) 19.5***	52
				2) $[Bmim][PF_6]$	40 °C, 96 h		1) 46.5 %***, 2) 43.3***	
					50 °C, 96 h		1) 60.7***, 2) 61.9***	
					60 °C, 96 h		1) 61.6***, 2) 63.0***	
					60 °C, 72 h		1) 55.6***, 2) 57.2***	
		Esculin				Mono- and diester of esculin	1) 85.8***, 2) 90.6***	
		Helicin				Mono- and diester of helicin	1) 89.9***, 2) 78.8***	
		Salicin				Mono-, di- and triester of salicin	1) 92.2***, 2)79.2***	
	Novozym 435®$^{\Delta}$	Esculin	Palmitic acid	$[TOMA][Tf_2N]$	40 °C, 96 h, 150 rpm	palmitoyl esculin	13.99***	58
				$[Bmim][PF_6]$			5.46***	
				$[Bmim][BF_4]$			3.81***	
				$[MeOcPy][BF_4]$			4.96***	
				$[Omim][BF_4]$			4.31***	
				$[Omim][PF_6]$			1.16***	
	Novozym 435®$^{\Delta}$	Esculin	Palmitic acid	$[Bmim][CF_3SO_3]$	60 °C, 48 h	palmitoyl esculin	15.2***	34
				$[Bmpy][N(CN)_2]$			11.2***	
				$[BMPyrr][N(CN)_2]$			6.3***	
				$[Bmim][PF_6]$			48.9***	
				$[Bmim][BF_4]$	60 °C, 144 h		27.8***	
				$[TOMA][TF_2N]$			98.3***	
				$[Bmim][CF_3SO_3]$	60 °C, 132 h		12.7***	
			Oleic acid	$[Omim][PF_6]$	60 °C, 40 h	Oleyl esculin	44.6***	

Table 2. (*Continued*)

Biocatalyst	Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
			[Bmim][BF_4]	60 °C, 48 h		34.6%***	
			[Bmim][CF_3SO_3]	60 °C, 132 h		17%***	
			[Emim][$OctSO_4$]	60 °C, 156 h		0.6%***	
			[Omim][BF_4]			88.9%***	
			[Ompy][BF_4]			37.1%***	
			[Bmim][PF_6]			81.6%***	
			[TOMA][TF_2N]			62.5%***	
Novozym 435®Δ	Rutin	Palmitic acid	[Bmim][CF_3SO_3]	60 °C, 132 h	Palmitoyl rutin	2.6%***	
			[Bmim][BF_4]	60 °C, 156 h		23.2%***	
			[Omim][BF_4]			42.4%***	
			[Ompy][BF_4]			20.5%***	
			[Bmim][PF_6]	60 °C, 48 h		2.3%***	
			[Omim][PF_6]	60 °C, 40 h		2.2%***	
		Oleic acid	[Bmim][CF_3SO_3]	60 °C, 132 h	Oleyl rutin	3%***	
			[Bmpy][$N(CN)_2$]	60 °C, 48 h		4.9%***	
			[BMPyrr][$N(CN)_2$]			12.9%***	
			[Bmim][BF_4]	60 °C, 156 h		14.8%***	
			[Omim][BF_4]			35.1%***	
			[Ompy][BF_4]			16.2%***	
			[Bmim][PF_6]	60 °C, 48 h		1.4%***	
Novozym 435®Δ	β-D-arabinofuranosyl cytosine	Vinyl proponiate	[Bmim][BF_4]	40 °C, 250 rpm	5′-*O*-propionyl 1-β-D-arabinofuranosylcytosine	17.8%***	56
			[Pmim][BF_4]			29.1%***	
			[Hmim][BF_4]			43.5%***	
			[Omim][BF_4]			60.5%***	
			[Bmim][PF_6]			65.1%***	
			[Bmim][Cl]			No reaction	
			[Bmim][Br]			No reaction	

57.2% in [Bmim][PF_6] after 72 h of reaction but with lauric acid and vinyl laurate as acyl donor the conversion rate was only 23% after 96 h (Fig. 2(B)). This was explained by the low solubility of the long chain acyl substrates in ionic liquids leading to a two-phase system, decreasing the availability of the substrates for the enzyme, and thus reducing the biocatalyzed acylation of flavonoids.[52,53]

Selectivity is a major incentive for choosing ILs instead of organic solvents as enzymatic reaction media.[5] Selectivity has been studied for the enzymatic acylation of unmodified sugar[49,54] and sugar derivatives.[26,55] For example, the enzymatic acetylation of glucose by vinyl acetate in ILs leading to the synthesis of 6-*O*-acyl-D-glucose (major product) and 3,6-*O*-diacyl-D-glucose (minor product) showed a better regioselectivity in all ILs tested (86% in [Bmim][BF_4] to 99.9% in [Emim][BF_4] of monoacetylation) except in [Bmim][PF_6] (from 36% to 38.5%) than in THF (53%) or acetone (76%).[49,54] The regioselective enzymatic monoacylation of methyl-6-*O*-trityl-β-D-glucose by vinyl acetate in [Bmim][PF_6] and [MOEmim][PF_6] showed that when the anomeric hydroxyl group as well as the primary hydroxyl group on the C_6 position are protected, Novozym 435® selectively catalyzes the esterification of the secondary hydroxyl groups on the C_2 (major product) and the C_3 of the glucose.[26] Yields and regioselectivity in these ILs are better than in THF and chloroform (90% to 98% of regioselectivity in ionic liquid and 77% to 95% in $CHCl_3$ according to the glycoside's nature). The same trend has been observed for the enzymatic acylation of flavonoids and nucleosides. The acylation of naringin by vinyl butyrate resulted in the production of 86.8% of monoester in [Bmim][BF_4], 63% in [Bmim][PF_6] and 45.5% in acetone.[52] However, ILs based on dca^-, Br^-, OAc^-, NO_3^- or lactate anions, did not allow the acylation of konjac glucomannan,[51] the acylation of ara-C[56] or the deacetylation of 3,4,6-tri-*O*-acetyl-D-glucal.[57] This confirms that, as mentioned above, ILs constituted of ions able to interact with proteins via strong hydrogen interactions can cause deactivation of enzymes.[22,58]

IL/organic solvent systems

In order to circumvent issues encountered in pure ILs systems like mass transfer limitation leading to lower reaction rates and yields,[15] enzymatic media composed of IL/organic solvent mixtures have been explored.

As shown in Table 3, several research teams have studied the efficiency of different IL–organic solvent mixtures at different ratios for biocatalyzed carbohydrate derivatives synthesis.[19,56,58–63] Depending on the organic solvent nature and the substrates, the conversion rates vary as a function of the ILs ratio. Polar organic solvents such as pyridine, DMSO and DMF are often used for the biocatalyzed acylation of nucleosides as they are good solubilizers of these compounds. However, the low substrate conversions observed are probably due to stripping off the essential water from the enzyme, which leads to its deactivation. Therefore, ILs have been added to the reaction medium to decrease the polar solvent content. This leads to an improvement of the rate as shown for the benzoylation of ara-C by Novozym 435® where the conversion reached 88.5% in [Bmim][PF_6]/pyridine (80/20, v/v, optimal ratio) while 36.4% only is observed in a mixture of hexane/pyridine (28/72, v/v).[60] THF, which is also used for the acylation of nucleosides, is miscible with many ILs.[56,61] The conversion of the β-1-D-arabinofuranosyl cytosine acylation with vinyl propionate increases with the proportion of IL in THF up to 20% (98.5% in [Bmim][PF_6]/THF (10/90, v/v) and 65.1% in pure [Bmim][PF_6]).[56] Above this ratio, the reaction rate and substrate conversion decreases significantly. A variety of polar and less polar ILs mixed with acetone at 25%, 50% and 75% of ILs have been tested for the synthesis of the flavonoïd ester 6-*O*-palmitoyl-esculin catalyzed by Novozym 435®.[58] The best conversion rates are reached for the minimum ILs concentration tested (25%). The mixture [TOMA][Tf_2N]/acetone (25/75, v/v) is the best medium tested with a 78% conversion reached after 96 h of reaction at 40 °C (Table 3). This conversion is much higher than in pure [TOMA][Tf_2N] (13.99%, Table 2) and equivalent to the one observed in pure acetone (78.17%), showing the possibility of decreasing the concentration of harmful volatile organic solvents while maintaining high yields. However, a higher conversion of 98.3%, (Table 3) has been reached recently in pure [TOMA][Tf_2N], certainly due to an increased flavonoïd solubility at a higher reaction temperature (60 °C instead of 40 °C).[34]

According to Li *et al.*,[56] the improvement observed at low concentrations of ILs is due to the interactions between ILs and charged groups of the enzyme that change the enzyme's structure to a more appropriate and more favorable conformation for the formation of the acyl-enzyme intermediate. With high ratios of ILs, high ionic strength can deactivate the enzyme and the high viscosity of the reaction media can increase the mass transfer limitation as observed for the benzoylation of floxuridine catalyzed by *Pseudomonas cepacia* lipase (PSL-C) in THF/[Bmim][PF_6] where 98% of conversion is reached within 82 h with 25% of [Bmim][PF_6] while 5% of [Bmim][PF_6] allows more than 99% conversion after 48.5 h.[61]

This explanation does not seem to be appropriate when tert-butanol is used as co-solvent. According to the results obtained for the enzymatic catalyzed synthesis of 6-*O*-lauroyl-D-glucose by the immobilized PSL-C, the ILs/tert-butanol mixtures showed a better yield with more than 50% of ILs. Mixtures composed of 60% of ILs give the best yield when vinyl laurate is used as acyl donor (62% for [Bmim][PF_6], 65% for [Bmim][BF_4]).[59] The enzymatic acylation of konjac glucomannan with vinyl acetate confirms this trend since the best yield (95.2%) is obtained with a mixture of [Bmim][BF_4]/tert-butanol (75/25, v/v).

Another type of enzyme, glycosidase, has been tested with ILs used as co-solvents in aqueous buffer systems for transglycosylation reactions with lactose[64,65] and glucose.[66] When glycosidases are used in aqueous medium, the equilibrium between transglycosylation and hydrolysis takes place and is responsible for low yields. Introduction of ILs into the enzymatic medium decreases the water concentration and thus shifts the equilibrium towards the transglycosylation reaction. For reaction media consisting of aqueous solutions of ILs, the chaotropic (water structure-breaking) and kosmotropic (water structure-forming) properties of ILs have to be taken into consideration.[67] According to Lang *et al.*,[65] the optimal structural stability of a protein comes from the appropriate combination of a kosmotropic anion and a chaotropic cation. That is the reason why the β-glycosidase CelB is compatible with [Mmim][$MeSO_4$] since $MeSO_4^-$ anion is kosmotrope and $Mmim^+$ cation is chaotrope whereas a strong inhibition of this enzyme is observed in the presence of 10–50% of [Bmim][BF_4],[65] in which BF_4^- anion is chaotrope and $Bmim^+$ cation is kosmotrope.[68]

The β-galactosidase activity decreases when increasing IL content with 74% of residual activity for 25% of [Mmim][$MeSO_4$] and 14% of residual activity for 50% of [Mmim][$MeSO_4$] (residual activity determined as a comparison with the enzymatic activity in pure buffer solution).[64] Therefore, 25% of ILs seemed to be the most appropriate percentage for the synthesis of N-acetyllactosamine (LacNAc).

Table 3. Biocatalysis of sugar derivatives in IL/solvent systems ($^{\Delta}$: immobilized enzyme)

Biocatalyst		Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
Lipases	Chirazyme® L2, C2$^{\Delta}$ (CAL-B)	Glucose	Vinyl laurate	[Bmim][BF_4]/Tert-butanol (60/40)	60 °C, 72 h	6-*O*-lauroyl-D-Glucose	75%**	50
			Vinyl myristate			6-*O*-myristoyl-D-Glucose	89%**	
			Palmitic acid			6-*O*-palmitoyl-D-Glucose	48%**	
	Chirazyme® L2, C2$^{\Delta}$ (CAL-B)	Glucose	Vinyl laurate	[Bmim][BF_4]/Tert-butanol (80/20)	60 °C, 72 h	6-*O*-lauroyl-D-Glucose	59%***	59
			Vinyl myristate			6-O-myristoyl-D-Glucose	11%***	
			Vinyl palmitate			6-*O*-palmitoyl-D-Glucose	8%***	
			Vinyl laurate	[Bmim][BF_4]/Tert-butanol (60/40)		6-*O*-lauroyl-D-Glucose	65%***	
			Palmitic acid			6-*O*-palmitoyl-D-Glucose	45%***	
			Vinyl laurate	[Bmim][PF_6]/Tert-butanol (80/20)		6-*O*-lauroyl-D-Glucose	10%***	
			Palmitic acid			6-*O*-palmitoyl-D-Glucose	9%***	
			Vinyl laurate	[Bmim][PF_6]/Tert-butanol (60/40)		6-*O*-lauroyl-D-Glucose	62%***	
			Palmitic acid			6-*O*-palmitoyl-D-Glucose	45%***	
	Novozym 435®$^{\Delta}$	Konjac glucomannan	Vinyl acetate	[Emim][BF_4]/Tert-butanol (85/15)	50 °C, 48 h	Acetylated KGM with various DS	87.6%**	51
				[Bmim][BF_4]/Tert-butanol (75/25)	250 rpm		95.2%**	
				[Omim][BF_4]/Tert-butanol (60/40)	Aw = 0.75		92.1%**	
				[Bmim][PF_6]/Tert-butanol (75/25)			89.6%**	
	Novozym 435®$^{\Delta}$	Ascorbic acid	Oleic acid	[Bmim][BF_4]/Hexane (90/10)	60 °C, 10 h	Oleyl ascorbate	44%**	90
				[Pentmim][BF_4]/Hexane (90/10)			65%**	
			Palmitic acid	[Bmim][BF_4]/Polypropoylene		Palmitoyl ascorbate	43%**	
				[Bmim][BF_4]/Hexane (90/10)			54%**	
				[Pentmim][BF_4]/Hexane (90/10)			62%**	
	Novozym 435®$^{\Delta}$	Esculin	Palmitic acid	[TOMA][Tf_2N]/Acetone	40 °C, 96 h,	6-*O*-palmitoyl-esculin	*** 78.02% (25/75), 51.31% (50/50), 48.57% (75/25)	58
				[Bmim][PF_6]/Acetone	150 rpm		*** 71.13% (25/75), 63.79% (50/50) 18.06% (75/25)	
				[Bmim][BF_4]/Acetone			*** 51.54% (25/75), 37.76% (50/50), 18.03% (75/25)	
				[MeOcPy][BF_4]/Acetone			*** 53.50% (25/75), 45.47% (50/50), 29.61% (75/25)	

Table 3. (*Continued*)

Biocatalyst	Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
			[Omim][BF_4]/Acetone			*** 26.22% (25/75), 22.23% (50/50) 12.77% (75/25)	
			[Omim][PF_6]/Acetone			*** 51.57% (25/75), 56.18% (50/50), 11.81% (75/25)	
			[Bmim][CF_3SO_3]/Acetone			*** 10.77% (25/75), 5.20% (50/50)	
			[MeEtPy][$C_4F_9SO_3$]/Acetone			*** 7.83% (25/75), 3.55% (50/50)	
			[MTOA][TAF]/Acetone	40 °C, 72 h,		*** 54.63% (1/99)	
			[Emim][$OctSO_4$]/Acetone	150 rpm		*** 36.65% (1/99)	
			[Emim][$MDEG_3SO_4$]/Acetone			*** 38.34% (1/99)	
Novozym 435®Δ	Ribavirin	Divinyl adipate	[Bmim][BF_4]/Acetone	50 °C, 12 h	Adipate ester of ribavirin	98.5%**	62
			[Bmim][BF_4]/Dioxane			21.3%**	
			[Bmim][BF_4]/Dichloromethane			5.2%**	
			[Bmim][BF_4]/THF			0.6%**	
CAL-BΔ (*Candida antartica*)	Ribavirin	Divinyl adipate	N-methylimidazole/Acetone (10/90)	50 °C, 4 h, 200 rpm	Adipate ester of ribavirin	96%**	63
Novozym 435®Δ	β-D-arabinofuranosyl cytosine	Vinyl propionate	[Bmim][BF_4]/THF(20/80)	40 °C, 250 rpm	5′-*O*-propionoyl-l 1-β-D-arabinofuranosylcytosine	95.2%***	56
			[Pmim][BF_4]/THF (20/80)			95.6%***	
			[Hmim][BF_4]/THF (10/90)			96%***	
			[Omim][BF_4]/THF (15/85)			97%***	
			[Bmim][PF_6]/THF (10/90)			98.5%***	
Novozym 435®Δ	β-D-arabinofuranosyl cytosine	Vinyl benzoate	[Emim][BF_4]/Pyridine (90/10)	40 °C, 250 rpm, Aw = 0.11	5′-*O*-benzoyl 1-β-D-arabinofuranosylcytosine	10.9%***	60
			[Bmim][[BF_4]/Pyridine (90/10)			13.1%***	
			[Hmim][BF_4]/Pyridine (80/20)			16.9%***	

Table 3. (*Continued*)

Biocatalyst		Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
				[Omim][BF_4]/Pyridine (70/30)			19.1%***	
				[Bmim][PF_6]/Pyridine (80/20)			88.5%***	
	Novozym 435®Δ	Floxuridine (FUdR)	Vinyl benzoate	[Bmim][PF_6]/THF (10/90)	50 °C, 200 rpm	5′-O-benzoyl-FUdR, 3′-*O*-benzoyl-FUdR, 5′,3′-*O*-dibenzoyl-FUdR	17%***	61
	Lipozyme TLL (*Thermomyces lanuginosus*)	Floxuridine (FUdR)	Vinyl benzoate	[Bmim][PF_6]/THF (10/90)			93%***	
	Lipozyme RML (*Rhizomucor miehei*)	Floxuridine (FUdR)	Vinyl benzoate	[Bmim][PF_6]/THF (10/90)			20%***	
	Lipozyme MML (*Mucor miehei*)	Floxuridine (FUdR)	Vinyl benzoate	[Bmim][PF_6]/THF (10/90)			10%***	
	PSL-C (*Pseudomonas cepacia*)	Floxuridine (FUdR)	Vinyl benzoate	[Hmim][PF_6]/THF (10/90)			57%***	
				[Hepmim][PF_6]/THF (10/90)			88%***	
				[Hmim][BF_4]/THF (10/90)			94%***	
				[Omim][BF_4]/THF (10/90)			76%***	
				[Emim][PF_6]/Acetone (10/90)			>99%***	
				[Bmim][PF_6]/Acetone (10/90)				
				[Hmim][PF_6]/Acetone (10/90)				
				[$(C_6)_3C_{14}P$][PF_6]/Acetone (10/90)				
				[Bmim][BF_4]/Acetone (10/90)				
				[Hmim][BF_4]/Acetone (10/90)				
				[HPyr][Tf_2N]/Acetone (10/90)				
				[$(C_6)_3C_{14}P$][Tf_2N]/Acetone (10/90)				
				[Bmim][$OctSO_4$]/Acetone (10/90)			29%***	
Glycosidases	β-galactosidase (*Bacillus circulans*)	Lactose	N-acetylglucosamine	[Mmim][$MeSO_4$]/Phosphate buffer (25/75)	23 °C, pH = 7.3	N-Acetyllactosamine	58%**	64
	β-glycosidase CelB (*Pyrococcus furiosus*)	Lactose	Lactose	[Mmim][$MeSO_4$]/Citrate buffer (45/55)	80 °C, pH = 5.5	Galactosyl lactose	~30%**	65

Table 3. (*Continued*)

Biocatalyst	Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc.	Products	Initial rate*, yield** (%) or conversion*** (%)	Ref.
		D-Xylose			Galactosyl xylose	40%**	
		Glycerol			Galactosyl glycerol	50%**	
		Isopropyl-β-D-thio-galactopyranoside			Galactosyl Isopropyl-β-D-thio-galactopyranoside	Data not given	
		1,2-Ethanediol			Galactosyl ethanediol		
		1-Propanol			Galactosyl propanol		
		1-Phenylethanol			Galactosyl phenylethanol		
		Benzylalcohol			Galactosyl benzylalcohol		
β-Glucosidase from prune (*Prunus domestica*) seed meal	D-Glucose	1) Tyrosol	[Bmim][[BF_4]/Phosphate buffer (15/85)	50 °C, 72 h,	Salidrosides	** 1) 21%, 2) 14%	66
		2) 4-nitrobenzyl alcohol	[Bmmim][[BF_4]/Phosphate buffer (15/85)	200 rpm, pH = 6		** 1) 21%, 2) 13%	
			[Hmim][[BF_4]/Phosphate buffer (10/90)			** 1) 20%, 2) 12%	
			[Bmim][[PF_6]/Phosphate buffer (10/90)			** 1) 18%, 2) 11%	
			[Bmim][C_8SO_4]/Phosphate buffer (15/85)			** 1) 21%, 2) 13%	
			[Mmim][$MeSO_4$]/Phosphate buffer (10/90)			** 1) 21%	
			[Bmim][I]/Phosphate buffer (10/90)			** 1) 22%, 2) 15%	
			[Bmim][Cl]/Phosphate buffer (2.5/97.5)			** 1) 20%, 2) 12%	
			[EOEmim][Cl]/Phosphate buffer (5/95)			** 1) 20%, 2) 12%	
			[AcOEmim][Cl]/Phosphate buffer (5/95)			** 1) 20%, 2) 12%	

Table 4. Biocatalysis of sugar derivatives in IL/IL systems ($^{\Delta}$: immobilized enzyme)

Biocatalyst		Substrates: Carbohydrate	Substrates: Substrate acceptor	Reaction conditions: Solvent	Reaction conditions: T°, Time, etc	Products	Initial rate* , yield** (%) or conversion*** (%)	Ref.
Lipases	Novozym 435®$^{\Delta}$	Glucose (supersaturated)	Vinyl laurate	[Bmim][TfO]/[Bmim][Tf_2N]	40 °C, 6 h	6-*O*-lauroyl-D-glucose		35
				25/75			3.75 μmol/min/g*	
				50/50			2.85 μmol/min/g* , (70%***)	
				75/25			1.89 μmol/min/g*	
				[Bmim][TfO]/[Bmim][PF_6]				
				50/50			1.94 μmol/min/g*	
		Glucose (supersaturated)	Lauric acid	[Bmim][Tf_2N]/[Bmim][TFO]	50 °C, 24 h			
				50/50			52%***	
	Novozym 435®$^{\Delta}$	Glucose (supersaturated)	Lauric acid	[Bmim][TfO]/[Omim][Tf_2N]	50 °C, 6 h	6-*O*-lauroyl-D-glucose		21
				90/10			4.29 μmol/min/g*	
				80/20			3.65 μmol/min/g*	
				70/30			3.31 μmol/min/g*	
				60/40			2.78 μmol/min/g*	
				50/50			2.38 μmol/min/g*	
					50 °C, 24 h		53.6%***	
				[Bmim][TfO]/[Bmim][Tf_2N]	50 °C, 6 h			
				90/10			2.86 μmol/min/g*	
				75/25			2.65 μmol/min/g*	
				50/50			2.15 μmol/min/g*	
		Glucose (supersaturated)		[Bmim][TfO]/[Omim][Tf2N]				
			Caprylic acid	50/50	50 °C, 24 h	6-*O*-capriloyl-D-glucose	59.6%***	
			Palmitic acid			6-*O*-palmitoyl-D-glucose	39.6%***	
	Novozym 435®$^{\Delta}$	Fructose (supersaturated)	Palmitic acid	[Bmim][TfO]/[Bmim][Tf_2N]	60 °C, 6 h	6-*O*-palmitoyl-D-fructose		36
				75/25			25.8 μmol/min/g*	
				50/50			20.3 μmol/min/g*	
				25/75			19.3 μmol/min/g*	

IL/IL systems

Until now, reaction media composed of a mixture of hydrophobic and hydrophilic ILs have been tested only for the lipase catalyzed synthesis of sugar esters. Higher sugar solubility are obtained in hydrophilic ILs such as [Bmim][TFO] but the enzyme stability is higher in hydrophobic ILs such as [Bmim][Tf_2N], [Omim][Tf_2N] or [Bmim][PF_6].[20,21] The purpose is thus to associate both type of ILs to design new reaction media combining their advantages. The hydrophilic IL [Bmim][TFO] was associated with [Bmim][Tf_2N], [Omim][Tf_2N] or [Bmim][PF_6] at different ratios for the Novozym 435® catalyzed synthesis of 6-*O*-lauroyl-D-glucose using a supersaturated glucose solution and lauric acid as reactants (Table 4). The system [Bmim][TFO]/[Omim][Tf_2N] (10/90) gives the highest initial rate (4.29 μmol min^{-1} $g_{Enz}{}^{-1}$, 50 °C, 6 h reaction time) compared with other ratios and ILs mixtures. This rate is much higher than in pure [Omim][Tf_2N] (0.72 μmol min^{-1} g^{-1}, 50 °C, 6 h reaction time)[21] and comparable with that obtained in pure [Bmim][TFO] (4.21 μmol min^{-1} $g_{Enz}{}^{-1}$, 40 °C, 6 h reaction time).[20] Furthermore, increasing the hydrophobic IL concentration in the reaction media increases the enzymatic stability. After reusing the enzyme five times, the residual activity of Novozym 435® was about 78% for the optimized system [Bmim][TFO]/[Omim][Tf_2N] (1/1, v/v, 50 °C, 24 h)[21] and 85% for the optimized system [Bmim][TFO]/[Bmim][Tf_2N] (1/1, v/v, 40 °C, 11 h), which is 1.3 times higher than in pure [Bmim][TFO].[20] Therefore, the addition of a hydrophilic IL into a hydrophobic IL allows the obtention of a high initial rate, while maintaining a high enzymatic stability.

The effect of the water activity (a_w) and water content on the enzymatic acylation of carbohydrates in ILs

Water is one of the most important impurities in ILs and a major factor affecting the micro-environment of the enzyme as well as its structure. Water is also a by-product of esterification and will thus affect the equilibrium position of the reaction displaced towards hydrolysis rather than synthesis.[69] Therefore, water has a strong influence on the enzymatic activity in non-aqueous media. In consequence, enzymes have an optimal water activity (a_w) which has to be cautiously considered.[70]

Several research teams determined the optimal water content and a_w for lipase catalyzed reactions in ILs. In general, enzymatic activity decreases with increasing a_w in organic solvent. However, a minimum value of a_w is required. Below the optimum a_w, lipase is not sufficiently hydrated and shows lower activity. This tendency is also observed in ILs. For example, the enzymatic acylation of konjac glucomannan at different a_w (from 0.11 to 0.97), show a maximum degree of substitution (DS) at 0.75 of a_w in all ILs tested and in tert-BuOH.[20,21,51]

In order to maintain a constant a_w in enzymatic acylations, the reaction medium can be equilibrated by saturated salt solution.[56,71] For example, the optimum a_w (0.07) is maintained with lithium bromide salt (LiBr) for the acylation of Ara-C in [Bmim][PF_6] catalyzed by immobilized CAL-B. For the enzymatic acylation of KGM in [Bmim][BF_4], sodium chloride (NaCl) keeps the optimum a_w at 0.75 and for the acylation of ascorbic acid in [Bmim][BF_4] the optimum a_w (0.11) is obtained with sodium iodide salt (NaI).

TOXICITY AND DEGRADABILITY

As seen above, ILs are actually being studied for the biocatalyzed synthesis of glycosylated derivatives as they can improve the efficiency and selectivity of the process. Furthermore, the use of ILs is also developed to reinforce the 'green' character of the synthesis. Thus their water and soil toxicity as well as their degradability must be considered.

Toxicity

ILs 'green' solvent label is mainly due to their very low volatility and thus their minimum release into the atmosphere via evaporation. Nevertheless, this property is not a sufficient reason to consider them as harmless to the environment. Although no pollution due to ILs has been recorded until now, ILs are poorly or non-biodegradable and can easily accumulate in soils and water. For the last few years and due to their numerous applications, research on the effect of ILs on the environment and human health has been constantly growing.[72]

Both cations and anions are involved in the ILs toxicity. Studies on rat and human cell lines showed that imidazolium cations are more toxic than pyridinium, pyrrolidinium and morpholinium cations.[72] Furthermore, the toxicity increases with the alkyl chain length and the number of alkyl chains linked to the cation.[19,72–74] The nature of the anion is also involved in ILs toxicity. Aquatic toxicity studies on the gram$^+$ bacterium *Vibrio fisheri* led to the anion toxicity order: $[Br]^- < [dca]^- < [Cl]^- < [BF_4]^- < [PF_6]^- < [Tf_2N]^-$.[72] The fluorinated anions are the most toxic because of their ability to rapidly generate hydrofluoric acid in an aquatic medium. The impact of the anion on the toxicity level of ILs is more important when the alkyl chain length on the cation is shorter.[72]

In general, ILs toxicity is lower than the toxicity of organic solvents.[75] However, some ILs such as [Bmim][Br] and [Bmpyr][Br] are estimated to be two and four orders of magnitude more toxic for the aquatic environment than organic solvents such as DMF, 2-propanol and methanol.[76] The toxicity mechanisms of ILs have scarcely been investigated. Up to now, an understanding of this mechanism is required to apprehend the toxicity issue at a biological level.[73]

Degradability

Because of their high chemical and thermal stability, ILs can accumulate in soils. Therefore, their treatment after use is an important environmental concern. Currently, two degradation methods are investigated: chemical degradation and biodegradation.

Chemical degradation

Literature reporting the use of chemical degradation for the destruction of ILs indicates that many research groups have focused on thermal and oxidative methods.

Thermal degradation studies of alkylimidazolium and alkylammonium cations show that the imidazolium cation is thermally more stable than the ammonium cation.[77] The nature of the anion also has an effect on the thermal stability and the isomeric structure of the alkyl side group affects the imidazolium thermal stability.[77] The imidazolium salts associated with fluorinated anions show higher thermal stability than with halides and follow the order $PF_6^- > Tf_2N > BF_4^- > Cl^-, Br^-$.[76] Since the imidazolium quaternary salts undergo pyrolysis via a S_N2 mechanism, this reaction takes place more easily with the most nucleophilic anions according to the order $Br^- > Cl^- > F^-$.[76]

Different oxidative degradation processes such as H_2O_2 or TiO_2 mediated oxidation, UV irradiation and ultrasound, separately or combined in pairs were also investigated.[78] For imidazolium based ILs, it was found that the degradation rate by UV irradiation is

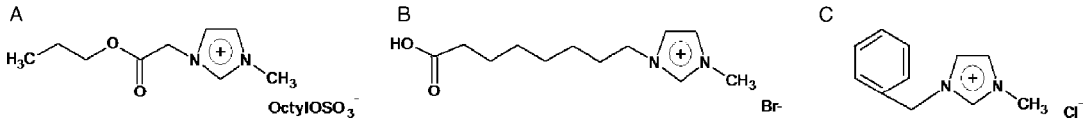

Figure 3. Examples of modified imidazolium cation for the development of more readily biodegradable ILs. A: Imidazolium cation including an ester group associated to an octylsulfate anion.[81] B: Imidazolium cation including a carboxylic group with a Br^- anion as counter ion.[84] C: Imidazolium cation including a phenyl ring with a Cl^- anion as counter ion.[84]

inversely correlated with the length of the alkyl chain substituted on the N_1 position. The Bmim (alkyl chain with four carbon atoms) cation gives the highest degradability rate (55% degraded in 360 min) whereas low rates were found for the Hmim (alkyl chain with 6 carbon atoms) and Omim (alkyl chain with 8 carbon atoms) cations. The same trend has been observed with oxidative processes using H_2O_2 and TiO_2. The degradation rate is improved by combining UV with either of these two reactants, the best one being H_2O_2[78] Ultrasound associated with H_2O_2/acetic acid gives the best degree of degradation leading to nearly complete degradation of imidazolium based ILs.[79] Changing the alkyl chain length and the nature of the anion does not affect the ultrasound assisted degradation process.

In order to remove ILs from water, a Fenton and a Fenton-like reaction (Fe(III) + H_2O_2) were used for particular alkylimidazolium and pyridinium ILs which are known to be resistant to bio- or thermal degradation.[80] The pyridinium cation is more resistant than imidazolium. The importance of the alkyl chain length on the cation is again verified. For imidazolium based ILs, the most stable is [Omim][Cl] (68% degradation in 90 min) and the less stable is [Bmim][Cl] (>97% degradation in 90 min).

Biodegradation

Until now, no imidazolium-based ILs could be classified as 'readily biodegradable' according to the Organization for Economic Cooperation and Development (OECD) standards (US EPA, 1998) for which 60–70% or greater biodegradation by activated sludge microbial inoculate is required in a 28 days period.[73,81] According to Garcia *et al.*,[82] no compounds based on 1-butyl-3-methyl imidazolium cations associated with Br^-, BF_4^-, PF_6^-, dca^- and Tf_2N^- anions show any significant degree of biodegradation after 28 days (<5%) except the $OctSO_4^-$ anion (25%). However, some ILs can be readily biodegradable, as is the case of the pyridinium based ILs as shown by the high biodegradation rate obtained by the test using the activated sludge microbial community for [Ompyr][Br] (96% in 28 days).[83]

The alkyl chain length on the cation also has an influence on the biodegradation rate. In opposition to the chemical degradation methods, the increase of the alkyl chain length on the imidazolium or pyridinium ring leads to an increase of the biodegradation rate.[83,84] Longer chains with four or more carbon atoms can be more easily accessible to bacteria as they have more potential sites for oxygenases.[83] However, the hydrophobic character of the cation tends to increase the ecotoxicity of ILs.[75,81,84] Therefore a compromise between toxicity and speed of biodegradability must be considered.[84] Investigations for the development of more readily biodegradable and non-toxic ILs are currently under way.[22,74,81,84,85] To be readily biodegradable, ILs should include potential sites for an enzymatic hydrolysis and for an oxygenase attack. For the former, groups such as ester or amides must be incorporated.[81,84] For the latter, ILs should include an oxygen atom in functional groups (hydroxyl, aldehyde, carboxylic acid), unsubstituted linear alkyl chains or phenyl rings.[73,84] Examples of some modified ILs according to the biodegradability requirements are presented in Fig. 3.

The addition of an ester group in the alkyl side chain of a dialkylimidazolium based IL improves its biodegradation properties (23–33% biodegradation in 28 days) compared with the negligible 0–1% (28 days) found for the conventional ILs [Bmim][Br] and [Bmim][BF_4].[73,81] However, incorporation of these biodegradable sites is sometimes not favorable for the physicochemical properties required by the ILs[73].

CONCLUSION AND OUTLOOK

ILs, also called 'tunable solvents', are a class of materials with a wide range of applications. The enzymatic catalyzed synthesis of sugar derivatives in ILs has recently received much attention because most ILs can solubilize higher concentrations of substrates than conventional organic solvents compatible with enzyme activity. In some cases, ILs lead to higher selectivities (regio-, stereo-, enantio-).[36,38,56] ILs are exploited pure as well as in mixtures of hydrophobic/hydrophilic ILs. ILs are also used in combination with organic solvents in order to minimize the impact of their high viscosity which lowers the mass transfer in the reaction medium.

Considered as 'green' solvents, toxicological studies and investigation of the environmental fate of ILs are currently in progress. Recent research focuses on the synthesis of biodegradable ILs because until now very few ILs could be classified as readily biodegradable.[73,81]

Finally, the promising results obtained with reaction systems containing ILs open up possibilities for the development of a 'greener' sugar derivatives biocatalyzed synthesis. In the near future, new and 'greener' ILs than the more classical imidazolium cations and fluorinated anions based ILs must be tested. To date, research has focused mainly on lipases and further work must be undertaken with other enzymes such as glycosidases, proteases or oxydases. For the development of 'greener' biocatalyzed synthesis other promising non-conventional methods such as microwave, ultrasound and supercritical CO_2 have also been investigated. These strategies may be combined with ILs to improve the enzymatic activity and stability but also to build a unique process for coupling synthesis and extraction.[86–89]

ACKNOWLEDGEMENTS

This work was funded through the ARC grant 'Superzym', financed by the French Community of Belgium which is gratefully acknowledged for its financial support. Magali Deleu and Antoine Debuigne thank the Fonds National de la Recherche Scientifique (FNRS) from Belgium for their Research Associate position.

REFERENCES

1 Chen S-T, Sookkheo B, Phutrahul S and Wang K-T, Enzymes in nonaqueous solvents. Applications in carbohydrate and peptide preparation. *Methods Biotechnol* **15**:373–400 (2001).
2 van Rantwijk F and Sheldon RA, Biocatalysis in ionic liquids. *Chem Rev* **107**:2757–2785 (2007).
3 Roosen C, Müller P and Greiner L, Ionic liquids in biotechnology: applications and perspectives for biotransformations. *Appl Microbiol Biotechnol* **81**:607–614 (2008).
4 Moniruzzaman M, Nakashima K, Kamiya N and Goto M, Recent advances of enzymatic reactions in ionic liquids. *Biochem Eng J* **48**:295–314 (2010).
5 Hernández-Fernández FJ, de los Ríos AP, Lozano-Blanco LJ and Godínez C, Biocatalytic ester synthesis in ionic liquid media. *Biochem Eng J* **85**:1423–1435 (2010).
6 Jain N, Kumar A, Chauhan S and Chauhan SMS, Chemical and biochemical transformations in ionic liquids. *Tetrahedron* **61**:1015–1060 (2005).
7 Khupse ND and Kumar A, Ionic liquids: new materials with wide applications. *Indian J Chem Sect A: Inorg Bio-Inorg Phys Theor* **49A**:635–648 (2010).
8 Dullius JEL, Suarez PAZ, Einloft S, de Souza RF, Dupont J, Fischer J, *et al*, Selective catalytic hydrodimerization of 1,3-butadiene by palladium compounds dissolved in ionic liquids. *Organometallics* **17**:815–819 (1998).
9 Murugesan S and Linhardt RJ, Ionic liquids in carbohydrate chemistry – current trends and future directions. *Curr Org Synth* **2**:437–451 (2005).
10 Sheldon RA, Lau RM, Sorgedrager MJ, van Rantwijk F and Seddon KR, Biocatalysis in ionic liquids. *Green Chem* **4**:147–151 (2002).
11 Dupont J, On the solid, liquid and solution structural organization of imidazolium ionic liquids. *J Brazil Chem Soc* **15**:341–350 (2004).
12 Zhao H, Methods for stabilizing and activating enzymes in ionic liquids – a review. *J Chem Technol Biotechnol* **85**:891–907 (2010).
13 Sigma-Aldrich (SIAL), Enabling technologies – ionic liquids. *Chemfiles* (2007).
14 Wilkes JS, Properties of ionic liquid solvents for catalysis. *J Mol Catal A: Chem* **214**:11–17 (2004).
15 Park S and Kazlauskas RJ, Biocatalysis in ionic liquids – advantages beyond green technology. *Curr Opin Biotechnol* **14**:432–437 (2003).
16 Kaar JL, Jesionowski AM, Berberich JA, Moulton R and Russell AJ, Impact of ionic liquid physical properties on lipase activity and stability. *J Am Chem Soc* **125**:4125–4131 (2003).
17 Mutschler J, Rausis T, Bourgeois J-M, Bastian C, Zufferey D, Mohrenz IV, *et al*, Ionic liquid-coated immobilized lipase for the synthesis of methylglucose fatty acid esters. *Green Chem* **11**:1793–1800 (2009).
18 Reichardt C, Polarity of ionic liquids determined empirically by means of solvatochromic pyridinium N-phenolate betaine dyes. *Green Chem* **7**:339–351 (2005).
19 Keskin S, Kayrak-Talay D, Akman U and Hortaçsu Ö, A review of ionic liquids towards supercritical fluid applications. *J Supercrit Fluids* **43**:150–180 (2007).
20 Lee SH, Dang DT, Ha SH, Chang W-J and Koo Y-M, Lipase-catalyzed synthesis of fatty acid sugar ester using extremely supersaturated sugar solution in ionic liquids. *Biotechnol Bioeng* **99**:1–8 (2008).
21 Ha S, Hiep N, Lee S and Koo Y-M, Optimization of lipase-catalyzed glucose ester synthesis in ionic liquids. *Bioprocess Biosyst Eng* **33**:63–70 (2010).
22 Lau RM, Sorgedrager MJ, Carrea G, van Rantwijk F, Secundo F and Sheldon RA, Dissolution of *Candida antarctica* lipase B in ionic liquids: effects on structure and activity. *Green Chem* **6**:483–487 (2004).
23 Klahn M, Lim GS, Seduraman A and Wu P, On the different roles of anions and cations in the solvation of enzymes in ionic liquids. *Phys Chem Chem Phys* **13**:1649–1662 (2011).
24 Zhao H, Jones CL and Cowins JV. Lipase dissolution and stabilization in ether-functionalized ionic liquids. *Green Chem* **11**:1128–1138 (2009).
25 van Rantwijk F, Madeira Lau R and Sheldon RA, Biocatalytic transformations in ionic liquids. *Trends Biotechnol* **21**:131–138 (2003).
26 Kim MJ, Choi MY, Lee JK and Ahn Y, Enzymatic selective acylation of glycosides in ionic liquids: significantly enhanced reactivity and regioselectivity. *J Mol Catal B – Enzym* **26**:115–118 (2003).
27 Kim K-W, Song B, Choi M-Y and Kim M-J. Biocatalysis in ionic liquids: markedly enhanced enantioselectivity of lipase. *Org Lett* **3**:1507–1509 (2001).
28 Lee S, Ha S, Lee S and Koo Y-M, Adverse effect of chloride impurities on lipase-catalyzed transesterifications in ionic liquids. *Biotechnol Lett* **28**:1335–1339 (2006).
29 Forsyth SA and MacFarlane DR, 1-Alkyl-3-methylbenzotriazolium salts: ionic solvents and electrolytes. *J Mater Chem* **13**:2451–2456 (2003).
30 Liu QB, Janssen MHA, van Rantwijk F and Sheldon RA, Room-temperature ionic liquids that dissolve carbohydrates in high concentrations. *Green Chem* **7**:39–42 (2005).
31 Zhao H, Baker GA, Song Z, Olubajo O, Crittle T and Peters D, Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. *Green Chem* **10**:696–705 (2008).
32 Shi Y-g, Li J-r and Chu Y-H, Enzyme-catalyzed regioselective synthesis of sucrose-based esters. *J Chem Technol Biotechnol* **86**:1457–1468 (2011).
33 Zhuo K, Chen Y, Chen J, Bai G and Wang J, Interactions of 1-butyl-3-methylimidazolium carboxylate ionic liquids with glucose in water: a study of volumetric properties, viscosity, conductivity and NMR. *Phys Chem Chem Phys* **13**:14542–14549 (2011).
34 Lue B-M, Guo Z and Xu X, Effect of room temperature ionic liquid structure on the enzymatic acylation of flavonoids. *Process Biochem* **45**:1375–1382 (2010).
35 Lee SH, Nguyen HM, Koo YM and Ha SH, Ultrasound-enhanced lipase activity in the synthesis of sugar ester using ionic liquids. *Process Biochem* **43**:1009–1012 (2008).
36 Ha S, Hiep N and Koo Y-M, Enhanced production of fructose palmitate by lipase-catalyzed esterification in ionic liquids. *Biotechnol Bioprocess Eng* **15**:126–130 (2010).
37 Kim HS, Pani R, Ha SH, Koo Y-M and Yingling YG, The role of hydrogen bonding in water-mediated glucose solubility in ionic liquids. *J Mol Liq* DOI: 10.1016/j.molliq.2011.11.008.
38 Lee SH, Ha SH, Hiep NM, Chang W-J and Koo Y-M, Lipase-catalyzed synthesis of glucose fatty acid ester using ionic liquids mixtures. *J Biotechnol* **133**:486–489 (2008).
39 Tan S and MacFarlane D, Ionic liquids in biomass processing. *Top Curr Chem* **290**:311–339 (2010).
40 Brennan T, Datta S, Blanch H, Simmons B and Holmes B, Recovery of sugars from ionic liquid biomass liquor by solvent extraction. *BioEnergy Res* **3**:123–133 (2010).
41 Janesko BG, Modeling interactions between lignocellulose and ionic liquids using DFT-D. *Phys Chem Chem Phys* **13**:11393–11401 (2011).
42 Gremos S, Zarafeta D, Kekos D and Kolisis F, Direct enzymatic acylation of cellulose pretreated in BMIMCl ionic liquid. *Bioresource Technol* **102**:1378–1382 (2011).
43 Liu H, Sale KL, Simmons BA and Singh S, Molecular dynamics study of polysaccharides in binary solvent mixtures of an ionic liquid and water. *J Phys Chem B* **115**:10251–10258 (2011).
44 Zakrzewska ME, Bogel-Łukasik E and Bogel-Łukasik R, Solubility of carbohydrates in ionic liquids. *Energy Fuels* **24**:737–745 (2010).
45 Kilpeläinen I, Xie H, King A, Granstrom M, Heikkinen S and Argyropoulos DS, Dissolution of wood in ionic liquids. *J Agric Food Chem* **55**:9142–9148 (2007).
46 Fort DA, Remsing RC, Swatloski RP, Moyna P, Moyna G and Rogers RD, Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-butyl-3-methylimidazolium chloride. *Green Chem* **9**:63–69 (2007).
47 Swatloski RP, Spear SK, Holbrey JD and Rogers RD, Dissolution of cellulose with ionic liquids. *J Am Chem Soc* **124**:4974–4975 (2002).
48 Kimizuka N and Nakashima T, Spontaneous self-assembly of glycolipid bilayer membranes in sugar-philic ionic liquids and formation of ionogels. *Langmuir* **17**:6759–6761 (2001).
49 Park S and Kazlauskas RJ, Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations. *J Org Chem* **66**:8395–8401 (2001).
50 Ganske F and Bornscheuer UT, Lipase-catalyzed glucose fatty acid ester synthesis in ionic liquids. *OrgLett* **7**:3097–3098 (2005).
51 Chen Z-G, Zong M-H and Li G-J, Lipase-catalyzed acylation of konjac glucomannan in ionic liquids. *J Chem Technol Biotechnol* **81**:1225–1231 (2006).
52 Katsoura MH, Polydera AC, Katapodis P, Kolisis FN and Stamatis H, Effect of different reaction parameters on the lipase-catalyzed selective acylation of polyhydroxylated natural compounds in ionic liquids. *Process Biochem* **42**:1326–1334 (2007).

53 Katsoura MH, Polydera AC, Tsironis L, Tselepis AD and Stamatis H, Use of ionic liquids as media for the biocatalytic preparation of flavonoid derivatives with antioxidant potency. *J Biotechnol* **123**:491–503 (2006).
54 Park S, Viklund F, Hult K and Kazlauskas Romas J, Ionic liquids create new opportunities for nonaqueous biocatalysis with polar substrates: acylation of glucose and ascorbic acid. *Ionic Liquids as Green Solvents: ACS Symposium Series* **856**:225–238 (2003).
55 Galletti P, Moretti F, Samori C and Tagliavini E, Enzymatic acylation of levoglucosan in acetonitrile and ionic liquids. *Green Chem* **9**:987–991 (2007).
56 Li X-F, Lou W-Y, Smith TJ, Zong M-H, Wu H and Wang J-F, Efficient regioselective acylation of 1-[β]-D-arabinofuranosylcytosine catalyzed by lipase in ionic liquid containing systems. *Green Chem* **8**:538–544 (2006).
57 Nara SJ, Mohile SS, Harjani JR, Naik PU and Salunkhe MM, Influence of ionic liquids on the rates and regioselectivity of lipase-mediated biotransformations on 3,4,6-tri-O-acetyl-glucal. *J Mol Catal B: Enzym* **28**:39–43 (2004).
58 Hu Y, Guo Z, Lue BM and Xu X, Enzymatic synthesis of esculin ester in ionic liquids buffered with organic solvents. *J Agric Food Chem* **57**:3845–3852 (2009).
59 Ganske F and Bornscheuer UT, Optimization of lipase-catalyzed glucose fatty acid ester synthesis in a two-phase system containing ionic liquids and t-BuOH. *J Mol Catal B: Enzym* **36**:40–42 (2005).
60 Tan Z-Y, Wu H and Zong M-H, Novozym 435-catalyzed regioselective benzoylation of 1-β-D-arabinofuranosylcytosine in a co-solvent mixture of C4MIm·PF6 and pyridine. *Biocatal Biotransform* **25**:408–413 (2007).
61 Li N, Ma D and Zong M-H, Enhancing the activity and regioselectivity of lipases for 3′-benzoylation of floxuridine and its analogs by using ionic liquid-containing systems. *J Biotechnol* **133**:103–109 (2008).
62 Liu BK, Wang N, Chen ZC, Wu Q and Lin XF, Markedly enhancing lipase-catalyzed synthesis of nucleoside drugs' ester by using a mixture system containing organic solvents and ionic liquid. *Bioorg Med Chem Lett* **16**:3769–3771 (2006).
63 Liu B-K, Wu Q, Xu J-M and Lin X-F, N-Methylimidazole significantly improves lipase-catalysed acylation of ribavirin. *Chem Comm* 295–297 (2007).
64 Kaftzik N, Wasserscheid P and Kragl U, Use of ionic liquids to increase the yield and enzyme Stability in the β-Galactosidase catalysed synthesis of N-acetyllactosamine. *Org Process Res Dev* **6**:553–557 (2002).
65 Lang M, Kamrat T and Nidetzky B, Influence of ionic liquid cosolvent on transgalactosylation reactions catalyzed by thermostable β-glycosylhydrolase CelB from *Pyrococcus furiosus*. *Biotechnol Bioeng* **95**:1093–1100 (2006).
66 Yang R-L, Li N and Zong M-H, Using ionic liquid cosolvents to improve enzymatic synthesis of arylalkyl β-d-glucopyranosides. *J Mol Catal B: Enzym* **74**:24–28 (2012).
67 Yang Z, Hofmeister effects: an explanation for the impact of ionic liquids on biocatalysis. *J Biotechnol* **144**:12–22 (2009).
68 Zhao H, Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids. *J Mol Catal B: Enzym* **37**:16–25 (2005).
69 Halling PJ, Thermodynamic predictions for biocatalysis in nonconventional media: theory, tests, and recommendations for experimental design and analysis. *Enzyme Microbiol Technol* **16**:178–206 (1994).
70 Lee S, Koo Y-M and Ha S, Influence of ionic liquids under controlled water activity and low halide content on lipase activity. *Korean J Chem Eng* **25**:1456–1462 (2008).
71 Adamczak M and Bornscheuer UT, Improving ascorbyl oleate synthesis catalyzed by *Candida antarctica* lipase B in ionic liquids and water activity control by salt hydrates. *Process Biochem* **44**:257–261 (2009).
72 Frade RF and Afonso CA, Impact of ionic liquids in environment and humans: an overview. *Hum Exp Toxicol* **29**:1038–1054 (2010).
73 Thuy Pham TP, Cho C-W and Yun Y-S, Environmental fate and toxicity of ionic liquids: a review. *Water Res* **44**:352–372 (2010).
74 Stolte S, Matzke M, Arning J, Boschen A, Pitner W-R, Welz-Biermann U, *et al*, Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. *Green Chem* **9**:1170–1179 (2007).
75 Gathergood N, Garcia MT and Scammells PJ, Biodegradable ionic liquids: Part I. Concept, preliminary targets and evaluation. *Green Chem* **6**:166–175 (2004).
76 Awad WH, Gilman JW, Nyden M, Harris RH, Sutto TE, Callahan J, *et al*, Thermal degradation studies of alkyl-imidazolium salts and their application in nanocomposites. *Thermochim Acta* **409**:3–11 (2004).
77 Azimova MA, Morton SA, Iii and Frymier PD, Comparison of three bacterial toxicity assays for imidazolium-derived ionic liquids. *J Environ Eng* **135**:1388–1392 (2009).
78 Stepnowski P and Zaleska A, Comparison of different advanced oxidation processes for the degradation of room temperature ionic liquids. *J Photochem Photobiol A* **170**:45–50 (2005).
79 Li X, Zhao J, Li Q, Wang L and Tsang SC, Ultrasonic chemical oxidative degradations of 1,3-dialkylimidazolium ionic liquids and their mechanistic elucidations. *Dalton Trans* 1875–1880 (2007).
80 Siedlecka E and Stepnowski P, The effect of alkyl chain length on the degradation of alkylimidazolium- and pyridinium-type ionic liquids in a Fenton-like system. *Environ Sci Pollut Res* **16**:453–458 (2009).
81 Gathergood N, Scammells PJ and Garcia MT, Biodegradable Ionic Liquids. Part 3. The first readily biodegradable ionic liquids. *Chem Inf* **37**:156–160 (2006).
82 Garcia MT, Gathergood N and Scammells PJ, Biodegradable ionic liquids. Part II. Effect of the anion and toxicology. *Green Chem* **7**:9–14 (2005).
83 Docherty K, Dixon J and Kulpa Jr C, Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community. *Biodegradation* **18**:481–493 (2007).
84 Stolte S, Abdulkarim S, Arning J, Blomeyer-Nienstedt A-K, Bottin-Weber U, Matzke M, *et al*, Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds. *Green Chem* **10**:214–224 (2008).
85 Matzke M, Stolte S, Arning J, Uebers U and Filser J, Ionic liquids in soils: effects of different anion species of imidazolium based ionic liquids on wheat (*Triticum aestivum*) as affected by different clay minerals and clay concentrations. *Ecotoxicology* **18**:197–203 (2009).
86 Gelo-Pujic M, Guibe-Jampel E, Loupy A, Galema SA and Mathe D, Lipase-catalysed esterification of some α-D-glucopyranosides in dry media under focused microwave irradiation. *J Chem Soc Perkin Trans 1*. 2777–2780 (1996).
87 Chen Z-G, Zong M-H, Gu Z-X and Han Y-B, Effect of ultrasound on enzymatic acylation of konjac glucomannan. *Bioprocess Biosyst Eng* **31**:351–356 (2008).
88 Zhao H, Baker GA, Song Z, Olubajo O, Zanders L and Campbell SM, Effect of ionic liquid properties on lipase stabilization under microwave irradiation. *J Mol Catal B: Enzym* **57**:149–157 (2009).
89 Blanchard LA, Hancu D, Beckman EJ and Brennecke JF, Green processing using ionic liquids and CO2. *Nature* **399**:28–29 (1999).
90 Park S, Viklund F, Hult K and Kazlauskas RJ, Vacuum-driven lipase-catalysed direct condensation of l-ascorbic acid and fatty acids in ionic liquids: synthesis of a natural surface active antioxidant. *Green Chem* **5**:715–719 (2003).