Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds

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We investigated the primary biodegradation of different *N*-imidazoles, imidazolium, pyridinium and 4-(dimethylamino)pyridinium compounds substituted with various alkyl side chains and their analogues containing functional groups principally based on OECD guideline 301 D. For the experiments we used two different types of inocula, a freeze-dried mix of bacteria and activated sludge microorganisms from a wastewater treatment plant. The aim of this study was to improve the knowledge base for the structural design of ionic liquids with respect to an increased biodegradability combined with a reduced (eco)toxicological hazard potential. We found a significant primary biodegradation for (eco)toxicologically unfavourable compounds carrying long alkyl side chains (C6 and C8). In contrast for (eco)toxicologically more recommendable imidazolium ionic liquids with short alkyl ($\leqslant$C6) and short functionalised side chains, no biological degradation could be found. The introduction of different functional groups into the side chain moiety thus offering a higher chemical reactivity did not lead to the expected improvement of the biological degradation. After an incubation period of 24 days for the 1-methyl-3-octylimidazolium cation we identified different biological transformation products carrying hydroxyl, carbonyl and carboxyl groups. Furthermore, shortened side chain moieties were identified indicating the degradation of the octyl side chain *via* β-oxidation. Moreover, we propose an electrochemical wastewater treatment as part of an alternative disposal strategy for non-biodegradable ionic liquids. We show for the first time that the 1-butyl-3-methylimidazolium cation was completely destroyed within four hours using an electrolysis double-cell (volume = 1.2 L) equipped with electrodes made of iridium oxide (anode), stainless steel (cathode), and a boron-doped diamond-coated bipolar electrode. The products formed electrochemically were easily accessible to biological degradation.

Introduction

In recent years ionic liquids (ILs) have gained a broad interest because of their applicability as solvents in different fields, *e.g.* in organic synthesis,[1] catalysis,[2] biocatalysis,[3] and electrochemistry.[4] This wide applicability of ionic liquids is mainly based on the beneficial physico-chemical properties (*e.g.* high thermal and electrochemical stability, high conductivity, extraction behaviour, *etc.*) of certain compounds out of this diverse substance class. Furthermore, the negligible vapour pressure of ionic liquids causes reduced air emission and non-flammability. In this respect, the operational safety of ionic liquids is improved as compared to conventional solvents. In general, the high structural variability of the head group (positively charged core structure), the substituent(s) and the corresponding anion leads to an enormous number of accessible ionic liquids. The combination of these different structural elements allows – at best – for an optimisation of physico-chemical properties of ionic liquids necessary for a defined technical application.

However, regarding the hazard assessment of ionic liquids this structural variability represents an almost insurmountable problem as it is impossible to generate a profound knowledge of the effects on man and the environment for every single compound. Different studies were conducted to evaluate the (eco)toxicity of certain ionic liquids in *in vitro* assays[5–10] and in some selected organism studies comprising, for example, bacteria,[11–14] algae,[15–18] earthworms,[19] waterfleas,[18,20–22] and zebrafish.[23] A complete overview of the (eco)toxicological data of ionic liquids has been given by Ranke *et al.*[24] and Zhao *et al.*[25]

These studies indicate that ionic liquids can cause adverse effects on organisms. Especially for cations substituted with long (C $\geqslant$ 8) alkyl side chains[16–18] or for anions showing lipophilicity or a susceptibility to hydrolysis[9,16] partially drastic effects have been observed.

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Nevertheless, by an appropriate choice of (eco)toxicologically favourable structural elements as short and functionalised side chains, avoiding the quinolinium and the 4-(dimethylamino)pyridinium head group and by using, for example, chloride, tetrafluoroborate or octylsulfate as the anion,[8,16,17,26] the (eco)toxicity of an ionic liquid can be reduced remarkably in the test systems investigated so far. This is an important result for the design of inherently safer and thus more sustainable substances. Additionally, according to principles of green chemistry (Paul Anastas and John Warner), chemicals should also be designed to break down to innocuous substances after their use so that they do not accumulate in the environment.[27] In contrast, the tendency of certain ionic liquid cations to be thermally and chemically very stable is mirrored in their stability to biological degradation processes. So far, only a few fundamental studies have investigated the biodegradability of ionic liquids.

In a theoretical study, Jastorff *et al.*[28] proposed biodegradation pathways for imidazolium cations and presented cytotoxicity data for some of the presumed and subsequently synthesised metabolites.[29] Stepnowski and Storoniak[30] calculated the energetic stability of radicals formed during the reaction with the cytochrome P450 enzyme system and identified preferred positions of biological transformation reactions for the 1-butyl-3-methylimidazolium cation. Scammells and co-workers[22,31,32] examined the degradation potential of different 1-butyl-3-methylimidazolium cations combined with Br^-, BF_4^-, PF_6^-, $N(CN)_2$, $(CF_3SO_2)_2N^-$ and octylsulfate as the counterion according to the Sturm and Closed-Bottle test protocols. No compound exhibited significant levels of biodegradation, and only for the octylsulfate-containing ionic liquid was an increased biodegradability observable. A series of imidazolium compounds substituted with amide side chains were analysed and showed poor levels of biodegradation, whereas for different ester group-containing side chains an enhanced biodegradability could be found.[31–33] However, no compound could be classified as readily biodegradable. In a later study the combination of the imidazolium moiety substituted with ester side chains and octylsulfate as the counterion resulted in readily biodegradable ionic liquids.[33]

Wells and Coombe[18] investigated the biodegradability of ammonium, imidazolium, phosphonium and pyridinium compounds by measuring the biological oxygen demand (BOD). No biodegradability of cations with short chains ($C \leqslant 4$) was observable within this test series. For phosphonium and imidazolium cations with longer chains (C12, C16 and C18) a strong inhibitory potential to the inoculum used was found, indicating the toxicity of these ionic liquids towards the microorganisms used.

Recently, Kulpa and co-workers[34] examined the biodegradability of *N*-methylimidazolium and 3-methylpyridinium compounds substituted with butyl, hexyl and octyl side chains and bromide as the anion. In dissolved organic carbon (DOC) die-away tests and in tests monitoring the changes in the total dissolved nitrogen (TDN) concentration a dependency between biodegradability and the side chain length was found. 1-Octylpyridinium bromide meets the OECD criterion for being classified as readily biodegradable, whereas 1-hexylpyridinium bromide exhibited a decreased degradation rate. Compared to the pyridinium ILs the mineralisation of the imidazolium ILs was lower. The 1-methyl-3-octylimidazolium cation showed significant degradation rates, but those were not high enough for a classification as readily biodegradable. For the pyridinium and imidazolium head groups carrying a butyl side chain no significant biodegradation was observable. The fact that only the long octyl side chain in ionic liquids brings about an improved biodegradability creates a conflict of aims between minimizing the toxicity and maximizing the biodegradability. Thus, the biodegradability seems to be a bottle-neck in the development of inherently safer ionic liquids.

Following our strategy of designing safer ionic liquids[28,29] we aimed to overcome this inherent problem and to enlarge the restricted knowledge in the field of biodegradation of ionic liquids, and we analysed systematically the influence of the structural elements 'head group' and 'side chain' (also containing functional groups) on the biodegradability of 27 compounds. Regarding this issue we follow a T-SAR (thinking in terms of structure–activity relationships) guided strategy to:

(i) systematically select test compounds and structural elements according to the 'test kit concept';[29,35]

(ii) apply a theoretical T-SAR algorithm to propose biological transformation products (metabolites);[28,36]

(iii) test the selected substances in biodegradation tests using inocula from a wastewater treatment plant according to a modified OECD test protocol;

(iv) ascertain the primary biodegradation of compounds and identify transformation products *via* HPLC–UV and HPLC–MS analysis;

(v) identify substructures in chemicals responsible for an improved or declined biodegradability;

(vi) incorporate the transformation products formed into the hazard assessment of ionic liquids and investigate the (eco)toxicity of the transformation products using test systems at different levels of biological complexity (*e.g.* enzymes, cells, micro-organisms and organisms);

(vii) use this knowledge in the prospective design of inherently safer chemical products.

For non-biodegradable ionic liquid cations (tested as halides) we propose an electrochemical wastewater treatment using boron-doped diamond (BDD)-coated electrodes to facilitate their breakdown. This electrochemical process is often applied to eliminate biologically persistent compounds.[37,38] In general, the BDD coating exhibits a high oxygen overpotential which promotes the electro-oxidation of organics *via* electrogenerated hydroxyl radicals due to their high standard redox potential of 2.8 V and reduces the side reaction of oxygen evolution.[39] The formed radicals can effectively transform non-biodegradable organic species to biodegradable organic compounds or final inorganic ones like CO_2 and H_2O.[37,40,41]

Selection of test kit compounds

The test kit comprises different cations combined with a halide (chloride, bromide or iodide) the as counterion. Three

aromatic head groups [4-(dimethylamino)pyridinium, pyridinium and 1-methylimidazolium] substituted with different alkyl side chains (C2, C4, C6, C8) and some mono-*N*-substituted imidazole compounds are included in the test kit. The pyridinium and imidazolium substances are selected to confirm the results from literature and for a validation of the primary degradation tests used. Furthermore, the HPLC–MS analysis allows for an identification of degradation products of these compounds and expands the currently available data.

The 4-(dimethylamino)pyridinium head group, which is not recommendable from an (eco)toxicological point of view,[8,17,42] was analysed because of its high lipophilicity. This head group being substituted with a hexyl side chain exhibits the same lipophilicity (corresponding to a HPLC-determined parameter) as an octyl-substituted imidazolium compound.[8] This fact allows for differentiation whether the side chain length or the lipophilicity of a compound is responsible for an increased biodegradability.

Additionally, the imidazolium core is substituted with short side chains containing ether (in different positions), terminal hydroxyl, carboxyl and nitrile functions. These compounds were selected because of their (eco)toxicological beneficial properties and for investigating if the increased chemical reactivity of these functionalised cations concomitantly increases the biodegradability.

Experimental

Chemicals

All tested ionic liquids were received from Merck KGaA (Darmstadt, Germany). Na_2SO_4, KH_2PO_4, K_2HPO_4, $Na_2HPO_4{\cdot}2H_2O$, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $FeCl_3{\cdot}7H_2O$ and H_2SO_4 were purchased from the Sigma-Aldrich Cooperation (Deisenhofen, Germany). Acetonitrile (HPLC grade), methanol, $HgCl_2$, NH_4Cl and sodium benzoate were obtained from Fluka (Buchs, Switzerland).

HPLC systems

The HPLC system used for the determination of primary biodegradation and the electrochemical degradation studies was a VWR Hitachi system containing the L-2130 HTA-pump, L-2130 degasser, L-2200 autosampler, L-2300 column oven, L-2450 diode array-detector and the EZChrom Elite software. The HPLC system utilised for analysing degradation products was a Hewlett Packard system Series 1100, with a gradient pump, online degasser, autosampler and a Bruker esquire ESI-MS ion trap detector.

For both systems a hydrophilic interaction liquid chromatography (HILIC) column (Atlantis HILIC Silica 5 μm, 4.6 × 150 mm) with guard column purchased from Waters (Eschborn, Germany) was used. The mobile phase consisted of 80% acetonitrile (HPLC grade) and 20% aqueous 5 mM HK_2PO_4/H_2SO_4 buffer. The system was operated at a flow rate of 1 mL min^{-1} and 10 μL portions of the samples were injected. A detection wavelength of 212 nm was used for quantification of the original compounds.

Primary biodegradation

The primary biodegradation test was conducted according to a modified version of OECD guideline 301 D.[43] Primary biodegradation of the test compounds was monitored *via* HPLC–UV for 31 days. The inoculum used was derived from the wastewater treatment plant Bremen-Seehausen (Germany). Five gram sludge flocs were suspended in 1 L mineral medium and pre-conditioned for 5 days under aerobic conditions. The mineral medium was composed of 8.5 mg L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.13 mg L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 1.7 mg L^{-1} NH_4Cl, 36.4 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 22.5 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ (pH 7.2). Solutions of the test substances were prepared at a concentration of 200 μM (corresponding to 14–61 mg L^{-1} depending on the molecular weight of the test substances) in inoculated test media (100 mL total volume). Blank samples (inoculated media without test the substance), abiotic controls (200 μM test substance in inoculated media poisoned with 50 mg L^{-1} $HgCl_2$) and positive controls (inoculated media with 200 μM imidazole) were also prepared. Replicates of test samples, blinds, abiotic and positive controls were kept in the dark at 20 ± 1 °C. The 100 mL test vessels were closed but not gas-tight. Losses due to evaporation were determined by weighing and were adjusted by the addition of test media. For every testing day 500 μL of all samples were taken, centrifuged (5000 rpm, 15 min) and subsequently analysed *via* HPLC. The percentage of degradation of each sample was calculated referring to the initial concentration.

Oxygen consumption biodegradation experiment

The biodegradation experiments using the sum parameter oxygen depletion was performed due to the DIN EN 1899-2 guideline.[44] The test solutions were added to the same test media used for the primary degradation test and were inoculated with a commercially available freeze-dried micro-organism mixture (Dr. Lange BioKIT LZC 555).[45] All experiments were performed in Karlsruhe-bottles, which are adapted to the oxygen electrode used (StirrOx G). Triplicates of each test solution, blanks and controls (using sodium benzoate) were analysed for each sampling day and were discarded after determination of the oxygen content. The oxygen depletion for the test solutions at a given time was corrected by the oxygen demand of the blank after the same time period.

For the determination of the chemical oxygen demand (COD) the cuvette high-speed tests LCK 314, 414, 514, 614 and 014 (Hach Lange GmbH, Düsseldorf, Germany) were used and measured with a photometer Cadas 200 (Hach Lange GmbH, Düsseldorf, Germany).

Electrochemical treatment

The electrochemical degradation investigations have been carried out with aqueous solutions of 1-butyl-3-methylimidazolium chloride (IM14 Cl) at a concentration of 230 μM (40 mg L^{-1}) which was supplemented with 0.06 M Na_2SO_4 and 0.15 M KOH. The conductivity of the mixture was measured to be 20 mS using a WTW inoLab station with a TetraCon conductivity electrode.

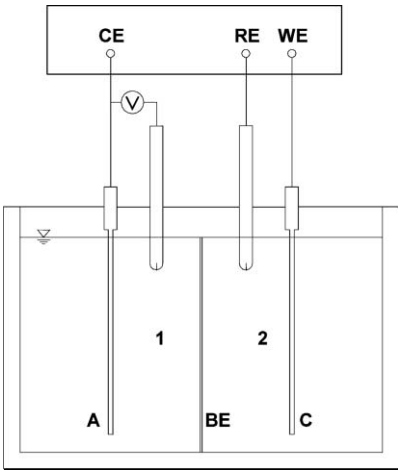


Fig. 1 Electrolysis reactor (A = anode, C = cathode, BE = bipolar BDD- mid-electrode, which divides the reactor into two chambers, RE = reference electrode, WE = working electrode, CE = counter electrode.

The electrochemical degradation experiments were carried out in an electrolysis cell (volume = 1.2 L) equipped with two electrodes made of iridium oxide (anode) and stainless steel (cathode). Additionally, a bipolar mid-electrode – purchased from 'pro aqua Diamantelektroden Produktion GmbH' (Niklasdorf, Germany) – made of polyvinylidenefluoride (PVDF) fitted with boron-doped diamonds was used. This electrode arrangement divided the reactor into two chambers (Fig. 1). The distances between the electrodes were 2 cm. The experiments were performed with a constant potential of 2.5 V at the steel cathode adjusted with a reference electrode placed next to the cathode. As the potentiostat an IMP83 PC-10-device of the company 'Jaissle Elektronik GmbH' (Waiblingen, Germany) and a power supply unit of the company 'Statron' (Zschopau, Germany) were employed.

Results

For biodegradation tests two different types of inocula were used. A commercially available freeze-dried micro-organism mixture (Dr. Lange BioKIT LZC 555) composed for the determination of sewage water qualities[45] and, secondly, activated sludge from a wastewater treatment plant (Bremen-Seehausen, Germany) was applied.

The freeze-dried bacteria mixture was chosen because this is a standardised product and should allow for a high reproducibility between different test runs. Furthermore, this mixture showed an acceptable biological matrix compared to the activated sludge and therefore caused less interference in the HPLC–UV and HPLC–MS analysis. However, no test substance was metabolised in the primary degradation tests using these freeze-dried bacteria. Thus, all results presented in the following section are related to tests using the activated sludge.

Toxicity and adsorption

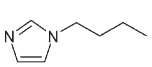
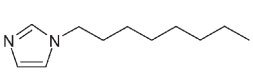
None of the test substances inhibited the biodegradability of the reference compound imidazole in the concentration range tested, indicating that the ionic liquids used are not toxic towards the micro-organisms.

The adsorption of the test substances to the activated sludge was checked with an abiotic control (sludge inactivated with $HgCl_2$). No test compound showed significant sorption to the inoculum. Consequently, an observed concentration decrease of a test substance in biotic samples can be exclusively attributed to biological degradation.

Primary biodegradation

Mono-*N*-substituted imidazole compounds. No decrease in the concentration within the test duration of 31 days could be found for *N*-methylimidazole (IM01), *N*-butylimidazole (IM04), and *N*-octylimidazole (IM08) (Table 1), whereas

Table 1 Structures, acronyms and primary biodegradation rates of alkylimidazoles

Structure	Acronym	Primary biodegradation (%)				
		Day 4	Day 9	Day 17	Day 24	Day 31
	IM00	100	100	100	100	100
	IM01	0	0	0	0	0
	IM01-2Me	0	0	9	93	100
	IM04	0	0	0	0	0
	IM08	0	0	0	0	0

N-methyl-2-methylimidazole (IM01-2Me) was completely degraded after 31 days (Table 1). The positive control, imidazole (IM00), was completely degraded after four days.

Imidazolium ionic liquids. Most of the imidazolium ionic liquids were not metabolised by the activated sludge microbial community (Table 2). In particular, imidazolium cores substituted with short alkyl side chains or short

Table 2 Structures, acronyms and primary biodegradation rates of 1-alkyl-3-methylimidazolium halides

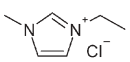
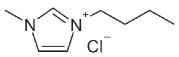
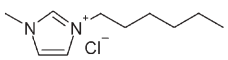
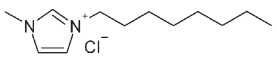
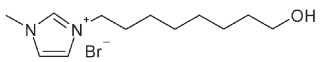
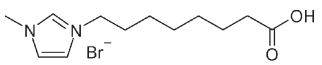
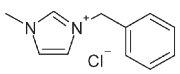
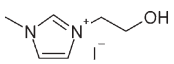
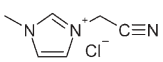
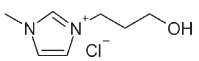
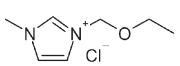
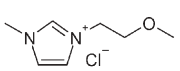
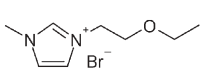
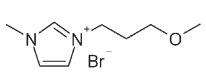
Structure	Acronym	Primary biodegradation (%)				
		Day 4	Day 9	Day 17	Day 24	Day 31
	IM12 Cl	0	0	0	0	0
	IM14 Cl	0	0	0	0	0
	IM16 Cl	0	0	5	8	11
	IM18 Cl	31	40	81	100	100
	IM18OH Br	25	58	100	100	100
	IM17COOH Br	10	18	100	100	100
	IM1-1Ph Cl	0	0	0	0	0
	IM12OH I	0	0	0	0	0
	IM11CN Cl	0	0	0	0	0
	IM13OH Cl	0	0	0	0	0
	IM11O2 Cl	0	0	0	0	0
	IM12O1 Cl	0	0	0	0	0
	IM2O2 Br	0	0	0	0	0
	IM13O1 Br	0	0	0	0	0

Table 3 Structures, acronyms and primary biodegradation rates of *N*-alkylpyridinium chlorides

Structure	Acronym	Primary biodegradation (%)				
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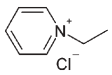
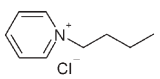
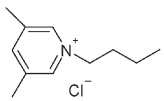
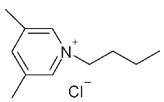
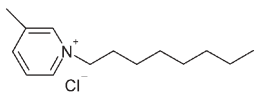
	Py2 Cl	0	0	0	0	0
	Py4 Cl	0	0	0	0	0
	Py4-3Me-5Me Cl	0	0	0	0	0
	Py8 Cl	0	25	58	100	100
	Py8-3Me Cl	0	25	33	97	100

side chains containing ether, hydroxyl and nitrile functions were highly resistant to biological degradation during the incubation period (Table 2). That also applies to the phenyl-linked imidazolium compound (IM1-1Ph Cl). Small losses (−11%) of 1-hexyl-3-methylimidazolium (IM16) were measured after 31 days compared to the initial concentration (Table 2). A complete primary degradation could be detected for 1-methyl-3-octylimidazolium (IM18) after 24 days, and after 17 days for the hydroxylated (IM18OH) and for the carboxylated (IM17COOH) derivative (Table 2).

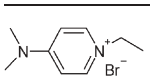
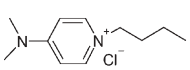
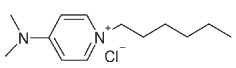
Pyridinium ionic liquids. The *N*-ethylpyridinium (Py2) and the *N*-butylpyridinium compounds (Py4 and Py4-3Me-5Me) did not undergo significant biodegradation, whereas for the *N*-octylpyridinium cations (Py8 and Py8-3Me) a total elimination was observed within 31 days (Table 3).

Dimethylaminopyridinium ionic liquids. In contrast to the *N*-hexyl-substituted 4-(dimethylamino)pyridinium head group (Py6-4NMe2) which was completely degraded within 31 days (Table 4), no biological breakdown could be observed for the *N*-ethyl- and *N*-butyl-derivatives (Py2-4NMe2 and Py4-4NMe2).

Identified transformation products of 1-octyl-3-methylimidazolium

The 24 day sample of the 1-octyl-3-methylimidazolium cation (IM18) was investigated for biodegradation products *via*

Table 4 Structures, acronyms and primary biodegradation rates of *N*-alkyl-4-(dimethylamino)pyridinium halides

Structure	Acronym	Primary biodegradation (%)				
		Day 4	Day 9	Day 17	Day 24	Day 31
	Py2-4NMe2 Br	0	0	0	0	0
	Py4-4NMe2 Cl	0	0	0	0	0
	Py6-4NMe2 Cl	6	12	55	93	100

HPLC–MS analysis. For this sample, no clear UV peaks were seen but different signals within the mass spectrometer were detectable. To ensure that the signals found within this sample correspond to transformation products a blank sample (inoculated test buffer after 24 days) and a sample from the beginning of the test were measured. None of the identified masses in the 24 day sample of IM18 were found in these reference analyses.

The identified transformation products and the concluded pathway for biodegradation of the IM18 compound are shown in Fig. 2. At different retention times the mass-to-charge ratio (m/z^+) 211 was detected, probably belonging to hydroxyl groups at different positions at the side chain of IM18. The observed retention time is similar to the investigated test compound IM18OH (14 min). Furthermore, different signals with m/z^+ = 209 could be determined, presumably pertaining to ketones or aldehydes after the oxidation of different hydroxylated compounds. The mass-to-charge ratio of 225 potentially corresponds to a carboxylated product of IM18 (like the test compound IM17COOH; retention time 12.3 min) or to substances containing two functional groups (ketone and hydroxyl). In accordance with this, m/z^+ = 225 occurred in a relatively wide retention time range of 2.5 min, indicating clear structural differences as occur between different carboxylic acids or compounds substituted with both ketone and hydroxyl functions.

Moreover, hydroxylated and/or carboxylated compounds with a reduced number (−2, −4 or −6) of carbon atoms in the side chain were found (Fig. 2). For these compounds the identified masses occurred as well-defined single peaks suggesting that no isomers with the same mass were formed. The chemical transformations identified were in accordance with the recently predicted ones.[28]

Adaptation and accumulation of degradation products

After the test period of 31 days the IM18 test bottles were used for a subsequent experiment. To these samples additional IM18 was given (final concentration of 200 μM). For these experiments with pre-adapted inocula an increased biodegradation rate was observed. Within four days a complete primary degradation of IM18 had taken place. This procedure was repeated for two other supplementations of the test compound (all at 600 μM). For all test runs an exhaustive primary degradation was observable after four days. In

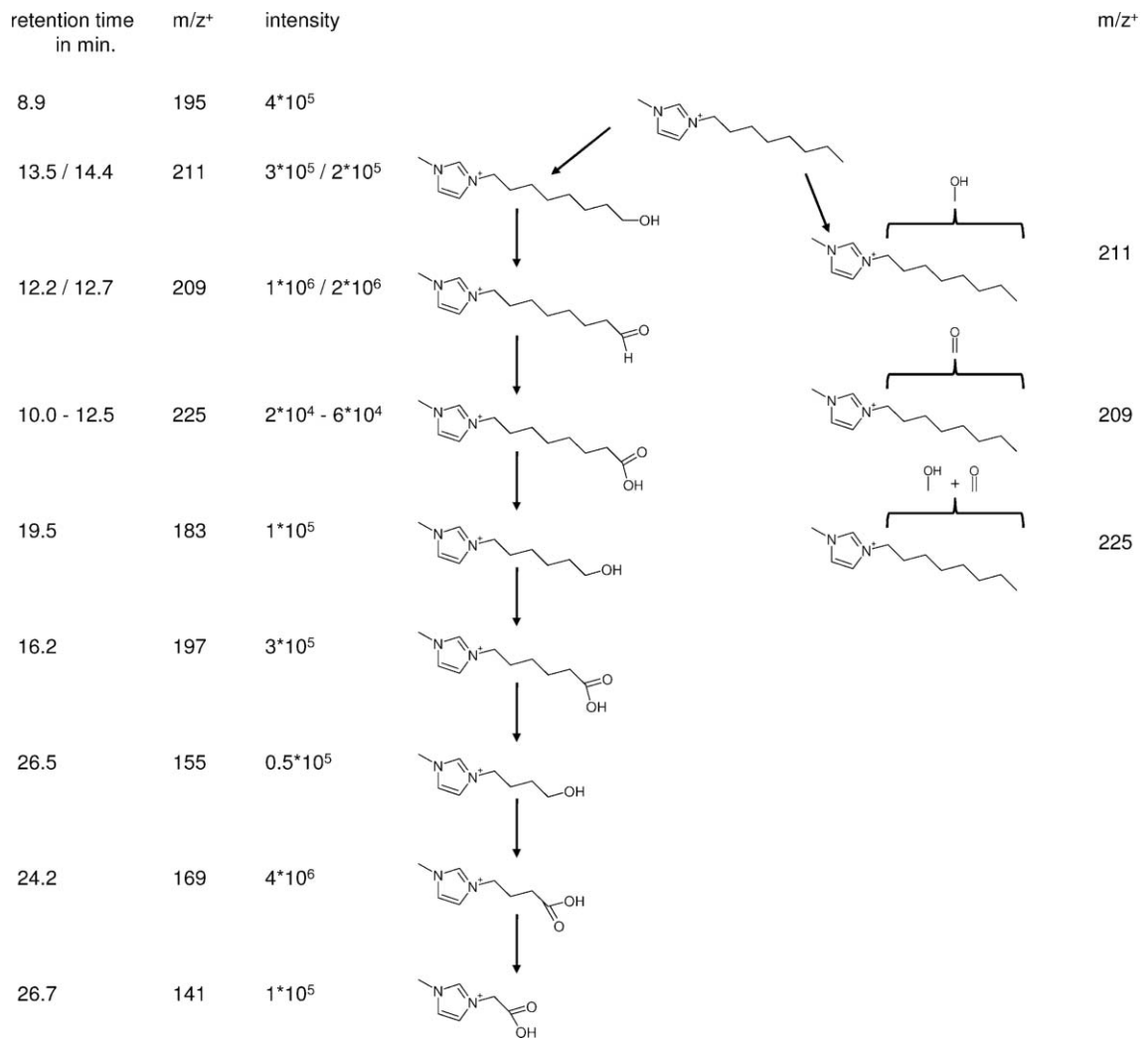


Fig. 2 Retention times, mass-to-charge ratios (positive mode), intensity of signals within the mass spectra and proposed chemical structures.

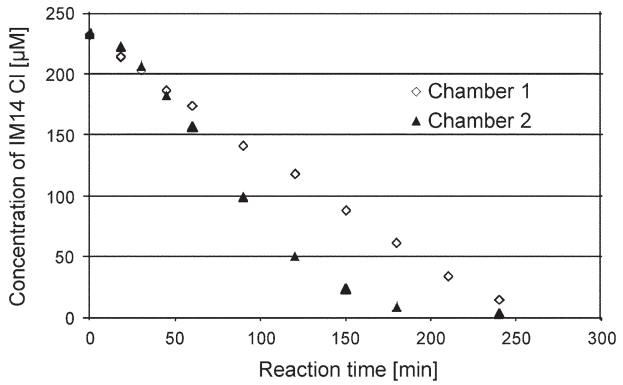


Fig. 3 HPLC-determined degradation of the 1-butyl-3-methylimidazolium cation during the electrolysis experiment.

general, no UV signal within the chromatogram of the spiked samples could be detected except for a peak at approximately 14 min (probably belonging to a hydroxylated product) with a concentration of 220 µM (if calculated as IM18OH). After an additional period of 31 days a remaining concentration of 10 µM was determined.

Electrochemical treatment of 1-butyl-3-methylimidazolium chloride

A nearly complete electrochemical dismantling of the 1-butyl-3-methylimidazolium cation (IM14) within the test duration of 240 min could be observed *via* HPLC–UV measurements (Fig. 3). For chamber 1 (iridium oxide anode/bipolar boron-doped cathode) a linear breakdown of IM14 was observable with a remaining ionic liquid concentration of 14 µM (substance loss 96%). The degradation course in the second chamber (bipolar-doped anode/steel cathode) was sigmoidal and led to a final IM14 concentration of 3 µM (substance loss 99%). The HPLC analysis of the different sampling points revealed various signals for both chambers – mainly with longer retention times on the HILIC column as found for the IM14 cation, indicating more polar chemical entities. After 240 min these signals of breakdown products were not or only marginally detectable. During electrochemical treatment an increasing electrical current (from 1.1 to 5.7 A) and temperature (19 to 58 °C) was observed. Furthermore, the chemical oxygen consumption (COD) – used to for indirectly measuring the amount of organic carbon in water – was reduced by around two-thirds for both chambers (chamber 1: t_0 = 69 mg L^{-1}, t_{240} = 23 mg L^{-1}; chamber 2: t_0 = 69 mg L^{-1}, t_{240} = 24 mg L^{-1}). The biodegradability of the solutions remaining after the electrochemical treatment (pH adjusted at 7 and inoculated with freeze-dried bacteria) was measured by the oxygen depletion assay. The biological oxygen demand (BOD) after 3, 6, and 8 days was determined for both chambers (Fig. 4). An oxygen consumption for both samples was detectable (chamber 1: 4.7 mg L^{-1} ≈ −60%, chamber 2: 4.1 mg L^{-1} ≈ −55%) associated with a decreased COD (chamber 1: 14 mg L^{-1} ≈ −41%, chamber 2: 14 mg L^{-1} ≈ −42%).

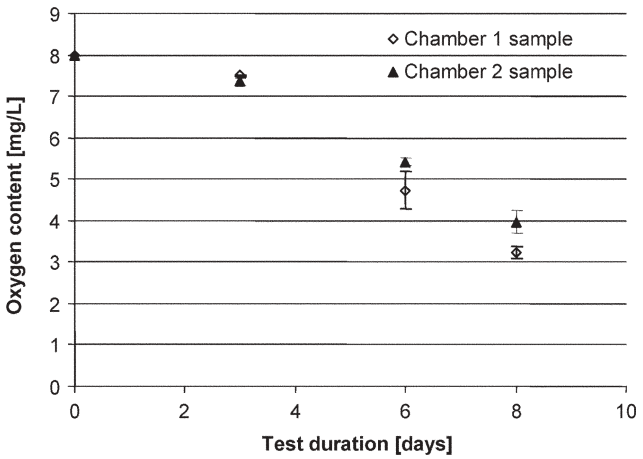


Fig. 4 Biological oxygen consumption of the remaining solutions after electrochemical treatment.

Discussion

Relevance of the primary biodegradation test

The test conditions employed for the primary biodegradation investigations are very similar compared to the ready biodegradability test according to OECD guideline 301 D. This allows for classifying structures as not readily biodegradable, when no clear decrease in the concentration of the test substance was observed.

In contrast, it is not possible within this primary degradation test to term compounds as readily biodegradable, even if a 100% degradation of the test substance is observed, because this 100% degradation is related only to the parent compound (primary degradation) and not to all possibly formed metabolites which might be resistant to further biodegradation.

In general, it is known from biodegradation experiments that after an adaptation period at the beginning of the tests a rapid biodegradation can occur within a few days. In our setup the timeframe between successive sampling points was too long to follow the degradation kinetics in detail.

Primary biodegradation of test compounds

The freeze-dried bacteria mixture was proven not to be suitable for evaluation of the general biodegradability of compounds according to OECD guidelines. This is related to the limited diversity of micro-organisms within this arranged mixture compared to the heterogeneous micro-organism community of the activated sludge.

The results obtained for the wastewater micro-organisms showed that the mono-*N*-substituted imidazole derivatives, which are considered to be poorly biodegradable,[46,47] could be confirmed for the *N*-alkylated imidazoles investigated here. An exception is *N*-methyl-2-methylimidazole (IM01-2Me), the observed primary degradation of which is unexpected. Either the microbial community used contained a specific micro-organism which could catabolise this substrate or the degradation was just related to the mother compound and not to the transformation products.

For imidazolium ionic liquids with short alkyl ($\leqslant$C6) and short functionalised side chains no biological degradation could be found. The introduction of functional groups linked to a higher chemical reactivity did not lead to the expected improvement of the biological degradation.

Our study confirmed the results found by Docherty *et al.*,[34] that imidazolium and pyridinium compounds with octyl chains are biodegradable. A complete primary degradation was observed within 24 days for IM18 and Py8. On the basis of the identified transformation products of IM18 we propose the breakdown pathway shown in Fig. 2. Here the transformation of the alkyl chain starts with the oxidation of the terminal methyl group (ω-oxidation) catalysed probably by mono-oxygenases, *e.g.* the cytochrome P450 system. The alcohol formed is subsequently oxidised by dehydrogenases *via* aldehydes to carboxylic acids (Fig. 2). The resulting carboxylic acids then can undergo β-oxidation and the two released carbon fragments can enter the tricarboxylic acid cycle as acetylCo-A.

The HPLC–MS results also indicate the formation of different non-terminal hydroxyl groups referring to identical masses but showing varying retention times. These secondary alcohol isomers could not be further degraded *via* β-oxidation. Their transformation process ended either with the formation of ketones or by additional hydroxylation steps. In contrast, for the shortened chain metabolites formed by the β-oxidation steps all identified compounds produced single peak signals, which means that no isomers were formed.

Additionally, we have found that the microbial community was able to adapt to the degradation of IM18. This could be observed by a clear reduction in the degradation time from 24 days down to four days after three additional supplementations of IM18. Potentially, this observation can also be addressed to an increased bacterial concentration within the sample. A total of 800 μM of IM18 was degraded and no transformation products were detected *via* HPLC–UV, indicating a complete biodegradation including the imidazolium core (responsible for UV absorption). Even if IM18 cannot be classified as readily biodegradable it is at least inherently biodegradable. Those results agree with results found by Docherty's group.[34]

The introduction of –OH and –COOH into the octyl chain resulted in an improved primary degradation with a shortened test duration of 17 days. For those compounds an ω-oxidation is not necessary, so the β-oxidation can occur immediately. Therefore, we assume that the ω-oxidation is the rate-limiting step within the biodegradation process of IM18.

Whereas no biodegradation of IM16 occurred, Py6-4NMe2 was totally biodegradable within 31 days. Recently, we investigated the lipophilicity of different compounds using an HPLC-derived parameter (k_0). We found similar lipophilicity values for IM18 (k_0 = 1.85)[26] and Py6-4NMe2 (k_0 = 1.80).[26] Therefore, it can be concluded that the chain length (C = 8) is not a mandatory criterion for biodegradation, but more important is a certain overall lipophilicity of the compound.

This observation can be explained in at least two ways. First, ionic liquids with longer alkyl chains (higher lipophilicity) have been proven to be more toxic. Therefore, they are able to produce selective pressure on the microbial community – micro-organisms being capable of degrading ionic liquids with longer alkyl chains are privileged whereas the others that are not able to metabolise ionic liquids are eliminated. This assumption has been proven by Docherty *et al.* who analysed the structure of the microbial community by DNA-PCR DGGE and found an enrichment of few bacteria species in the samples treated with IM18.[34]

A second explanation is based on an uptake or even an increased uptake into the organisms, which is also related to the lipophilicity of the compounds. Owing to this (higher) uptake the substances can be metabolised by appropriate enzyme systems.

In contrast, IM08 was not degraded even though it is mainly protonated under test conditions (pH = 7) and therefore should posses a similar lipophilicity compared to IM18. Also, the cytotoxicity of IM18 (100 μM) and IM08 (150 μM) is comparable. According to our above-mentioned hypotheses this result was not expected and IM08 should have been biodegradable.

In general, the identified transformation products of IM18 are compounds with shorter side chains and functionalised groups. Previous studies from our group showed that compounds with short and functionalised side chains exhibit lower toxicities towards mammalian cells,[8] marine bacteria, limnic green algae[17] and duckweed.[17] Therefore, we propose for most of the transformation products lower hazard potentials compared to IM18, which has a high aquatic toxicity (especially to algae)[16]. Nevertheless, some restrictions have to be made because the aldehydes, which are intermediates in the oxidation pathway from the –CH_2OH group to the –COOH group, have not been analysed regarding their (eco)toxicity so far and in principle the formation of highly reactive epoxides is thinkable.

Further investigations in this field are desirable to examine the detailed biodegradation pathways, detailed kinetics and the metabolites of ionic liquids showing primary biodegradation. With respect to the design of inherently safer ionic liquids it is necessary to find structural modifications which improve biodegradability without increasing lipophilicity, which correspondingly would increase the toxicity.

Both IM18OH and IM17COOH are biodegradable and they exhibit a reduced cytotoxicitiy (EC_{50} IM18OH Br = 230 μM; EC_{50} IM17COOH Br = 3000 μM) as compared to IM18 Cl (EC_{50} IM18 Cl = 60 μM). However, it still has to be investigated if the physico-chemical properties of these ionic liquids (mp $<$100 °C) are useful for technical applications and if their toxicities to algae are also in an acceptable range.

The biodegradability of, for example, morpholinium and piperidinium moieties have not been investigated so far, but their *N*-alkylation probably will lead to poorly biodegradable compounds.

Based on the experiences gathered so far, the introduction of oxo-groups into the ring *N*-heterocycles (Fig. 5) should increase biodegradability, because this substructure represents a target site for the cleavage of the C–N bond by an amidohydrolase.[47] But again, the changes in the physico-chemical properties have to be investigated.

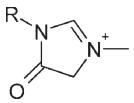


Fig. 5 Introduction of an oxo-group into the core structure represents a potential target site for the cleavage of the C–N bond by an amidohydrolase.

Electrochemical treatment

An electrochemical treatment – as an alternative disposal strategy for non-biodegradable compounds – was applied exemplarily for an aqueous solution of 1-butyl-3-methylimidazolium chloride. An almost complete primary degradation of the IM14 cation was determined *via* HPLC–UV measurements within 4 h. The degradation rate of IM14 in the second chamber was higher compared to the first chamber. This observation is probably caused by an increased formation of hydroxyl radicals at the surface of the anodic side (toward chamber 2) of the bipolar boron electrode. In general, no UV signals after the electrochemical reaction could be found in the chromatogram leading to the conclusion that the positively charged imidazolium core structure (considered mainly to be responsible for the non-biodegradability) has been destroyed. The chemical oxygen demand was decreased in both chambers indicating an electrochemical burning of the test compounds and of the transformation products formed. The remaining solution after the electrolysis process has been analysed in a biodegradation test. For both chambers a clear oxygen consumption through the test micro-organisms used was found after eight days, suggesting an improved biodegradability of the transformation products in comparison to IM14.

These investigations represent a first feasibility study that an electrochemical treatment could be an appropriate technique to remove ionic liquids from wastewater. Detailed examinations evaluating the applicability of this technique – including an optimisation of the performance, the identification of transformation products and a toxicity assessment for the generated solution – are in preparation.

Conclusion

We used a primary biodegradation test to analyse the biodegradability of 27 different compounds with different head groups and side chains. Our results strongly indicate that a certain lipophilicity of the test compounds is an essential criterion for biodegradable ionic liquid cations. However, a high lipophilicity corresponds to an increased (eco)toxicity in different test systems. Thus, a conflict of goals between (eco)toxicologically favourable compounds (short and functionalised side chains) on the one hand and inherently biodegradable substances on the other hand needs to be solved for the development of more sustainable ionic liquids.

The electrochemical wastewater treatment is proposed as an alternative disposal strategy for non-biodegradable ionic liquid cations. Nevertheless, the design of inherently biodegradable ionic liquids should be preferred due to a reduced hazard for man and the environment. Furthermore, here the energy consumption and the need for further chemicals and apparatus is reduced compared to an electrochemical wastewater treatment.

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