Novel biocompatible cholinium-based ionic liquids—toxicity and biodegradability†

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The synthesis, characterisation and toxicological assessment of a new group of environmentally friendly ionic liquids are presented. Focussing on the toxic effect of the anion, the ionic liquids were designed by combining the benign cholinium cation, $[NMe_3(CH_2CH_2OH)]^+$, with a range of linear alkanoate anions ($[C_nH_{2n+1}CO_2]^-$, n = 1-9), as well as two structural isomers (n = 3 or 4). The toxicity of these ionic liquids was evaluated using filamentous fungi as model eukaryotic organisms. Surprisingly, most of the tested species showed active growth in media containing extremely high ionic liquid concentrations, up to molar ranges in some cases. The biodegradability of these ionic liquids was assessed, and new biotechnological applications for them are proposed, *e.g.* as solvents for biopolymers. This study leads to the better understanding of the anion influence on the ionic liquid toxicity, but its core is the recognition that conscious design of ionic liquids can be used to deliver truly biocompatible salts without adversely affecting one of the most striking of their properties—their outstanding solvent ability.

Introduction

The chemistry of ionic liquids has developed dramatically during the last decade. Their generic, yet not universal, properties, such as negligible vapour pressure, nonflammability, chemical and thermal stability, and outstanding solvation ability, enabled rapid advance in numerous applications.[1] The potential of ionic liquids is further emphasised because their physical and chemical properties may be finely tuned by varying the cation and the anion.[2] Their dual nature is, relative to conventional molecular organic solvents, a clear advantage.

In order to improve old, or to create novel, ionic liquid based processes, aiming at cost-efficiency and sustainability, an interdisciplinary approach is essential. So-called risk-conscious design and "thinking in terms of structure–activity relationships" should be applied before implementing any new materials on a large scale. Thus, a better, structure-based, understanding of the environmental fate of ionic liquids is critical.[3] Unfortunately, this is a complex equation which crosses numerous unknown abiotic and biotic factors. The enormous diversity of ionic liquids is also a major concern from the ecotoxicological point of view, since testing such a vast number of ionic liquids and organisms is inconceivable.

In the expanding studies on the toxicity and biodegradability of ionic liquids, they were not *a priori* accepted as environmentally benign.[4] It is widely accepted that the head group of the cation has a deciding *role* in toxicity,[5,6] that longer side chains have a more severe impact on living cells,[7] and that incorporation of an ester group significantly increases their biodegradability.[8] The anion plays an essential *role* in the ionic liquid physicochemical properties,[2] and it is known to contribute to the overall toxicity,[9] but its effect is usually neglected. The predictive value of certain trends, both for the cation and the anion, is unquestionable; however, generalisations should be taken cautiously, given the current limited understanding of the modes of toxicity of ionic liquids, their biodegradation pathways, and their behaviour concerning biosorption, bioaccumulation, *etc*. This complexity can be simplified if ionic liquids are synthesised from carefully selected naturally-derived materials, which are safer, carrying reduced toxicity and enhanced biodegradability.[10]

Although a wide range of cholinium ionic liquids have been known for some time,[11-17] the alkanoates have been largely ignored. We present here a new group of cholinium ionic liquids. Cholinium, a quaternary ammonium cation, $[NMe_3(CH_2CH_2OH)]^+$, which is an essential micronutrient,[18] was chosen as the benign cation and combined with alkanoates of systematically elongated chains. In order to better understand the anion influence on ionic liquid toxicity, the toxicities of the obtained ionic liquids were evaluated using filamentous fungi—previously demonstrated to be an excellent test system.[19]

Experimental section

Chemicals

The materials used in the ionic liquid syntheses, including source and grade, were as follows: cholinium hydrogencarbonate

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(Aldrich, 75 wt.% in H_2O), ethanoic acid (Aldrich, 99-100% puriss), propanoic acid (Aldrich, 99.5%), butanoic acid (Lancaster, 99+%), 2-methylpropanoic acid (Aldrich, 99%), pentanoic acid (Aldrich, 99%), 2,2-dimethylpropanoic acid (Aldrich, 99%), hexanoic acid (Alfa Aesar, 99%), octanoic acid (Sigma, 99%) and decanoic acid (Aldrich, 96%). Sodium salts of the corresponding carboxylic acids were prepared *via* addition of the acid to sodium methoxide (Sigma Aldrich 25 wt.% in methanol), vacuum-filtration, and washing the precipitated salt with cold methanol, followed by drying under high vacuum (80 °C, >14 h).

Other chemicals used in the study were: cholinium chloride (choline; Sigma, ≥98%), sodium dodecyl sulfate (SDS, Amersham Biosciences, >99%), benzalkonium chloride (BAC, predominantly $[C_{12}H_{25}N(CH_3)_2\ (CH_2C_6H_5)]Cl$, but also contains C_{14} and C_{16} homologues, Aldrich), ethanol (Panreac, absolute PA) and propanone (Fisher Scientific, analytical grade).

Ionic liquids

The ionic liquids were prepared by dropwise addition of the corresponding acid (1 : 1) to aqueous cholinium hydrogencarbonate, stirring at ambient temperature and pressure. Water was then removed under reduced pressure, first using a rotary evaporator (*e.g.* 70 °C, 30 min), and then stir-heating *in vacuo* (65-70 °C, 24 h, *ca.* 0.01 mbar).

The ionic liquids were characterised by ^1H and ^{13}C NMR (Bruker Avance III spectrometer, 400 MHz) spectroscopy at 25 °C. Their purity was confirmed by electrospray ionisation mass spectrometry (ESI-MS) (Waters LCT Premier fitted with electrospray). The spectroscopic analyses are provided as Supporting Information.† The water contents, determined by Karl-Fischer titration, were below 0.1 wt%. The obtained salts fulfilled the requirements of the present study.

Thermal properties analysis

The thermal stabilities and decomposition temperatures of the ionic liquids were measured using a thermal gravimetric analyser (TGA Q5000 V3.10 Build 258). All samples were recorded in aluminium pans under a dinitrogen atmosphere. Samples were heated up to 300 °C at a heating rate of 5 °C min^{-1} until complete thermal degradation was achieved. Universal Analysis, version 4.4A software, was used to determine the onset (T_{onset}) and the decomposition (T_{dec}) temperatures, as the temperatures at which the baseline slope changes during the heating, and at which 50% of weight loss was observed, respectively. Additionally, their melting (T_m) and glass transition temperatures (T_g) were determined by differential scanning calorimetry (DSC) (DSC Q2000 V24.2 Build 107). Heating and cooling cycles were conducted at the rate of 5 °C min^{-1}. Results are presented in Table 1.

Fungal isolates

The following fungal isolates were used, all belonging to the Instituto de Biologia Experimental e Tecnológica (IBET) culture collection: *Penicillium brevicompactum* Dierckx (IBETPeA), *P. glandicola* (Oudem) Seifert and Samson (IBETPeB), *P. corylophilum* Dierckx (IBETF6), and *P. diversum* Raper and Fennell (IBETPeE). They had previously been isolated from cork samples purchased from several Portuguese cork industries.[20,21]

Table 1 The thermal properties of the ionic liquids: onset (T_{onset}), decomposition (T_{dec}), melting (T_m) and glass transition (T_g) temperatures.

Ionic liquid	T_{onset}[a]/°C	T_{dec}[a]/°C	T_m/°C	T_g/°C
cholinium ethanoate	169	210	80	
cholinium propanoate	113	172	n.d.	−74
cholinium butanoate	97	166	45	
cholinium 2-methylpropanoate	110	172	35	
cholinium pentanoate	165	203	31	
cholinium 2,2-dimethylpropanoate	112	177	57	
cholinium hexanoate	106	169	52	
cholinium octanoate	107	166	26	
cholinium decanoate	116	168	50	

[a] T_{onset} and T_{dec} defined as the temperatures at which the baseline slope changes during the heating, and at 50% weight loss, respectively. Please note these are from scanning TGA, and do not represent isothermal stabilities.

Toxicity tests

The toxicity of ionic liquids to the fungal isolates was evaluated by determining their minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC), distinguishing between growth inhibition and death, respectively.

The minimal culture medium containing glucose (1.0 g l^{-1}), K_2HPO_4 (1.0 g l^{-1}), $NaNO_3$ (3.0 g l^{-1}), $ZnSO_4{\cdot}7H_2O$, (0.01 g l^{-1}), $CuSO_4{\cdot}5H_2O$ (0.005 g l^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g l^{-1}), $FeSO_4{\cdot}7H_2O$ (0.01 g l^{-1}) and KCl (0.5 g l^{-1}), was dissolved in distilled water, sterilised in an autoclave (20 min; 121 °C), and finally supplemented with the testing compounds. Final concentrations of ionic liquids and control compounds in growth media were in the range from 2.5 mM up to 2 M (distributed stepwise from 0.5 mM to 0.1 M).

Each liquid medium (1 cm^3) was inoculated with a suspension of fungal spores, prepared as previously described,[22] in order to obtain the final concentration of 10^5 spores per cm^3, and divided into four wells (0.25 cm^3 each) of a 96-well microtitre plate. Cultures were incubated in the dark, at 25 °C, for 14 days. Fungal growth (or lack thereof) was followed by measuring the absorbance (600 nm) of the medium and, when necessary, at the end of incubation gauging by eye the formation of mycelium (turbidity) and/or spores. The lowest concentration that inhibited the formation of mycelium was taken as the MIC.

Additionally, all the samples where no active growth was detected were used as inocula and spread, with a ~2 µl loop, onto malt extract agar medium (Oxoid, UK). The plates were incubated in the dark, at 25 °C, for 7 days. The lowest concentration of the test compound which results in unviable spores was taken as MFC. MIC and MFC values should not be interpreted as absolute ones, but rather as an indication of the inhibitory and the fungicidal upper concentrations limits.

The control samples were incubated under the same conditions: negative control (ionic liquid free medium), cationic surfactant control (BAC), anionic surfactant control (SDS), anion effect control (sodium salts), common organic solvents (ethanol and propanone) and blank samples (non-inoculated). Cholinium chloride (choline) was also tested to measure the cation effect.

Biodegradability assessment of the ionic liquids

Penicillium corylophilum was chosen for studies on ionic liquid biodegradability. Concentrations of tested compounds used in this assay were below the previously determined MICs. Fungal cultures of 20 cm^3 were inoculated as described above, and incubated in the dark, at 25 °C, under agitation (90 rpm), for 28 days. The aliquot of 1 cm^3 was taken from the cultures, filtered (0.2 μm), freeze-dried to remove water, dissolved in 0.7 cm^3 of deuteriated water, and analysed by ^{1}H NMR spectroscopy. Ionic liquid biodegradability in the static fungal cultures (toxicity tests) was analysed by ^{1}H NMR spectroscopy, and in some cases the anion biodegradability confirmed by liquid chromatography.[23]

Results and discussion

In the present work, a new group of ionic liquids, composed by the benign cholinium cation[18] and alkanoate anions, was investigated (Fig. 1). The physical and thermal properties of cholinium ethanoate and propanoate have been reported previously,[24] but these salts are seldom studied. The linear chains in the alkanoate anions were systematically elongated, from ethanoate to decanoate, and two branched isomers, 2-methylpropanoate and 2,2-dimethylpropanoate, were also analysed. This constitutes, *pace* cholinium ethanoate,[19] the very first systematic study on their toxicity.

Previously, it was demonstrated that several phylogenetically closely related fungal isolates, all belonging to the *Penicillium* genus, were distinctively grouped according to their growth susceptibility in media containing ionic liquids.[19] Importantly, a high degree of correlation between the species genetic proximity and their response to the ionic liquid environment was also inferred.[19] One representative fungal isolate from each susceptibility group was therefore selected for this work. They were, set by order of tolerance: *P. brevicompactum*, *P. glandicola*, *P. corylophilum* and *P. diversum*. These fungal isolates were able to grow in media containing relatively high concentrations of cholinium alkanoates (Table 2), and yet showed very distinct behaviours, *e.g.* MIC values varied from 2.5 mM to 1.5 M (Fig. 2). The fungi tolerance ranking defined here is consistent with that previously reported,[19] further highlighting the robustness and the predictive value of the testing system, indifferent to the chemical nature of the salt.

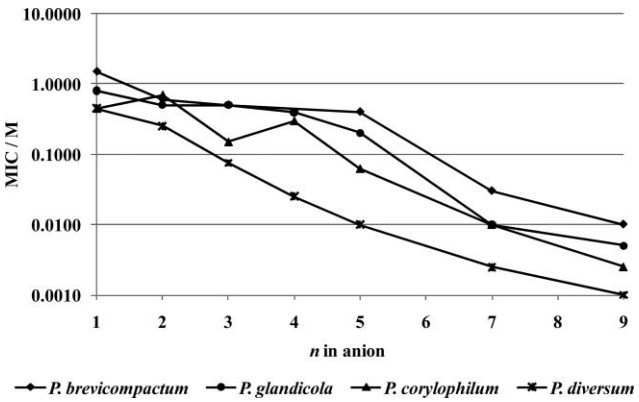

Fig. 2 Minimal inhibitory concentrations (MIC) of the cholinium alkanoates ($[NMe_3(CH_2CH_2OH)][C_nH_{2n+1}CO_2]$, n = 1-9 linear chain length): comparison of $\log_{10}$(MIC) values of cholinium alkanoates in four fungal isolates, *Penicillium brevicompactum*, *P. glandicola*, *P. corylophilum*, *P. diversum*. MIC values plotted in logarithmic scale.

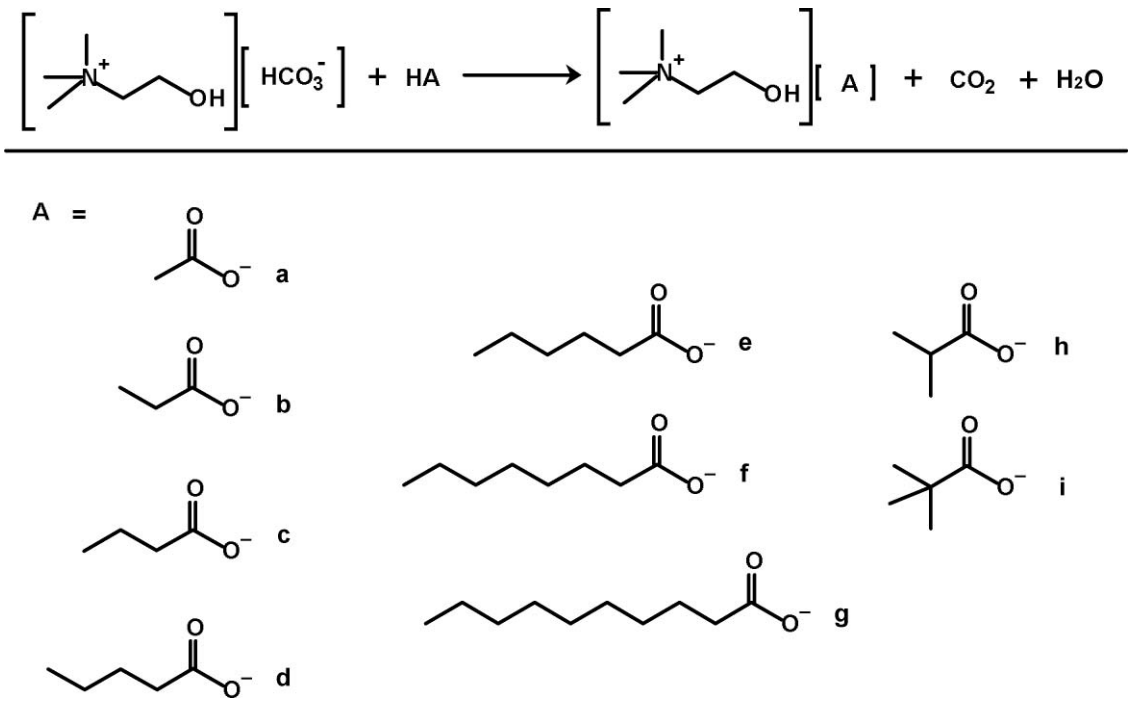

Fig. 1 Chemical route for obtaining the cholinium alkanoate ionic liquids, $[NMe_3(CH_2CH_2OH)][C_nH_{2n+1}CO_2]$ (n = 1-9) and chemical structures of the anions used: (a) ethanoate, (b) propanoate, (c) butanoate, (d) pentanoate, (e) hexanoate, (f) octanoate, (g) decanoate, (h) 2-methylpropanoate, (i) 2,2-dimethylpropanoate.

Table 2 Minimal inhibitory and fungicidal concentrations (MIC and MFC, respectively) of cholinium alkanoates (M) in four fungal isolates (*Penicillium brevicompactum*, *P. glandicola*, *P. corylophilum*, *P. diversum*). Results obtained for control compounds (sodium salts of corresponding carboxylic acids, cholinium chloride, SDS, BAC, ethanol and propanone) are also presented.

Fungal species	*P. brevicompactum*		*P. glandicola*		*P. corylophilum*		*P. diversum*	
Test compounds	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
cholinium ethanoate	>1.5	>2	0.8	0.9	0.45	0.5	0.45	0.5
cholinium propanoate	0.6	0.6	0.5	0.7	0.7	0.7	0.25	0.375
cholinium butanoate	0.5	1	0.5	0.75	0.15	0.15	0.075	0.075
cholinium 2-methylpropanoate	0.5	1.5	0.6	0.75	0.25	0.25	0.1	0.2
cholinium pentanoate	n.d.	n.d.	0.4	0.6	0.3	0.3	0.025	0.05
cholinium 2,2-dimethylpropanoate	0.75	1.5	0.4	0.7	0.75	1.5	0.075	0.15
cholinium hexanoate	0.4	0.5	0.2	0.2	0.0625	0.07	0.01	0.025
cholinium octanoate	0.03	0.03	0.01	0.02	0.01	0.03	0.0025	0.005
cholinium decanoate	0.01	0.01	0.005	0.0075	0.0025	0.005	0.001	0.0025
sodium ethanoate	1.25	1.5	0.75	0.75	0.4	0.5	0.2	0.5
sodium propanoate	1	1.25	0.6	0.75	0.3	0.4	0.075	0.15
sodium butanoate	0.25	>1	0.4	0.6	0.4	0.5	0.05	0.075
sodium 2-methylpropanoate	0.75	>1	0.4	0.6	0.4	0.4	0.075	0.15
sodium pentanoate	n.d.	n.d.	n.d.	n.d.	0.1	0.1	0.03	0.05
sodium 2,2-dimethylpropanoate	0.5	1	0.25	0.4	0.25	0.25	0.05	0.075
sodium hexanoate	0.1	0.15	0.05	0.05	0.05	0.05	0.01	0.025
sodium octanoate	0.02	0.025	0.0075	0.02	0.02	0.03	0.0025	0.005
sodium decanoate	0.015	0.02	0.001	0.005	0.01	0.015	0.005	0.005
cholinium chloride	>2	>2	1.25	>2	1.25	>2	0.8	>2
SDS	<0.001	<0.001	<0.0001	<0.0001	<0.0001	0.125	<0.0001	<0.0001
BAC	<0.0025	<0.0025	<0.00025	<0.00025	0.0025	0.005	<0.001	<0.001
Ethanol	1	1	n.d.	n.d.	2	2	0.5	1
propanone	>4	>4	n.d.	n.d.	>4	>4	>4	>4

n.d. not determined. MIC and MFC concentrations (M) were reproducible in the four replicates. Data consistency was further confirmed re-testing, randomly, ~20% of the fungal cultures. The concentrations ranged from 2.5 mM to 2 M and were distributed stepwise increasing from 0.5 mM to 0.1 M.

The selection of tested compounds enabled us to investigate the specific influence of the anion on the overall toxicity of the ionic liquid. It becomes apparent that the anion toxicity is defined by the length of the linear chain and can be ranked as follows:

ethanoate < propanoate < butanoate < pentanoate < hexanoate < octanoate < decanoate

A similar effect was reported for the cation, where high correlation between toxicity and the side chain in the cation has been observed.[25] The dodecanoate anion was apparently slightly less toxic than decanoate (data not shown), but was not considered in the present study because it has induced the formation of a precipitate.

MIC and MFC values followed the same trend (Table 2). Fungal ionic liquid susceptibility *versus* the length of the linear chain in the anion is depicted in Fig. 3 for *P. brevicompactum* and *P. diversum* (the most and least tolerant fungal isolates, respectively). In more than 40% of the cases, the reported MIC and MFC values were very similar, suggesting that growth inhibition and the spores' viability are somehow correlated events.

2-Methylpropanoate and 2,2-dimethylpropanoate are branched isomers of butanoate and pentanoate, respectively. They exhibited, relative to the linear isomers, lower inhibitory and fungicidal effects, especially evident in the latter isomer (Table 2). Although MIC and MFC values in the non-linear isomers were in good agreement for the same species, no trend could be defined. Likewise, no correlation can be observed between their toxicities and that of propanoate (equal linear chain length).

The toxicity of cholinium alkanoates, as a function of the anion, may be partially understood by QSAR analyses using different molecular descriptors, such as the 1-octanol/water partition coefficient, $\log_{10}(K_{ow})$.[26] In studies with ionic liquids which are not hydrophobic, $\log_{10}(K_{ow})$ can be measured from thermodynamic data[27] or indirectly determined by chromatographic methods if the anion is a constant (the anion governs miscibility with the HPLC mobile phase).[28] Most studies have focussed on the cation, leaving the effect of the anion neglected. Nevertheless, a correlation between toxicity and lipophilicity of the cation or the anion has been observed elsewhere.[29] In the present study, the $\log_{10}(K_{ow})$ values of the isolated anions were predicted using algorithms[30] available on the ChemSpider website, www.chemspider.com. These values established a relative scale of lipophilicity for the anions studied, increasing linearly, from -0.285 ± 0.184 to 3.965 ± 0.185, with the increase of the number of carbon atoms in the linear anions. The branched isomers constitute an outlier group, reporting, relative to their linear isomers, slightly lower $\log_{10}(K_{ow})$ values. The lipophilic trend shown by this set of data describes perfectly the observation that toxicity increases with the elongation of the linear chain in the anion. In addition, branching, which generally reduces lipophilicity,[31] resulted in lower toxicity. This interpretation thus constitutes a reasonable view of the ionic liquids' toxicity, but experimental standardised methods, and probably other molecular descriptors, are necessary for better understanding of the specificity of their toxicity mechanisms.

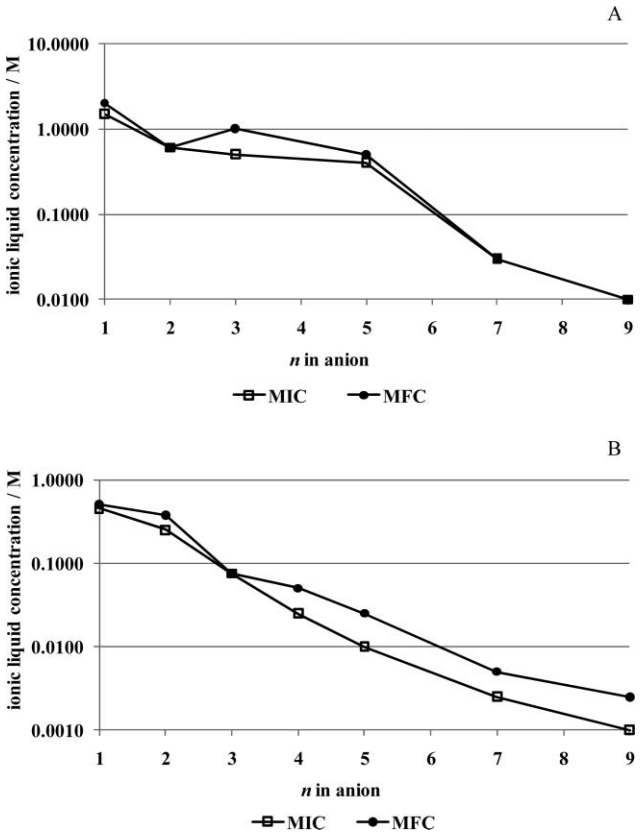

Fig. 3 Minimal inhibitory and fungicidal concentrations (MIC and MFC, respectively) of the cholinium alkanoates ($[NMe_3(CH_2CH_2OH)][C_nH_{2n+1}CO_2]$, n = 1-9 linear chain length): comparison of $\log_{10}$(MIC) and $\log_{10}$(MFC) values of cholinium alkanoates for the most (A) and the least (B) tolerant fungal isolates, *P. brevicompactum* and *P. diversum*, respectively. MIC and MFC values plotted in logarithmic scale.

The cationic and anionic surfactant controls, SDS and BAC, and the common organic solvents, ethanol and propanone, were either extremely more toxic or less toxic, respectively (Table 2). The sodium salts used as controls showed the same trend, but were slightly more toxic than the ionic liquids, reiterating the biocompatible nature of cholinium in this test system (Table 2). Fungal susceptibility to the ionic liquids and the salt controls *versus* the length of the linear chain in the anion is depicted in Fig. 4 for *P. glandicola*. In comparison, cholinium chloride, which measured individually the cation contribution, showed the lowest ionic liquid toxicity. This reinforces the statement that ionic liquid toxicity is a very complex equation that cannot be explained exclusively by the properties of the individual counter ions.

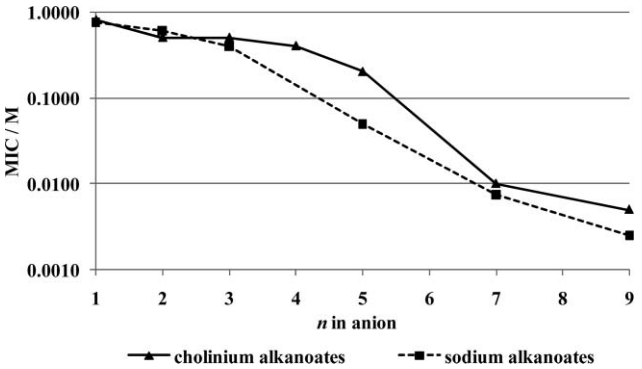

Fig. 4 Comparison of the toxic effects, $\log_{10}$(MIC) (minimal inhibitory concentration, MIC) of the tested cholinium alkanoates ($[NMe_3(CH_2CH_2OH)][C_nH_{2n+1}CO_2]$, n = 1-9 linear chain length) and the sodium alkanoates in *Penicillium glandicola*. MIC values plotted in logarithmic scale.

The biodegradation of ionic liquids in *P. corylophilum* cultures, after four weeks of incubation, was monitored by NMR spectroscopy (Table 3). The peaks attributed to the cholinium cation were conserved in the spectral analyses, thus suggesting that it was only partially degraded (Fig. 5). Under aerobic conditions, choline has been previously reported to undergo almost complete biodegradation (93%).[32] The anions demonstrated distinct biodegradability potentials, which were confirmed by liquid chromatography (data not shown). Very efficient degradation was observed in the longer linear chain anions, butanoate, pentanoate, hexanoate and octanoate, shown by the disappearance of the anion peaks in the spectra. In contrast, the short linear chain anions (ethanoate and propanoate) were not fully degraded, most probably due to their high concentration in media (0.375 and 0.5 M). This was supported by the chromatographic analyses of *P. corylophilum* static cultures (toxicity tests). The anion biodegradability was observed to be highly concentration dependent, *e.g.* ethanoate, 0.125 and 0.25 M, was degraded after two weeks up to 52% and 16%, respectively. The branched chain anions, 2,2-dimethylpropanoate and 2-methylpropanoate, both less toxic than their corresponding linear isomers, were more resistant to fungal attack, leading to null or partial degradation. Chain branching is generally considered to increase resistance to aerobic biodegradation.[32] The distinctive biodegradability potential of isomers is well proven while comparing the biodegradation levels of butanoate and 2-methylpropanoate in media containing the same ionic

Table 3 Biodegradation assessment of ionic liquids using fungal isolate *Penicillium corylophilum*. Concentrations of tested compounds used in this assay were below the previously determined minimal inhibitory concentrations (MIC) of ionic liquids

Ionic liquid	MIC/M	Tested conc/M	Anion degradation (^{1}H NMR spectral analyses)
cholinium ethanoate	0.45	0.375	not detected
cholinium propanoate	0.7	0.5	not detected
cholinium butanoate	0.15	0.1	complete degradation
cholinium 2-methylpropanoate	0.25	0.1	partial degradation
cholinium pentanoate	0.3	0.1	complete degradation
cholinium 2,2-dimethylpropanoate	0.75	0.5	not detected
cholinium hexanoate	0.0625	0.05	complete degradation
cholinium octanoate	0.01	0.01	complete degradation

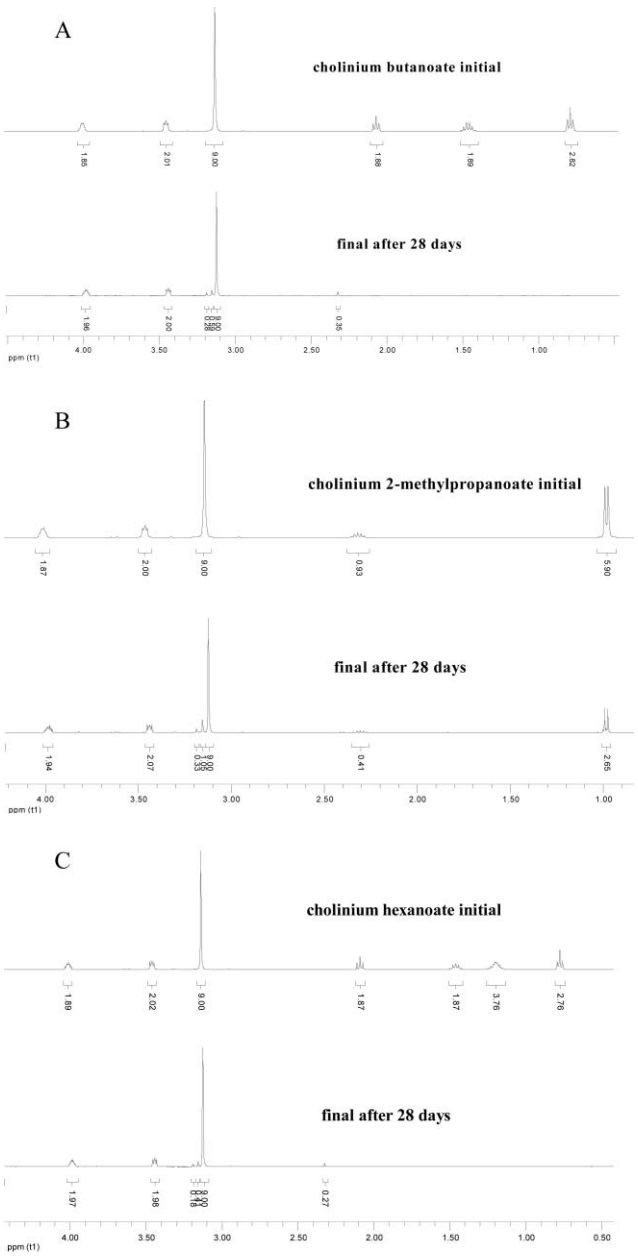

Fig. 5 Biodegradability assessment using *Penicillium corylophilum*. ^{1}H NMR spectra at the beginning and the end of the incubation period (28 days) for (A) cholinium butanoate (0.1 M), (B) cholinium 2-methylpropanoate (0.1 M), and (C) cholinium hexanoate (0.05 M).

liquid concentration (0.1 M) (Fig. 5A, B). In the static cultures (toxicity tests), only partial biodegradation of the ionic liquids was detected (data not shown). This suggests that the cultivation conditions (*e.g.* agitation, dioxygen availability and incubation time) play a critical role, influencing biodegradability.

Conclusions

The benign cholinium cation, combined with a range of alkanoate anions, allowed the synthesis of a novel set of highly promising biocompatible ionic liquids, $[NMe_3(CH_2CH_2OH)][C_nH_{2n+1}CO_2]$ ($n = 1$-9). Their toxicity evaluation, using filamentous fungi as model eukaryotic organisms, demonstrated that fungi can actively grow in media containing, in some cases, concentrations up to molar range. The cholinium alkanoates were less toxic than their corresponding sodium salts. It becomes apparent that the anion toxicity is defined by its lipophilicity, as a consequence of the length of the linear chain and its branching. The cation was not completely mineralised by the fungi, and the isomers demonstrated also distinct biodegradability potentials, with branched isomers being more resistant to *P. corylophilum* attack. The methodology used for toxicity assessment has proven to be a rather robust one. Nevertheless, advanced studies on the ionic liquids' interaction with the spore coats and other cellular boundary structures are necessary to further predict their environmental risks. Cholinium alkanoates are not only environmentally benign and biodegradable, but also extraordinarily good solvents for some very recalcitrant plant biocomposites, in some cases reaching dissolution yields of 60 wt%.[23] There are no doubts that this family of ionic liquids, which promises high value for future biotechnological applications, will be harnessed further in future.

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