Ionic liquids in whole-cell biocatalysis: a compromise between toxicity and efficiency

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Received: 9 October 2017 / Accepted: 13 December 2017 / Published online: 8 January 2018

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Abstract

Comparison of chemical catalysis by metal complexes, enzymatic catalysis and whole-cell biocatalysis shows well-addressed advantages of the latter approach. However, a critical limitation in the practical applications originates from the high sensitivity of microorganisms to the toxic effects of organic solvents. In the present review, we consider toxic solvent properties of ionic liquid/water systems towards the development of efficient applications in practical organic transformations.

Keywords Ionic liquids · Toxicity · Biological activity · Catalysis · Biocatalysis

Abbreviations

AMMOENGTM Cocosalkylpentaethoxymethylammonium
 100 methylsulfate

IL cations

[C _n MIM]	1-alkyl-3-methylimidazolium
[HOC ₂ MIM]	(2-hydroxy)ethyl-3-methylimidazolium
[C _n C ₁ MIM]	1-alkyl-2,3-dimethylimidazolium
[PPMIM]	1-phenylpropyl-3-methylimidazolium
[AMIM]	1-allyl-3-methylimidazolium
[BzMIM]	1-benzyl-3-methylimidazolium
[HOOC ₂ MIM]	1-carboxymethyl-3-methylimidazolium
[C _n Py]	<i>N</i> -alkylpyridinium
[C _n MPy]	<i>N</i> -alkyl-3-methylpyridinium
[C _n MPyr]	<i>N</i> -alkyl- <i>N</i> -methylpyrrolidinium
[C _n MPip]	<i>N</i> -alkyl- <i>N</i> -methylpiperidinium
[(C ₁ OC ₃)C ₁ Pip]	<i>N</i> -methyl- <i>N</i> -(1-methoxypropyl)-piperidinium
[C _n C _n C _n C _n N]	<i>N,N,N,N</i> -tetraalkylammonium
[Chol]	cholinium, <i>N</i> -(2-hydroxy)ethyl- <i>N,N,N</i> -trimethylammonium

[(HOC ₂)(C ₁) ₂ N]	<i>N,N</i> -dimethyl- <i>N</i> -ethanolammonium
[(HOC ₂) ₂ C ₄ C ₂ N]	<i>N,N</i> -di(2-hydroxy)ethyl- <i>N</i> -butyl- <i>N</i> -ethylammonium
[(HOC ₃)C ₂ (C ₁) ₂ N]	<i>N</i> -(3-hydroxy)propyl- <i>N</i> -ethyl- <i>N,N</i> -dimethylammonium
[(HOC ₃)C ₄ (C ₁) ₂ N]	<i>N</i> -(3-hydroxy)propyl- <i>N</i> -butyl- <i>N,N</i> -dimethylammonium
[(HOC ₂) ₃ C ₁ N]	Tris(2-hydroxyethyl)methylammonium
[C _n C _n C _n C _n N]	Tetraalkylphosphonium
[(HOC ₃) ₃ C ₁₀ P]	Decyltris(3-hydroxypropyl)phosphonium
[(C ₁) ₄ C ₂ Gua]	<i>N,N,N,N'</i> -tetramethyl- <i>N''</i> -ethylguanidinium
[CABHEM]	PEG-5 cocomonium

IL anions

[BF ₄]	Tetrafluoroborate
[PF ₆]	Hexafluorophosphate
[OTf]	Trifluoromethanesulfonate
[NTf ₂]	Bis(trifluoromethylsulfonyl)imide
[(C ₂ F ₅) ₃ PF ₃]	Tris(pentafluoroethyl)trifluorophosphate
[N(CN) ₂]	Dicyanamide
[SCN]	Thiocyanate
[NO ₃]	Nitrate
[OAc]	Acetate
[C ₂ COO]	Propanoate
[C ₅ COO]	Hexanoate
[C ₁ PO ₃]	Methylphosphonate
[C ₂ PO ₄]	Dimethylphosphate
[i-(C ₈) ₂ PO ₄]	Bis(2,4,4-trimethylpentyl)phosphinate

This article is part of a Special Issue on 'Ionic Liquids and Biomolecules' edited by Antonio Benedetto and Hans-Joachim Galla.

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[C ₂ SO ₃]	Ethylsulfonate
[C _n SO ₄]	Alkylsulfate
[C _n OC _n SO ₄]	2-alkoxyalkylsulfate
[C ₁ (OC ₂) ₃ SO ₄]	2-[2-(2-methoxy)ethoxy]ethoxyethylsulfate
[Tos]	Tosylate, <i>p</i> -toluenesulfonate
[MDEGSO ₄]	Ethylenglycolmonomethylethersulfate
[SbF ₆]	Hexafluoroantimonate
[Lac]	Lactate
[Sacch]	Saccharinate
[Doc]	Docusate
[Lin]	Linoleate

Introduction

Transition metal catalysis dominates modern industrial and chemical synthesis, both in terms of production volumes and diversity of reactions (hydrocarbon processing, fuel production, fine chemical synthesis, pharmaceutical applications, etc.) (Egorova and Ananikov 2016). Such chemical catalysis has indisputable advantages: it shows good selectivity and can be carried out in a variety of solvents (Table 1). However, known disadvantages include sustainability issues (North and Clark 2016), high cost of metals (Pt, Pd, Rh, Ru, Au, etc.) and the impossibility of easy self-reproduction of catalysts.

From this viewpoint, enzymatic catalysis can be seen as a successful alternative to metal catalysis in terms of good sustainability, chemical selectivity and solvent tolerance (Table 1). However, the cost of enzymatic catalysts remains high, and enzymes cannot directly self-replicate.

In this regard, whole-cell biocatalysis presents an excellent opportunity, since it is characterized by high sustainability level, excellent chemical selectivity, relatively low cost and an ability to easily self-replicate. However, the well-addressed critical limitation is a poor solvent tolerance: usually whole-cell systems perform best in aqueous buffered media, while such biocatalytic systems are not usually compatible with regular organic solvents (Table 1). This is a critical limitation from a practical point of view and overcoming this problem may solve a number of challenges in modern research and development.

As shown in Table 1, biocatalytic approaches are inherently very efficient and can be divided into isolated enzymes and whole cells (Fig. 1). The former are beneficial in one-step reactions, whereas the latter allow the carrying out of complex multi-step processes. Whole-cell biocatalysis, in turn, can be roughly divided into biotransformation and fermentation processes, though intermediate cases are known (de Carvalho 2011; Schrewe et al. 2013; Ladkau et al. 2014; Wachtmeister and Rother 2016; de Carvalho 2017; Lin and Tao 2017).

One of the main advantages of enzymes is their ability to function *in vitro*, under conditions unsuitable for cell growth. Such conditions can be optimized for particular chemical

transformation for facilitating product purification. Among the other advantages of enzymes are the reaction specificity and simplicity of the methodology. However, enzymatic protocols often demand preliminary isolation and immobilization procedures, as well as usage of cofactors. Whole-cell biocatalysis provides the means of running highly selective multi-step reactions with cofactor regeneration. Its disadvantages relate to catalyst instability or product inhibition, formation of toxic by-products and mass transport issues (Fig. 2) (de Carvalho 2011; Schrewe et al. 2013; Lin and Tao 2017).

The nature of target compounds also imposes its restrictions on the application of whole-cell biocatalysts. Due to hydrophobicity of many products, studies on whole-cell biocatalysis in unconventional, non-aqueous media have recently been attracting much attention. Often, biphasic systems consisting of an aqueous (buffer) phase and an organic phase are used (Wachtmeister and Rother 2016; de Carvalho 2017).

In both chemical catalysis and biocatalysis, the issue of environmental toxicity of catalytic media is of great importance. Since common organic solvents can be dangerous to the environment, recyclable media have been attracting significant attention in recent decades (Zhang 2006). In contrast to chemical catalysts, biocatalytic systems, especially whole-cell ones, can be very sensitive to organic solvents. Therefore, ionic liquids and deep eutectic solvents have been suggested as safer alternatives to common organic reagents (Weuster-Botz 2007; Gorke et al. 2010; Quijano et al. 2010; Xu et al. 2016a).

Ionic liquids (ILs), or molten salts, are thermally stable, nonflammable and nonvolatile media prone to micro- and nanostructuring. ILs usually consist of bulky organic cations and weakly coordinating anions (Fig. 3). Remarkable tunability and solvent properties of ILs are being widely employed in dissolution of biomolecules and extraction (Seitkalieva et al. 2015; Egorova et al. 2017; Seitkalieva et al. 2017). The striking solvent properties of ILs can be explained by spatial micro- and nano-heterogeneity of the media which has been established by both theoretical and experimental studies. IL ions in the liquid phase self-assemble into relatively stable amphiphilic nanostructures which are realized via ionic interactions and hydrogen bonds (Wang and Voth 2005; Triolo et al. 2007; Hayes et al. 2015; Egorova et al. 2017).

Table 1 Selected key characteristics of different types of catalysis

Characteristics	Catalysis		
	Chemical	Enzymatic	Whole-cell
Sustainability	Low	High	High
Cost	High	High	Low
Selectivity	Good	High	High
Self-replication	No	No	Yes
Solvent tolerance	High	Good	Low

From the economical viewpoint, ILs also seem rather plausible candidates. In recent years, the cost of IL production has been decreasing, and production prices of US\$1–5 per kg of ammonium or imidazolium IL have been reported (Chen et al. 2014; George et al. 2015; Sun et al. 2017). Moreover, IL recycling in biocatalytic applications has been demonstrated (Dennewald et al. 2011).

In spite of their presumable “safety”, ILs can possess significant biological activity (Egorova and Ananikov 2014; Egorova et al. 2017). Therefore, their application in biocatalysis should be a compromise between two factors: (1) solvent properties that benefit the desired chemical reaction/separation, and (2) toxic effects of solvents that inhibit the whole-cell approach. In this review, we briefly discuss both aspects of IL usage in modern whole-cell biocatalysis, while our main attention is paid to the second aspect dealing with the toxicological properties of ILs studied in various whole-cell biocatalytic systems. Our research interests are focused on the toxicity of ILs, and here we analyze the perspectives of application of whole-cell biocatalysts in demanding chemical reactions from the viewpoint of reaction media (solvents).

Ionic liquids in whole-cell biocatalysis

To date, ILs have found applications in various types of whole-cell biocatalytic reactions, among which are biofuel production (Arai et al. 2010; Ouellet et al. 2011; Frederix et al. 2016; Hashmi et al. 2017; Pérez de los Ríos et al. 2017), enantioselective reduction of ketones (Howarth et al. 2001; Pfruender et al. 2004, 2006; Lou et al. 2006; Bräutigam et al. 2007; Hussain et al. 2007; Schroer et al. 2007; Zhang et al. 2008; Bräutigam et al. 2009; Lou et al. 2009a, b and c; Wang et al. 2009, 2013; Dennewald et al. 2011, 2012; Xiao et al. 2012; Zhang et al. 2012; Silva et al. 2013; Zampieri et al. 2013; Li et al. 2015; Xu et al. 2016b, 2017), enantioselective sulfoxidation (Gao et al. 2014), enantioselective hydrolysis (Matsumoto et al. 2014), enantioselective hydroxylation (Cornmell et al. 2008; Wu et al. 2011; Mao et al. 2012, 2013), dehydrogenation (He et al. 2011), double-bond hydrogenation (Lenourry et al. 2005; Castiglione et al. 2017), glycosylation (Schmideder et al. 2016), and nucleoside acylation (Yang et al. 2014) (see Table 2).

Fig. 1 Biocatalysis is carried out by isolated enzymes or by whole cells. In the latter case, non-native (biotransformation) or native (fermentation) substrates can be used

Fig. 2 Main advantages and disadvantages of whole-cell biocatalysis

As evidenced by the accumulated reports, enantioselective reduction of ketones is one of the most popular reactions in IL-assisted whole-cell biocatalysis. In this case, ILs are usually employed as a non-aqueous phase in the two-phase systems in which the reaction is carried out (Fig. 4). Biocatalysis is carried out in nearly native conditions in the water phase, in which the toxic effect of the added solvent can be minimized. Extraction of organic product to IL phase simplifies the overall process and facilitates separation. Diffusion of reagents between two phases is an important parameter to connect different stages into a continuous process.

Such IL-containing systems have been successfully used for the asymmetric bioreduction of 2-octanone to (*R*)-2-octanol (organism: recombinant *E.coli*; IL: [C₆MPyr][NTf₂]) (Fig. 4) (Bräutigam et al. 2009; Dennewald et al. 2011, 2012); of 2,5-hexanedione to (2*R*,5*R*)-hexanediol (organism: recombinant *E.coli*; IL: [C₄MIM][NTf₂]) (Schroer et al. 2007); of 6-Br-β-tetralone to (*S*)-6-Br-β-tetralol (organisms: *Rhodococcus erythropolis*, *Trichosporon capitatum*; IL: [C₂MIM][Tos]) (Hussain et al. 2007); of 4'-methoxyacetophenone to (*R*)- or (*S*)-1-(4-methoxyphenyl)ethanol (organisms: *Trigonopsis variabilis*, *Rhodotorula* sp.; ILs: [HOC₂MIM][NO₃] (co-solvent), [C₄MIM][PF₆]) (Lou et al. 2009b, c; Wang et al. 2009); of 4-chloroacetophenone to (*R*)-1-(4-chlorophenyl)ethanol (organism: *Lactobacillus kefir*; IL: [C₄MIM][NTf₂]) (Pfruender et al. 2004); and of ethyl acetoacetate to ethyl (*R*)-3-hydroxybutyrate (organism: *Acetobacter* sp.; IL: [C₄MIM][PF₆]) (Wang et al. 2013).

Fig. 3 Examples of common cations and anions used in ionic liquids

Another promising direction of IL application in whole-cell biocatalysis is the production of biofuels, such as biodiesel and bioethanol, from biomass. Due to their unique dissolution properties, ILs have been used for the pretreatment of biomass (Zakrzewska et al. 2011; Wang et al. 2012). Possible positive and negative effects of ILs on biofuel-producing organisms have been studied in several microbial systems. A study on $[C_4MIM][OAc]$ -pretreated sugarcane bagasse produced no definite evidences of advantages (disadvantages) of the IL application (Hashmi et al. 2017). In *Saccharomyces cerevisiae*, residual $[C_2MIM][OAc]$ in biomass hydrolyzate inhibited the yeast growth (Ouellet et al. 2011). A $[C_2MIM][OAc]$ pretreatment of switchgrass was efficient for lignocellulose disruption, but toxic for *Escherichia coli*. The authors reported the development of $[C_2MIM]$ -resistant *E. coli* strain possibly providing new advantages in biofuel production (Frederix et al. 2016). The design and selection of IL-resistant microbial strains seem to be the main route to successful application of ILs in modern whole-cell biocatalysis. This subject will be further discussed in the next section.

Currently, *Escherichia coli* and *Saccharomyces cerevisiae* are the most studied microorganisms to which IL-containing systems have been applied. Representative examples of such applications are provided in Table 3.

Indeed, in a carefully optimized process, ILs exhibit a noticeable positive impact on whole-cell biocatalysis and greatly enhance practical usage. The nanoheterogeneous nature of ILs can enhance their application in biocatalysis. In particular, IL-water systems can contain nanobubbles, nanochannels and other structures, depending on the water content (Kashin et al. 2016; Seitkalieva et al. 2017). Protein molecules and even whole bacterial cells can possibly exist in such aqueous

compartments or surrounded by water shells without redundant contacts with the IL media (Benedetto and Ballone 2016; Egorova et al. 2017). However, further optimization and application to a diverse range of chemical reactions is limited by the toxic effect of added ILs, which is discussed in more detail in the next section.

Toxicity of ILs towards microbial cells

There are three broad categories of studies on biological effects imposed by ILs on microbial cells: environmental (concerning toxicity towards ecological indicator species), medical (concerning inhibition of antibiotic-resistant pathogenic microorganisms) and biotechnological (concerning IL advantages in biocatalysis). Such separation often seems rather artificial, because eventually all ILs regardless of their application can enter the environment. For example, $[C_4MIM][PF_6]$ was found to enhance the horizontal transfer of antibiotic resistance genes thus posing an indirect danger to human health (Luo et al. 2014). Therefore, the toxic aspect of these reagents should not be underestimated.

Most publications on the application of ILs in whole-cell biocatalysis address their toxic effects to some degree. These findings are summarized in Table 4. Usually, they do not allow unambiguous recommendations on the choice of ILs for a particular application; however, some general conclusions made for other biological objects are also valid for biocatalytic microorganisms. Thus, the IL toxic effect is thought to depend on the length of the alkyl chain in the cation, as well as on the nature of the cation and anion; the organism nature also plays an important role in the development of toxic effects (Egorova

Table 2 IL-assisted whole-cell biocatalytic reactions

Reaction	Organism	Ref.
Biofuel production	<i>Rhizopus oryzae</i> , <i>Aspergillus oryzae</i> , <i>Escherichia coli</i> , <i>Saccharomyces cerevisiae</i> , <i>Scheffersomyces stipitis</i>	(Arai et al. 2010; Ouellet et al. 2011; Frederix et al. 2016; Hashmi et al. 2017; Pérez de los Ríos et al. 2017)
Enantioselective reduction of ketones	<i>Escherichia coli</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida parapsilosis</i> , <i>Rhodotorula</i> sp., <i>Trigonopsis variabilis</i> , <i>Lactobacillus kefir</i> , <i>Acetobacter</i> sp., <i>Acinetobacter</i> sp., <i>Cryptococcus</i> sp., <i>Candida albicans</i> , <i>Rhodotorula glutinis</i> , <i>Geotrichum candidum</i> , <i>Micrococcus luteus</i> , <i>Aureobasidium pullulans</i> , <i>Trichosporon capitatum</i> , <i>Rhodococcus erythropolis</i>	(Howarth et al. 2001; Pfruender et al. 2004, 2006; Lou et al. 2006; Bräutigam et al. 2007; Hussain et al. 2007; Schroer et al. 2007; Zhang et al. 2008; Bräutigam et al. 2009; Lou et al. 2009a, b, c; Wang et al. 2009, 2013; Dennewald et al. 2011, 2012; Xiao et al. 2012; Zhang et al. 2012; Silva et al. 2013; Zampieri et al. 2013; Li et al. 2015; Xu et al. 2016b, 2017)
Hydrolysis of glycyrrhizin	<i>Penicillium purpurogenum</i> , <i>Escherichia coli</i> , <i>Pichia pastoris</i>	(Chen et al. 2012)
Enantioselective hydrolysis	<i>Rhodotorula glutinis</i>	(Matsumoto et al. 2014)
Enantioselective hydroxylation	<i>Escherichia coli</i> , <i>Penicillium raistrickii</i> , <i>Aspergillus ochraceus</i> , <i>Rhizopus nigricans</i>	(Commell et al. 2008; Wu et al. 2011; Mao et al. 2012, 2013)
Enantioselective sulfoxidation	<i>Escherichia coli</i>	(Gao et al. 2014)
Dehydrogenation	<i>Arthrobacter simplex</i>	(He et al. 2011)
C=C hydrogenation	<i>Escherichia coli</i> , <i>Sporomusa termitida</i>	(Lenourry et al. 2005; Castiglione et al. 2017)
Glycosylation	<i>Escherichia coli</i>	(Schmieder et al. 2016)
Nucleoside acylation	<i>Pseudomonas fluorescens</i>	(Yang et al. 2014)

and Ananikov 2014). Figure 5 shows the map of biocompatibility of various ILs for *Escherichia coli* compiled on the basis of the available data.

Further analysis involves different microorganisms and shows the relative tolerance of frequently used biocatalytic systems towards the most studied ILs (Fig. 6). According to the existing data, cholinium ILs and imidazolium ILs with short alkyl chains represent the systems of choice for application in whole-cell biocatalysis. As for the IL anions, chloride seems the best choice for the selected microorganisms. Regarding the organisms, the *Penicillium* genus shows the highest IL tolerance, whereas *S.cerevisiae* demonstrates high sensitivity to most ILs.

Toxicity of ILs presumably manifests due to their interaction with the cell membrane. ILs with long alkyl chains

supposedly penetrate into the lipid bilayer thus disturbing its structure (Jeong et al. 2012; Petkovic et al. 2012; Benedetto and Ballone 2016; Benedetto 2017; Egorova et al. 2017). Upon the insertion of IL cations, the membrane starts bending which supposedly leads to morphological damage (Yoo et al. 2016). In the case of imidazolium ILs, the imidazolium ring interacts with the lipid head group, whereas the alkyl side chain forms contacts with the lipid tail. Longer alkyl chains facilitates the deeper penetration of IL into the lipid bilayer (Yoo et al. 2014).

Presumably, in the case of microorganisms, the damage to the cell membrane is one of the main reasons of IL toxicity. Thus, it was shown that $[C_nMIM][Cl]$ with long alkyl chains ($n = 6, 8, 10$) caused membrane damage, whereas cholinium ILs were less toxic, though their toxicity increased upon increasing

Fig. 4 Enantioselective whole-cell bioreduction of 2-octanone to (*R*)-2-octanol in a biphasic system consisting of an aqueous buffer and an IL. LB-ADH alcohol dehydrogenase from *Lactobacillus brevis*; CB-FDH formate dehydrogenase from *Candida boidinii*. Reproduced with permission from Bräutigam et al. (2009)

Table 3 Application of ILs in whole-cell biocatalysis by *E.coli* and *S.cerevisiae*

Reaction	IL system	Ref.
<i>Escherichia coli</i> (recombinant)		
Benzaldehyde, HCN $\rightarrow$ (<i>S</i>)-mandelic acid, (<i>S</i>)-mandeloamide	Two-phase system (aqueous phase - 20% (v/v) [C ₄ MPyr][NTf ₂] or [C ₄ MIM][PF ₆])	(Baum et al. 2012)
(<i>R</i>)-carvone $\rightarrow$ (2 <i>R</i> ,5 <i>R</i>)-dihydrocarvone	Two-phase system (aqueous phase - 20% (v/v) [C ₆ MPyr][NTf ₂] or [C ₆ MIM][PF ₆] or [C ₆ Py][NTf ₂])	(Castiglione et al. 2017)
3-chloro-1-phenyl-1-propanone $\rightarrow$ (<i>S</i>)-3-chloro-1-phenyl-1-propanol	Two-phase system (aqueous phase - 20% (v/v) [C ₄ MIM][NTf ₂])	(Choi et al. 2011)
4-chloroacetophenone $\rightarrow$ (<i>R</i>)-1-(4-chlorophenyl)ethanol; ethyl 4-chloroacetoacetate $\rightarrow$ ethyl (<i>S</i>)-4-chloro-3-hydroxybutyrate; phenacyl chloride $\rightarrow$ (<i>S</i>)- α -chloro-1-phenylethanol	Two-phase system (aqueous phase - 20% (v/v) [C ₄ MIM][NTf ₂] or [C ₄ MIM][PF ₆])	(Bräutigam et al. 2007)
Toluene $\rightarrow$ toluene <i>cis</i> -glycol	Two-phase system (aqueous phase - 23% (v/v) [(C ₈) ₃ C ₁ N][NTf ₂] or [C ₁₄ (C ₆) ₃ P][NTf ₂])	(Cornnell et al. 2008)
2-Octanone $\rightarrow$ (<i>R</i>)-2-octanol	Two-phase system (aqueous phase - 20–70% (v/v) [C ₆ MPyr][NTf ₂])	(Bräutigam et al. 2009; Dennewald et al. 2011, 2012)
Biomass $\rightarrow$ limonene	Biomass pretreatment ([C ₂ MIM][OAc])	(Frederix et al. 2016)
Asymmetric sulfoxidation of sulfides	Two-phase system (aqueous phase: [C ₁₄ (C ₆) ₃ P][NTf ₂] = 10:1)	(Gao et al. 2014)
Geraniol $\rightarrow$ geranyl glucoside	Two-phase system (aqueous phase - 5% (v/v) [C ₆ Py][NTf ₂])	(Schmiededer et al. 2016)
2,5-hexanedione $\rightarrow$ (2 <i>R</i> ,5 <i>R</i>)-hexanediol	Two-phase system (aqueous phase - 5% (v/v) [C ₄ MIM][NTf ₂])	(Schroer et al. 2007)
Baeyer–Villiger bioconversion	Three-phase system tank bioreactor (air – water – 5–20% (v/v) [C ₄ MPyr][NTf ₂])	(Melgarejo-Torres et al. 2011)
<i>Saccharomyces cerevisiae</i>		
Biomass $\rightarrow$ ethanol	Biomass pretreatment ([C ₂ MIM][OAc], [C ₄ MIM][OAc]); as extraction phase ([C ₈) ₃ C ₁ N][NTf ₂], [C ₁₄ (C ₆) ₃ P][Cl])	(Ouellet et al. 2011; Hashmi et al. 2017; Pérez de los Rios et al. 2017)
Ketone bioreduction	[C ₄ MIM][PF ₆] : water system (10:1)	(Howarth et al. 2001)
Benzaldehyde, glucose $\rightarrow$ (<i>R</i>)-phenylacetylcarbinol	Two-phase system (aqueous phase +5% (v/v) [C ₄ MIM][PF ₆])	(Kandar et al. 2015)
Acetyltrimethylsilane $\rightarrow$ (<i>S</i>)-1-trimethylsilylethanol	Two-phase system (aqueous phase: [C ₄ MIM][PF ₆] = 6:1 (v/v); aqueous phase +10% (v/v) [C ₄ MIM][BF ₄] as co-solvent)	(Lou et al. 2006)
4-chloroacetoacetate $\rightarrow$ (<i>S</i>)-4-chloro-3-hydroxybutanoate	Two-phase system (aqueous phase +20% (v/v) [C ₄ MIM][PF ₆])	(Pfruender et al. 2006)
L-phenylalanine $\rightarrow$ 2-phenylethanol	Two-phase system (aqueous phase +20% (v/v) [C ₄ MIM][NTf ₂] or [C ₃ MPy][NTf ₂] or [(C ₈) ₃ C ₁ N][NTf ₂])	(Sendovski et al. 2010)
Reduction of (<i>Z</i>)-C ₆ H ₅ CH=CXC(=O)CH ₃ (X = Cl, Br)	Two-phase system (aqueous phase +0.5% (v/v) [C ₄ MIM][PF ₆])	(Zampieri et al. 2013)

the length of the alkanoate anion. [C_nMIM][Cl] also up-regulated the expression of genes involved in the biosynthesis of saturated fatty acids (Jing et al. 2014a, b; Hartmann et al. 2015). A study on the accumulation of [P_{6,6,6,14}][NTf₂] in *Escherichia coli* found the IL in the membrane fraction, but not in the cytoplasm (Cornnell et al. 2008).

Another important issue is the ability of ILs to penetrate into the cellular nucleus (Chattoraj et al. 2016). ILs can bind to nucleic acids via electrostatic interactions with phosphate groups and via hydrogen bonds with nucleobases. Various

ILs have been shown to stabilize DNA in its native B structure (Chandran et al. 2012; Jumbri et al. 2014; Egorova et al. 2017). Some ILs (e.g., guanidinium ILs) also cause DNA compaction (Satpathi et al. 2015; Benedetto and Ballone 2016). Though no direct evidence has yet been obtained, such a strong impact of ILs on the DNA structure suggests the possibility of harmful effects on cell viability and metabolism.

From the viewpoint of the development of resistant microbial strains, studies on transcriptome and proteome of IL-treated microorganisms are of primary importance. Such

Table 4 Summary of toxicity effects of ILs towards whole-cell biocatalysts (MNTC - maximum non-toxic concentration)

IL	Effect	Ref.
<i>Escherichia coli</i>		
[C ₂ MIM][Cl]	Inhibited cell growth at concentrations >200 mM (some mutations increased tolerance); imposed no effect at 2% (v/v) in 24 h	(Wood et al. 2011; Frederix et al. 2014, 2016)
[C ₂ MIM]X X = [Br], [C ₂ SO ₄], [C ₁ (OC ₂) ₃ SO ₄], [Tos]	Imposed no significant effect at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₂ MIM][BF ₄]	Inhibited cell growth at >500 mg*L ⁻¹ in solid culture or at >4000 mg*L ⁻¹ in suspension culture; 8-h EC ₅₀ 30,000–40,000 mg*L ⁻¹	(Lee et al. 2005)
[C ₂ MIM][OAc]	Inhibited cell growth at concentrations >150 mM (some mutations increased tolerance)	(Frederix et al. 2016)
[C ₂ MIM][C ₁ SO ₄]	Inhibited cell growth at >100 mg*L ⁻¹ in solid culture or at >1000 mg*L ⁻¹ in suspension culture; 8-h EC ₅₀ 2000–3000 mg*L ⁻¹	(Lee et al. 2005)
[C ₂ MIM]X X = [C ₈ SO ₄], [Lac]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₂ MIM][OTf]	8-h EC ₅₀ 10,000–30,000 mg*L ⁻¹	(Lee et al. 2005)
[C ₂ MIM][NTf ₂]	Inhibited cell growth at 0.25–40% (v/v)	(Cornmell et al. 2008; Wood et al. 2011)
[C ₂ MIM][Doc]	Imposed some effect at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₄ MIM]X X = [Cl], [C ₁ SO ₄]	Imposed no effect at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₄ MIM]X X = [I], [OAc]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₄ MIM]X X = [Br], [C ₂ SO ₄], [C ₃ SO ₄], [i-C ₃ SO ₄], [i-C ₄ SO ₄], [C ₁ OC ₂ SO ₄], [C ₂ OC ₂ SO ₄], [Lac], [Doc]	Imposed some effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₄ MIM][BF ₄]	8-h EC ₅₀ 8500–9500 mg*L ⁻¹	(Lee et al. 2005)
[C ₄ MIM][PF ₆]	According to some reports, 20% (v/v) did not impose significant effects in 4–5 h; according to one report, 9% (v/v) inhibited cell growth in 4 h; membrane integrity decreased moderately after 5 h incubation in 20% (v/v); 8-h EC ₅₀ 3500–4500 mg*L ⁻¹	(Lee et al. 2005; Pfruender et al. 2006; Bräutigam et al. 2007; Baum et al. 2012; Gao et al. 2014)
[C ₄ MIM][NTf ₂]	According to one report, 20% (v/v) did not impose significant effects in 5 h; according to other reports, inhibited cell growth at 0.25–40% (v/v); membrane integrity decreased significantly after 5 h incubation in 20% (v/v); 8-h EC ₅₀ 100–200 mg*L ⁻¹	(Lee et al. 2005; Pfruender et al. 2006; Bräutigam et al. 2007; Cornmell et al. 2008; Wood et al. 2011; Baum et al. 2012; Gao et al. 2014)
[C ₄ MIM][(C ₂ F ₅) ₃ PF ₃]	Membrane integrity decreased dramatically after 5 h incubation in 20% (v/v)	(Bräutigam et al. 2007)
[C ₆ MIM]X X = [Cl], [Br], [I], [Sacch]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₆ MIM][PF ₆]	Membrane integrity decreased moderately after 5 h incubation in 20% (v/v); inhibited cell growth at >5000 mg*L ⁻¹ in solid culture or at >2000 mg*L ⁻¹ in suspension culture; 8-h EC ₅₀ 50–1050 mg*L ⁻¹	(Lee et al. 2005; Bräutigam et al. 2007)
[C ₆ MIM][SbF ₆]	8-h EC ₅₀ 50–1050 mg*L ⁻¹	(Lee et al. 2005)
[C ₆ MIM][NTf ₂]	Membrane integrity decreased significantly after 5 h incubation in 20% (v/v); 8-h EC ₅₀ 100–200 mg*L ⁻¹ ; inhibited cell growth at 2% (v/v) in 24 h	(Lee et al. 2005; Bräutigam et al. 2007; Wood et al. 2011)
X[Doc]	Imposed some effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [C ₆ MIM], [C _n MPy] (n = 4, 6)	Inhibited cell growth at 0.25–40% (v/v)	(Cornmell et al. 2008; Wood et al. 2011)
X[Cl]		
X = [C ₈ MIM], [(C ₈) ₃ C ₁ N]		

Table 4 (continued)

IL	Effect	Ref.
[C ₈ MIM]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Br], [I], [NTf ₂], [Sacch]		
[C ₈ MIM][PF ₆]	8-h EC ₅₀ 100–200 mg*L ⁻¹	(Lee et al. 2005)
[C ₈ MIM][C ₁ SO ₄]	Inhibited cell growth at >200 mg*L ⁻¹ in solid culture or at >50 mg*L ⁻¹ in suspension culture; 8-h EC ₅₀ 1000–2000 mg*L ⁻¹	(Lee et al. 2005)
[C ₁₀ MIM][Cl]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[PPMIM][SbF ₆]	8-h EC ₅₀ 1000–5000 mg*L ⁻¹	(Lee et al. 2005)
[PPMIM][OTf]	8-h EC ₅₀ 1000 mg*L ⁻¹	(Lee et al. 2005)
[C ₄ MPy][i-(C ₈) ₂ PO ₄]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₁₀ Py][NTf ₂]	Inhibited cell growth at 0.25–40% (v/v)	(Cornmell et al. 2008)
[C ₄ MPyr]X	Imposed no significant effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Cl], [C ₁ SO ₄], [(C ₁) ₂ PO ₄]		
X[Br]	Imposed some effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [C ₄ MPyr], [C ₄ MPip]		
[C ₄ MPyr]X	Membrane integrity decreased significantly after 5 h incubation in 20% (v/v)	(Bräutigam et al. 2007)
X = [NTf ₂], [(C ₂ F ₅) ₃ PF ₃]		
[C ₄ MPyr]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Lac], [Doc]		
[C ₆ MPyr][Br]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₆ MPyr][NTf ₂]	Membrane integrity decreased significantly after 5 h incubation in 20% (v/v)	(Bräutigam et al. 2007)
[C ₆ MPip]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Br], [C ₁ SO ₄]		
[C ₈ MPip]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Br], [OAc]		
X[NTf ₂]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [(C ₁ OC ₃)C ₁ Pip], [(HOC ₂) ₂ C ₁₂ C ₁ N]		
[C ₄ (C ₁) ₃ N][(C ₁) ₂ PO ₄]	Imposed no significant effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X[Br]	Imposed no significant effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [C ₄ C ₂ (C ₁) ₂ N], [(HOC ₃)C ₂ (C ₁) ₂ N]		
[C ₄ C ₂ (C ₁) ₂ N]X	Imposed some effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [C _n SO ₄] (n = 2, 4)		
[C ₈ C ₂ (C ₁) ₂ N][C ₂ SO ₄]	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[C ₈ C ₄ (C ₁) ₂ N]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Br], [I], [NTf ₂], [Sacch], [i-(C ₈) ₂ PO ₄], [Lin]		
[C ₁₀ C ₄ (C ₁) ₂ N][NTf ₂]	Imposed some effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[(C ₈) ₂ (C ₁) ₂ N]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [C ₁ SO ₄], [C ₂ SO ₄], [C ₆ SO ₄], [Tos]		
[(C ₈) ₃ C ₁ N]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Br], [Sacch], [i-(C ₈) ₂ PO ₄]		
[(C ₈) ₃ C ₁ N][NTf ₂]	Biocompatible, according to some reports (20% (v/v) did not impose significant effects in 5 h); according to one report, 9% (v/v) inhibited cell growth in 4 h	(Pfruender et al. 2006; Cornmell et al. 2008; Wood et al. 2011; Gao et al. 2014)
[Chol][OAc]	Imposed no significant effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[Chol]X	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [C ₂ COO], [C ₅ COO]		
[(HOC ₂)C ₂ (C ₁) ₂ N][C ₂ SO ₄]	Imposed no effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[(HOC ₃)C ₄ (C ₁) ₂ N][Cl]	Imposed no significant effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
[(HOC ₂) ₂ C ₄ C ₂ N]X	Imposed no significant effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
X = [Br], [C ₂ SO ₄]		

Table 4 (continued)

IL	Effect	Ref.
$[(\text{HOC}_2)_3\text{C}_1\text{N}][\text{C}_1\text{SO}_4]$	Imposed no effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
$[(\text{C}_8)_3\text{C}_1\text{P}][\text{C}_1\text{SO}_4]$	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
$[\text{C}_{14}(\text{C}_6)_3\text{P}][\text{Cl}^-]$	Inhibited cell growth at 0.25–40% (v/v)	(Cornmell et al. 2008)
$[\text{C}_{14}(\text{C}_6)_3\text{P}][\text{NTf}_2^-]$	Biocompatible	(Cornmell et al. 2008; Gao et al. 2014)
$[\text{C}_{14}(\text{C}_6)_3\text{P}][\text{Doc}]$	Imposed no effect on cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
$[\text{C}_{14}(\text{C}_8)_3\text{P}][\text{Doc}]$	Inhibited cell growth at 2% (v/v) in 24 h	(Wood et al. 2011)
$[(\text{C}_1)_4\text{C}_2\text{Gua}][(\text{C}_2\text{F}_5)_3\text{PF}_3]$	Membrane integrity decreased dramatically after 5 h incubation in 20% (v/v)	(Bräutigam et al. 2007)
<i>Saccharomyces cerevisiae</i>		
$[\text{C}_2\text{MIM}][\text{Cl}^-]$	MNTC <6.7 mM	(Santos et al. 2014)
$[\text{C}_2\text{MIM}][\text{NTf}_2^-]$	MNTC <2.5 mM	(Santos et al. 2014)
$[\text{C}_2\text{MIM}][\text{OAc}^-]$	Inhibited cell growth at >0.5% (v/v) and reduced glucose consumption at 2% (v/v); inhibited cell growth at 30–35 mM under both aerobic and anaerobic conditions	(Ouellet et al. 2011; Mehmood et al. 2015)
$[\text{C}_2\text{MIM}][\text{C}_2\text{SO}_3^-]$	MNTC <4.5 mM	(Santos et al. 2014)
$[\text{C}_2\text{MIM}][\text{C}_2\text{SO}_4^-]$	MNTC <4.2 mM	(Santos et al. 2014)
$[\text{C}_2\text{MIM}][\text{C}_1\text{PO}_3^-]$	Inhibited cell growth at >0.5% (v/v)	(Mehmood et al. 2015)
$[\text{C}_4\text{MIM}][\text{PF}_6^-]$	Biocompatible (20% (v/v) did not impose significant effects in 20 h, but imposed pronounced effect in 72 h); according to another study, inhibited growth significantly at low concentrations	(Lou et al. 2006; Pfruender et al. 2006; Sendovski et al. 2010; Song et al. 2011; Zampieri et al. 2013; Kandar et al. 2015; Pérez de los Ríos et al. 2017)
$[\text{C}_4\text{MIM}][\text{NTf}_2^-]$	Biocompatible (20% (v/v) did not impose significant effects in 20 h, but imposed some effect in 72 h); MNTC <2.5 mM	(Pfruender et al. 2006; Sendovski et al. 2010; Santos et al. 2014; Pérez de los Ríos et al. 2017)
$[\text{C}_4\text{MIM}][\text{BF}_4^-]$	Moderately inhibited cell growth at 3% (v/v)	(Lou et al. 2006)
$[\text{C}_n\text{MIM}][\text{PF}_6^-]$ ($n = 6, 8$)	Inhibited cell growth at 20% (v/v) in 72 h	(Sendovski et al. 2010)
$[\text{C}_8\text{MIM}][\text{BF}_4^-]$	Inhibited cell growth at 3% (v/v)	(Pérez de los Ríos et al. 2017)
$[\text{C}_8\text{MIM}][\text{NTf}_2^-]$	Biocompatible	(Pérez de los Ríos et al. 2017)
$[\text{C}_{10}\text{MIM}][\text{PF}_6^-]$	Completely inhibited cell growth at 20% (v/v) in 72 h	(Sendovski et al. 2010)
$[\text{BzMIM}][\text{BF}_4^-]$	Completely inhibited cell growth at 20% (v/v) in 72 h	(Sendovski et al. 2010)
$[\text{C}_2\text{Py}][\text{NTf}_2^-]$	Inhibited cell growth at 3% (v/v)	(Pérez de los Ríos et al. 2017)
$[\text{C}_3\text{MPip}][\text{NTf}_2^-]$	Imposed no effect on cell growth at 20% (v/v) in 72 h	(Sendovski et al. 2010)
$[(\text{C}_8)_3\text{C}_1\text{N}][\text{NTf}_2^-]$	Biocompatible (20% (v/v) did not impose significant effects in 20–72 h)	(Pfruender et al. 2006; Sendovski et al. 2010; Pérez de los Ríos et al. 2017)
$[\text{Chol}][\text{Cl}^-]$	MNTC 224 mM	(Santos et al. 2014)
$[\text{Chol}][\text{NTf}_2^-]$	MNTC 10 mM	(Santos et al. 2014)
$[(\text{C}_4)_3\text{C}_1\text{P}][\text{C}_1\text{SO}_4]$	MNTC 1.95 mM	(Santos et al. 2014)
$[\text{C}_{14}(\text{C}_6)_3\text{P}][\text{Cl}^-]$	Biocompatible	(Pérez de los Ríos et al. 2017)
$[\text{C}_{14}(\text{C}_6)_3\text{P}]\text{X}$ $\text{X} = [\text{Br}^-], [\text{N}(\text{CN})_2^-]$	Inhibited cell growth at 3% (v/v)	(Pérez de los Ríos et al. 2017)
$[\text{C}_{14}(\text{C}_6)_3\text{P}][\text{i}-(\text{C}_8)_2\text{PO}_4^-]$	Completely inhibited cell growth at 20% (v/v) in 72 h	(Sendovski et al. 2010)

Table 4 (continued)

IL	Effect	Ref.
<i>Clostridium sporogenes</i>		
[C ₄ MIM][C ₂ SO ₄]	Inhibited cell growth at 2.0% (w/v)	(Dipeolu et al. 2009)
[C ₄ MIM][BF ₄]	Inhibited cell growth at 0.25–2.0% (w/v)	(Dipeolu et al. 2009)
[(HOC ₂)(C ₁) ₂ N][OAc]	Inhibited cell growth at 2.0% (w/v)	(Dipeolu et al. 2009)
[Chol][C ₂ PO ₄]	Stimulated cell growth at 0.25–1.0% (w/v)	(Dipeolu et al. 2009)
AMMOENG TM 100	Inhibited cell growth at 0.1–2.0% (w/v)	(Dipeolu et al. 2009)
<i>Propionibacterium freudenreichii</i> subsp. <i>freudenreichii</i>		
[C ₄ MIM]X X = [BF ₄], [C ₁ SO ₄], [SCN], [Tos], [NO ₃] [C ₆ MIM][BF ₄]	48-h IC ₅₀ 0.5–1.0% (w/v)	(Hajfarajollah et al. 2014)
[C ₆ MIM]X X = [Tos], [NO ₃] [C ₈ MIM][C ₁ SO ₄]	48-h IC ₅₀ 0.05% (w/v)	(Hajfarajollah et al. 2014)
[C ₈ MIM][SCN]	48-h IC ₅₀ 0.5% (w/v)	(Hajfarajollah et al. 2014)
[C ₈ MIM][NO ₃]	48-h IC ₅₀ 0.1–0.5% (w/v)	(Hajfarajollah et al. 2014)
[C ₈ MIM][NO ₃]	48-h IC ₅₀ 0.005% (w/v)	(Hajfarajollah et al. 2014)
<i>Rhodococcus erythropolis</i>		
[C ₂ MIM][Tos]	Imposed no significant effect at 50% (v/v)	(Hussain et al. 2007)
[C ₄ MIM]X X = [BF ₄], [PF ₆] [C ₄ MIM]X X = [C ₈ SO ₄], [MDEGSO ₄] [(C ₈) ₃ C ₁ N][NTf ₂]	Imposed no significant effect at 50% (v/v)	(Hussain et al. 2007)
[CABHEM][C ₁ SO ₄]	Inhibited cell growth at 50% (v/v)	(Hussain et al. 2007)
<i>Candida parapsilosis</i>		
[C _n MIM][NTf ₂] (n = 2, 4)	Biocompatible (IL system reduced toxicity of the substrate 4-(trimethylsilyl)-3-butyne-2-one and the product (S)-4-(trimethylsilyl)-3-butyne-2-ol and of the substrate acetyltrimethylsilane and the product (R)-1-trimethylsilylethanol)	(Lou et al. 2009a; Zhang et al. 2012)
[C _n MIM][PF ₆] (n = 4, 4(i), 5, 6)	Biocompatible (IL system reduced toxicity of the substrate 4-(trimethylsilyl)-3-butyne-2-one and the product (S)-4-(trimethylsilyl)-3-butyne-2-ol and of the substrate acetyltrimethylsilane and the product (R)-1-trimethylsilylethanol)	(Lou et al. 2009a; Zhang et al. 2012)
X[NTf ₂] X = [C ₆ MIM], [C ₄ MPyr], [C ₄ MPip], [C ₆ (C ₄) ₃ P] [C ₇ MIM][PF ₆]	Biocompatible (IL system reduced toxicity of the substrate acetyltrimethylsilane and the product (R)-1-trimethylsilylethanol)	(Zhang et al. 2012)
	Biocompatible (IL system reduced toxicity of the substrate 4-(trimethylsilyl)-3-butyne-2-one and the product (S)-4-(trimethylsilyl)-3-butyne-2-ol and of the substrate acetyltrimethylsilane)	(Lou et al. 2009a; Zhang et al. 2012)
<i>Candida albicans</i>		
[C ₄ MIM][PF ₆]	No formation of inhibition halo was observed with pure IL in 24 h	(Zampieri et al. 2013)
<i>Rhodotorula</i> sp.		
[C _n MIM][NTf ₂] (n = 2, 4)	Imposed some effect on cell viability at 20% (v/v) in 24 h; reduced substrate toxicity (4'-methoxyacetophenone)	(Wang et al. 2009)
[C ₂ MIM][NO ₃]	Imposed no significant effect on cell viability at 10% (v/v) in 24 h	(Lou et al. 2009b)
X[Cl] X = [HOC ₂ MIM], [C ₄ MIM] [HOC ₂ MIM][PF ₆]	Imposed some effect on cell viability at 10% (v/v) in 24 h	(Lou et al. 2009b)
	Imposed no significant effect on cell viability at 10% (v/v) in 24 h	(Lou et al. 2009b)

Table 4 (continued)

IL	Effect	Ref.
[HOC ₂ MIM][NO ₃]	Imposed no significant effect on cell viability at 10% (v/v) in 24 h; reduced substrate toxicity (4'-methoxyacetophenone)	(Lou et al. 2009b)
[HOC ₂ MIM][OTf]	Inhibited cell growth at 10% (v/v) in 24 h	(Lou et al. 2009b)
X[NO ₃] X = [C ₄ MIM], [C ₄ C ₁ MIM]	Imposed some effect on cell viability at 10% (v/v) in 24 h	(Lou et al. 2009b)
[C _n MIM][PF ₆] (n = 4, 5, 6, 7)	Imposed no significant effect on cell viability at 20% (v/v) in 24 h; reduced substrate toxicity (4'-methoxyacetophenone)	(Wang et al. 2009)
[C _n MIM][Br] (n = 4, 5, 6, 7)	Imposed some effect on cell viability at 10% (v/v) in 24 h	(Lou et al. 2009b)
<i>Rhodotorula glutinis</i>		
[C ₄ MIM][PF ₆]	No formation of inhibition halo was observed with pure IL n 24 h	(Zampieri et al. 2013)
<i>Trigonopsis variabilis</i>		
[C ₂ MIM][NO ₃]	Imposed some effect on cell viability at 5% (v/v) in 24 h; reduced substrate toxicity (4'-methoxyacetophenone)	(Lou et al. 2009c)
[HOC ₂ MIM][Cl]	Imposed some effect on cell viability at 5% (v/v) in 24 h; reduced substrate toxicity (4'-methoxyacetophenone)	(Lou et al. 2009c)
[HOC ₂ MIM][PF ₆]	Imposed no significant effect on cell viability at 5% (v/v) in 24 h	(Lou et al. 2009c)
[HOC ₂ MIM][NO ₃]	Imposed no significant effect on cell viability at 5% (v/v) in 24 h; reduced substrate toxicity (4'-methoxyacetophenone)	(Lou et al. 2009c)
[HOC ₂ MIM][OTf]	Inhibited cell growth at 5% (v/v) in 24 h	(Lou et al. 2009c)
[C ₄ MIM][Cl]	Imposed some effect on cell viability at 5% (v/v) in 24 h	(Lou et al. 2009c)
[C _n MIM][Br] (n = 4, 5, 6, 7)	Imposed some effect on cell viability at 5% (v/v) in 24 h	(Lou et al. 2009c)
X[NO ₃] X = [C ₄ MIM], [C ₄ C ₁ MIM]	Imposed some effect on cell viability at 5% (v/v) in 24 h	(Lou et al. 2009c)
<i>Synechocystis</i> sp.		
X[NTf ₂] X = [C ₄ MPyr], [C ₁₄ (C ₆) ₃ P]	Imposed no significant effect on cell viability at 25% (w/v) in 24 h	(Lovejoy et al. 2012)
X[Cl] X = [Chol], [(C ₄) ₄ P]	Imposed no significant effect on cell viability at 25% (w/v) in 24 h	(Lovejoy et al. 2012)
[Chol][NTf ₂]	Imposed some effect on cell viability at 25% (w/v) in 24 h	(Lovejoy et al. 2012)
[(HOC ₃) ₃ C ₁₀ P][Br]	Imposed some effect on cell viability at 25% (w/v) in 24 h	(Lovejoy et al. 2012)
<i>Penicillium raistrickii</i>		
[C ₄ MIM][PF ₆]	Inhibited glucose uptake at 20% in 10 h	(Mao et al. 2012)
[C ₄ MIM][NTf ₂]	Imposed little inhibitory effect on glucose uptake at 20% in 10 h	(Mao et al. 2012)
[C ₆ MIM]X X = [PF ₆], [NTf ₂]	Significantly inhibited glucose uptake at 20% in 10 h	(Mao et al. 2012)
<i>Penicillium diversum</i>		
[C ₂ MIM]X X = [Cl], [C ₂ SO ₄], [SCN], [OAc], [Lac]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[Cl] X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[OAc] X = [C ₂ Py], [C ₄ MPip], [Chol]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[Lac] X = [C ₂ Py], [Chol]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
[C ₄ MPyr][Lac]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)

Table 4 (continued)

IL	Effect	Ref.
[Chol][Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
[Chol][NTf ₂]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
<i>Penicillium variable</i>		
[C ₂ MIM]X	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [OAc], [Lac]		
X[Cl]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr]		
X[OAc]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
[C ₂ Py][Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
[C ₄ MPyr][Lac]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc], [Lac]		
<i>Penicillium restrictum</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [OAc], [Lac]		
[C ₂ MIM][SCN]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
[C ₄ MIM][Cl]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
[C ₂ Py]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [OAc], [Lac]		
[C ₄ Py][Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
[C ₄ MPyr]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [Lac]		
[C ₄ MPip][OAc]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc], [Lac]		
<i>Penicillium corylophilum</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [OAc], [Lac]		
[C ₄ MIM][Cl]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr]		
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ Py], [C ₄ MPyr]		
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc]		
[Chol][Lac]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
<i>Penicillium janczewskii</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [Lac]		
[C ₂ MIM][OAc]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr], [Chol]		
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip], [Chol]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr], [Chol]		
[Chol][NTf ₂]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)

Table 4 (continued)

IL	Effect	Ref.
<i>Penicillium brevicompactum</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [Lac]		
[C ₂ MIM][OAc]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr]		
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr]		
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc], [Lac]		
<i>Penicillium adametzii</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [OAc], [Lac]		
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr]		
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr]		
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc], [Lac]		
<i>Penicillium glandicola</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [OAc], [Lac]		
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ MPyr]		
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr]		
[C ₄ Py][Cl]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc], [Lac]		
<i>Penicillium olsonii</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [OAc], [Lac]		
[C ₂ MIM][SCN]	Inhibited cell growth at 50 mM	(Petkovic et al. 2009)
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr]		
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr]		
[Chol]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [NTf ₂], [OAc], [Lac]		
<i>Penicillium glabrum</i>		
[C ₂ MIM]X	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [Cl], [C ₂ SO ₄], [SCN], [OAc], [Lac]		
X[Cl]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₄ MIM], [C ₄ Py], [C ₄ MPyr]		
X[OAc]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPip]		
X[Lac]	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
X = [C ₂ Py], [C ₄ MPyr]		

Table 4 (continued)

IL	Effect	Ref.
[Chol]X X = [Cl], [NTf ₂], [OAc], [Lac] <i>Aspergillus ochraceus</i>	Imposed no inhibitory effect on cell growth at 50 mM	(Petkovic et al. 2009)
[C ₃ MIM][PF ₆]	Imposed little inhibitory effect on glucose consumption at 20% in 12 h; alleviated toxicity of the substrate 16 α , 17-epoxyprogesterone	(Mao et al. 2013)
X[NTf ₂] X = [C ₃ MIM], [C ₄ MIM], [C ₆ MIM], [AMIM] [C _n MIM][PF ₆] (n = 4, 6)	Significantly inhibited glucose consumption at 20% in 10 h	(Mao et al. 2013)
<i>Aspergillus brasiliensis</i>		
[C ₂ MIM][Cl]	MNTC 426 mM	(Santos et al. 2014)
[C ₂ MIM][NTf ₂]	MNTC 160 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC >581 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC >540 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC 150 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 448 mM	(Santos et al. 2014)
[Chol][NTf ₂]	MNTC 74 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 196 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC 8 mM	(Santos et al. 2014)
<i>Lactobacillus kefir</i>		
[C ₄ MIM][PF ₆]	20% (v/v) imposed no damage to the cell membrane in 5 h	(Pfruender et al. 2004)
[C ₄ MIM][NTf ₂]	20% (v/v) imposed no damage to the cell membrane in 5 h and alleviated the harmful effect of the substrate 4-chloroacetophenone	(Pfruender et al. 2004)
[(C ₈) ₃ C ₁ N][NTf ₂]	20% (v/v) imposed no damage to the cell membrane in 5 h	(Pfruender et al. 2004)
<i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i>		
[C ₂ MIM][Cl]	MNTC 53 mM	(Santos et al. 2014)
X[NTf ₂] X = [C ₂ MIM], [Chol] [C ₂ MIM][C ₂ SO ₃]	MNTC <5 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC 72 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC 67.5 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC <4.7 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 112 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 24.5 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC 8 mM	(Santos et al. 2014)
<i>Bacillus subtilis</i>		
[C ₂ MIM][Cl]	MNTC 426 mM	(Santos et al. 2014)
[C ₂ MIM][NTf ₂]	MNTC <5 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC 581 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC 270 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC <4.7 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 28 mM	(Santos et al. 2014)
[Chol][NTf ₂]	MNTC 10 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 24.5 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC <3.75 mM	(Santos et al. 2014)

Table 4 (continued)

IL	Effect	Ref.
<i>Bacillus coagulans</i>		
[C ₂ MIM]X X = [Cl], [OAc]	Significantly inhibited cell growth at 236 mM	(Simmons et al. 2014)
<i>Streptomyces drozdowiczii</i>		
[C ₂ MIM][Cl]	MNTC 27 mM	(Santos et al. 2014)
X[NTf ₂] X = [C ₂ MIM], [Chol]	MNTC <5 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC 36 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC 34 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC <4.7 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 112 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 24 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC <3.75 mM	(Santos et al. 2014)
<i>Pseudomonas aeruginosa</i>		
[C ₂ MIM][Cl]	MNTC 852.5 mM	(Santos et al. 2014)
[C ₂ MIM][NTf ₂]	MNTC 10 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC 145 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC 135 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC 300 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 448 mM	(Santos et al. 2014)
[Chol][NTf ₂]	MNTC 74 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 98 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC 15 mM	(Santos et al. 2014)
<i>Pseudomonas fluorescens</i>		
[C _n MIM][BF ₄] (n = 2, 4, 6, 8)	Inhibited cell growth at 5% (v/v) in 48 h	(Yang et al. 2014)
[C ₄ MIM][PF ₆]	Inhibited cell growth at 5% (v/v) in 48 h	(Yang et al. 2014)
<i>Yarrowia lipolytica</i>		
[C ₂ MIM][Cl]	MNTC 235 mM	(Santos et al. 2014)
[C ₂ MIM][NTf ₂]	MNTC 10 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC 145 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC 270 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC 5 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 448 mM	(Santos et al. 2014)
[Chol][NTf ₂]	MNTC 37 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 196 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC 7.8 mM	(Santos et al. 2014)
<i>Kluyveromyces marxianus</i>		
[C ₂ MIM][Cl]	MNTC <6.7 mM	(Santos et al. 2014)
X[NTf ₂] X = [C _n MIM] (n = 2, 4), [Chol]	MNTC <2.5 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC <4.5 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC <4.2 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC 224 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 98 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC <3.75 mM	(Santos et al. 2014)

Table 4 (continued)

IL	Effect	Ref.
<i>Rhizopus oryzae</i>		
[C ₂ MIM][Cl]	MNTC >852.5 mM	(Santos et al. 2014)
[C ₂ MIM][NTf ₂]	MNTC 20 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₃]	MNTC 290 mM	(Santos et al. 2014)
[C ₂ MIM][C ₂ SO ₄]	MNTC >540 mM	(Santos et al. 2014)
[C ₄ MIM][NTf ₂]	MNTC >300 mM	(Santos et al. 2014)
[Chol][Cl]	MNTC >896 mM	(Santos et al. 2014)
[Chol][NTf ₂]	MNTC 74 mM	(Santos et al. 2014)
[Chol][OAc]	MNTC 392 mM	(Santos et al. 2014)
[(C ₄) ₃ C ₁ P][C ₁ SO ₄]	MNTC 8 mM	(Santos et al. 2014)
<i>Acetobacter</i> sp.		
X[Cl]	Imposed some effect on metabolic activity	(Xiao et al. 2012)
X = [HOC ₂ MIM], [C _n MIM] (n = 2, 4)	at 5% (v/v) in 24 h; alleviated toxicity of the substrate 4-(trimethylsilyl)-3-butyn-2-one; increased membrane permeability	
[HOC ₂ MIM][NO ₃]	Imposed no significant effect on metabolic activity at 5% (v/v) in 24 h; alleviated toxicity of the substrate 4-(trimethylsilyl)-3-butyn-2-one; increased membrane permeability	(Xiao et al. 2012)
[HOC ₂ MIM][OTf]	Inhibited cell growth at 5% (v/v) in 24 h; increased membrane permeability	(Xiao et al. 2012)
[C _n MIM]X (n = 2, 4) X = [Br], [NO ₃]	Imposed some effect on metabolic activity at 5% (v/v) in 24 h; alleviated toxicity of the substrate 4-(trimethylsilyl)-3-butyn-2-one; increased membrane permeability	(Xiao et al. 2012)
[C ₄ MIM][PF ₆]	Imposed no significant effect on metabolic activity in 12 h; alleviated toxicity of the substrate ethyl acetoacetate	(Wang et al. 2013)
[C _n MIM][NTf ₂] (n = 4, 6)	Imposed some effect on metabolic activity in 12 h; alleviated toxicity of the substrate ethyl acetoacetate	(Wang et al. 2013)
[C _n MIM][PF ₆] (n = 4(i), 5, 6, 7)	Imposed some effect on metabolic activity in 12 h; alleviated toxicity of the substrate ethyl acetoacetate	(Wang et al. 2013)
[C ₄ C ₁ MIM][NO ₃]	Imposed some effect on metabolic activity at 5% (v/v) in 24 h; increased membrane permeability	(Xiao et al. 2012)
[C _n MIM][Br] (n = 5, 6, 7)	Imposed some effect on metabolic activity at 5% (v/v) in 24 h; increased membrane permeability	(Xiao et al. 2012)
<i>Acinetobacter</i> sp.		
[C ₂ MIM]X X = [Cl], [BF ₄], [NO ₃]	Imposed some effect on metabolic activity in 22 h; increased membrane permeability	(Xu et al. 2016b)
[HOC ₂ MIM]X X = [Cl], [OTf]	Imposed some effect on metabolic activity in 22 h; increased membrane permeability	(Xu et al. 2016b)
X[NO ₃] X = [HOC ₂ MIM], [HOOC ₂ MIM]	Imposed no significant effect on metabolic activity in 22 h; increased membrane permeability	(Xu et al. 2016b)
[C ₂ C ₁ MIM][NO ₃]	Imposed some effect on metabolic activity in 22 h; increased membrane permeability	(Xu et al. 2016b)
[C _n MIM][Cl] (n = 4, 7)	Inhibited metabolic activity in 22 h; increased membrane permeability	(Xu et al. 2016b)
<i>Geotrichum candidum</i>		
[C ₄ MIM][PF ₆]	No formation of inhibition halo was observed with pure IL in 24 h	(Zampieri et al. 2013)
<i>Micrococcus luteus</i>		
[C ₄ MIM][PF ₆]	No formation of inhibition halo was observed with pure IL in 24 h	(Zampieri et al. 2013)

Table 4 (continued)

IL	Effect	Ref.
<i>Aureobasidium pullulans</i>		
X[BF ₄]	Inhibited metabolic activity in 4 h	(Zhang et al. 2008)
X = [C ₄ MIM], [C ₈ Py]	Imposed no effect on metabolic activity in 4 h	(Zhang et al. 2008)
X[PF ₆]		
X = [C ₄ MIM], [C ₄ C ₁ MIM]	Inhibited metabolic activity in 4 h	(Zhang et al. 2008)
[C ₄ MIM][OTf]		

works are scarce, but some interesting results have been obtained thus far.

In *Aspergillus nidulans*, exposure to [Chol][Cl] or [C₂MIM][Cl] led to significant changes in the expression of metabolism-related genes. The ILs activated the mechanisms of detoxification, but these responses depended on the IL nature. Thus, primary metabolism was activated by [Chol][Cl], but was inhibited by [C₂MIM][Cl], presumably because choline could be utilized by cells. Both ILs enhanced the production of acetyl-CoA, which was the precursor of various secondary metabolites. In *Neurospora crassa*, [Chol][Cl] and [C₂MIM][Cl] also induced the expression of various stress-responsive proteins and proteins participating in the biosynthesis of unusual metabolites (Martins et al. 2013; Alves et al. 2016). The authors suggested that ILs could be applied for diversifying or regulating the repertoire of metabolites produced by microbes.

The lignocellulolytic bacterium *Enterobacter lignolyticus* (strain SCF1) was tolerant up to 0.5 M [C₂MIM][Cl], possibly

due to an increase of the content of cyclopropane fatty acids in the membrane, down-regulation of membrane porins and up-regulation of multidrug efflux pumps and osmoprotectant transporters. According to the authors, the response differed from general stress and was unique for the IL studied. They suggested an IL tolerance model, which included: (1) reducing cell permeability via fast cell membrane remodeling and porin down-regulation; (2) decreasing the intracellular IL concentration via multidrug efflux pump up-regulation; and (3) alleviating harmful osmotic pressure effects of IL via enhanced scavenging of biocompatible solutes (Khudyakov et al. 2012). When two IL resistance genes encoding an inner membrane transporter and its IL-inducible repressor from *Enterobacter lignolyticus* were transferred to biofuel-producing *Escherichia coli*, it acquired the IL resistance (Ruegg et al. 2014). Overall, these findings suggest a promising opportunity for designing IL-tolerant biocatalytic microorganisms.

Fig. 5 Biocompatibility of selected ILs for *E. coli* (based on data from Table 4)

Fig. 6 Biocompatibility of most studied ILs with most demanded microorganisms (based on data from Table 4 and Egorova and Ananikov (2014) and Radosevic et al. (2015))

Conclusions

In modern chemistry, whole-cell biocatalysis can become a ‘magic’ alternative to traditional chemical synthesis (Table 1). However, low aqueous solubility of target compounds and the necessity to use organic solvents hinder its successful widespread application. ILs with their unique solvent properties have been proposed as a more efficient and safe option for biocatalysis. However, in spite of the first encouraging results, we have not gained enough information for the rational design of IL-containing whole-cell biocatalytic systems. There is evidence of the possibility of selecting IL-resistant microorganisms, which can enjoy the benefits of ILs without suffering from their toxic effects. Nevertheless, such endeavors should be undertaken with extreme caution, because acquired resistance of a microbial species to a chemical can pose potential dangers to human health. Thus, prior to cultivating IL-tolerant strains of bacteria and fungi, attention should be paid to the mechanisms of IL toxicity which have not yet been fully elucidated.

Whereas the advantages of ILs can undoubtedly be used in whole-cell biocatalytic systems, the biological activity of these compounds should also be assessed with the utmost care. Indeed, more detailed studies on the biological activity and toxic effects of ILs towards microorganisms should be carried out. The progress in this area will to a large extent determine the practical impact of water/IL systems for whole-cell biocatalysis.

Acknowledgements This work was supported by the Russian Foundation for Basic Research (grant 16-29-10804).

Compliance with ethical standards

Conflict of interest Ksenia S. Egorova declares that she has no conflict of interest. Valentine P. Ananikov declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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