# Anaerobic biodegradability of ionic liquid cations under denitrifying conditions

**Jennifer Neumann,[a] Olav Grundmann,[b] Jorg Thöming,[c] Michael Schulte[d] and Stefan Stolte*[a]**

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Biodegradability and ecotoxicity of ionic liquids (ILs) are key properties for determining the greenness of IL applications, and have been increasingly investigated during the last few years. Former studies on the biodegradability of ILs were solely focused on the aerobic side. Nevertheless, the anaerobic biodegradation of many compounds plays an important role in the environment. Anaerobic respiration, especially nitrogen reduction, is widespread in the environment and is commonly used for waste water treatment. Therefore, we investigated in this study, whether ILs can be biodegraded under nitrogen reducing conditions. The primary anaerobic biodegradability of nine different imidazolium, pyridinium and dimethylaminopyridinium based IL cations was monitored *via* HPLC-UV over a time period of 11 months. Only for the 1-(8-hydroxyoctyl)-3-methyl-imidazolium cation (IM18OH), and a degradation could be observed and several metabolites were identified using LC-MS. Co-metabolism is sometimes the only way to degrade difficult substances. However, a possible co-metabolism of the substances by using acetate was not observed. All in all, the biodegradability of the tested ILs seems to be even worse under denitrifying conditions compared to aerobic ones. Nevertheless, the present paper aims to fill the gap concerning the biodegradability of ILs in waste water treatment plants. It gives a first insight into the biological degradation of ILs in the absence of oxygen, and provides further data for an appropriate hazard assessment.

## Introduction

### The popularity of ILs in the light of green chemistry

Ionic liquids (ILs) have gained popularity as designer solvents with high operational safety, particularly due to their low vapour pressure and non-flammability. Operational safety together with the various ways in which anions, cation headgroups and side chains of ILs can be combined, open up a wide range of ILs and applications in industrial processes: be it as organic solvents,[1,2] catalysts,[3] enzyme stabilisers,[4] sensors,[5,6] or potential pharmaceutical ingredients,[7] the application possibilities are manifold. However, increasing application possibilities and usage of ILs also results in a higher amount of final IL waste and its potential release into the environment. The ILs in use should therefore be of high intrinsic safety to reduce environmental hazards according to the principles of green chemistry published by Anastas *et al.* in 1998.[8] A review focusing on the issue of sustainability in the design of ILs has already been published by Ranke *et al.* in 2007.[9] The authors emphasise the welcoming effect of biodegradable ILs for environmental purposes to diminish the risk of bioaccumulation, which is even more important the higher the toxicity and the exposure of an IL is. T-SAR (thinking in terms of structure–activity relationships) based prospective design[10] aims at finding and using chemical structures, which fulfil the principles of green chemistry to avoid environmental hazards whilst maintaining an appropriate technical performance, before large-scale industrial applications for ILs are built up.[11,12]

### Aerobic biodegradability of ionic liquids—the main results until now

Following this principle of green chemistry and having stressed the necessity for the determination of biodegradation parameters by Jastorff *et al.* in 2005,[12] the determination of the aerobic biodegradation of ILs has already started. So far, these experiments have been limited to stringent tests for the determination of the ready biodegradability under aerobic conditions. The results indicate that some structural characteristics of ILs enhance or reduce the possibility for an enzymatic cleavage of ILs. One of the main results leads to the conclusion that the biodegradability depends on the length of the alkyl side chain of the cationic head group. The longer the alkyl side chain ($>C_6$) the better the biodegradability is.[13-15] However, this effect does not seem to be endlessly valid due to an increasing toxicity to microorganisms with increasing alkyl side chain length.[16]

[a]*UFT - Centre for Environmental Research and Sustainable Technology, Department 3: Sustainable Chemistry, University of Bremen, Leobener Straße, D-28359, Bremen, Germany. E-mail: stefan.stolte@uni-bremen.de*
[b]*MPI - Max-Planck-Institute for Marine Microbiology, Celsiusstraße 1, 28359, Bremen, Germany*
[c]*UFT - Centre for Environmental Research and Sustainable Technology, Department 4: Chemical Engineering - Recovery & Recycling, University of Bremen, Leobener Straße, D-28359, Bremen, Germany*
[d]*Merck KGaA, Life Science Solutions, Frankfurter Straße 250, D-64293, Darmstadt, Germany*

 

Functional groups, such as amides or esters, were introduced into the alkyl side chain of ILs to make this structural element more susceptible to enzymatic cleavage. The amide functionalised side chains showed no improved biodegradability, whereas the incorporation of an ester group could enhance the biodegradability of the alkyl side chains.[17,18]

Headgroup molecules, such as N-substituted imidazole derivatives, are in general supposed to be not readily biodegradable as it could be shown for, *e.g.*, 1-methyl-, 1-vinyl-, 4-nitro imidazole.[19] For imidazolium based ILs, this observation could be confirmed.[14] Whereas, for pyridinium rings combined with longer alkyl side chains[13] and for 1-undecyloxymethyl-3-hydroxypyridinium saccharinate full mineralisation could be found.[20]

For the determination of the biodegradability of ILs, it is furthermore important to consider not only IL cations but IL anions, too. Biodegradable anions are usually organic compounds such as octyl sulfates,[18] acetate and naphthenic acids like 3-cylcohexylpropionate.[21] For inorganic anions, like $BF_4^-$ or $PF_6^-$, which cannot serve as a carbon source in microbial degradation, other degradation processes might be relevant, *e.g.* hydrolysis or photolysis.[9]

### Exploring further metabolic pathways

These former studies have shown that ILs, which undergo primary aerobic biodegradation, do exist, but there is still a large number of ILs that did not show ready biodegradability in the conducted experiments. Among those were short-chain IL cations, such as the 1-ethyl-3-methylimidazolium cation (IM12), which have—although ecotoxicologically preferred—very low biodegradability at the same time. In such a case, a conflict arises between ecotoxicity and biodegradability.[14] One way out of this conflict might be the biodegradation of ILs through other metabolic pathways than the aerobic one. For example, ILs reaching natural surroundings might not only face aerobic, but also anaerobic environmental conditions, *e.g.* in aquifers, eutrophic lakes, soils or sediments. Some microorganisms, which are well-adapted to anoxic milieus, could be able to degrade ILs under these conditions, *e.g.* by anaerobic respiration or fermentation. A combination of both environmental conditions is commonly used for the purification of water in water treatment plants.

### Abiotic degradation

Another way out of the conflict between higher ecotoxicity and lower biodegradability has been examined on a technical level by several authors.[22,23] Advanced oxidation processes (AOPs) shall lead to the oxidation of persistent substances into various break-down products, which are hopefully less toxic than the initial substrate. This technique is mainly used for industrial waste water treatment. It has been shown that ionic liquids can be oxidised electrochemically[14] and by UV photolysis in the presence of hydrogen peroxide[24,25] amongst others. In these recent studies, it has been demonstrated that the oxidation by AOP modifies the imidazolium ring, not the alkyl chain of the molecule. Therefore, a complete degradation could be proven.

### The anaerobic biodegradation pathway

The anaerobic microbial degradation process is applied in water treatment and soil remediation, amongst others. It has already been shown to be successful for a range of substances that are recalcitrant for aerobic biodegradation processes.[26] Examples are the anaerobic biodegradation of highly chlorinated hydrocarbons, such as tetrachloroethane (PCE)[27] and the reductive dechlorination of polychlorinated biphenyls (PCBs) in sediments[28-30] or landfill leachate.[31] Although the latter substance group is partly accessible to aerobic biodegradation, highly chlorinated members, which are not readily biodegradable under aerobic conditions, can be made accessible to aerobic biodegradation by anaerobic pre-treatment.[30]

The aerobic biodegradation pathway has further been shown to be unsuccessful for the decomposition of azo dyes, whereas these substances seem to be biodegradable under anaerobic conditions especially when a second organic substrate is added.[32] Such a co-metabolism or co-oxidation process has been defined as an oxidation in which the substrate is oxidised without using the energy derived.[33] It had been reported that several xenobiotics, which are not easily biodegradable, can be degraded by microorganisms *via* co-metabolism, *e.g.* pesticides,[34,35] nonylphenols[36] and aminoaromatic acids.[37]

Anaerobic biodegradation might also be important for substances that are even more biodegradable under aerobic conditions, *e.g.* linear hydrocarbons and aromatic compounds. Once they are exposed to the environment, they face anaerobic conditions in deep aquifers, soils or sediments.[38,39] Additionally, the formation of metabolites during the biodegradation process has to be considered within a proper hazard assessment. Independent of the toxicity of the parent compound, metabolites exhibit their own, sometimes even higher, toxicity as it has been observed, *e.g.*, for the biodegradation products of nonylphenols.[40]

Although the necessity of biodegradable ILs in the context of water treatment facilities has been emphasised several times,[9,16] attempts to further determine the anaerobic metabolic pathways for ILs have not been reported, yet. The present paper aims to fill this gap concerning the biodegradability of ILs in waste water treatment plants and therefore gives a first insight into the biological degradation in the absence of oxygen and the presence of nitrate (denitrification), and provides further data for an appropriate hazard assessment of ILs.

## Experimental

### Chemicals

Most of the tested ILs were received from Merck KGaA (Darmstadt, Germany), as well as the salts for the mineral salt medium. The synthesis of 1-(8-hydroxyoctyl)-3-methylimidazolium bromide had already been described.[41] Acetonitrile (HPLC grade) was obtained from Fluka (Buchs, Switzerland). Methanol for the HPLC measurements was bought from Acros Organics BVBA (Geel, Belgium).

### Selection of ionic liquids

The ionic liquids used in the present study were selected according to established knowledge gained from the primary

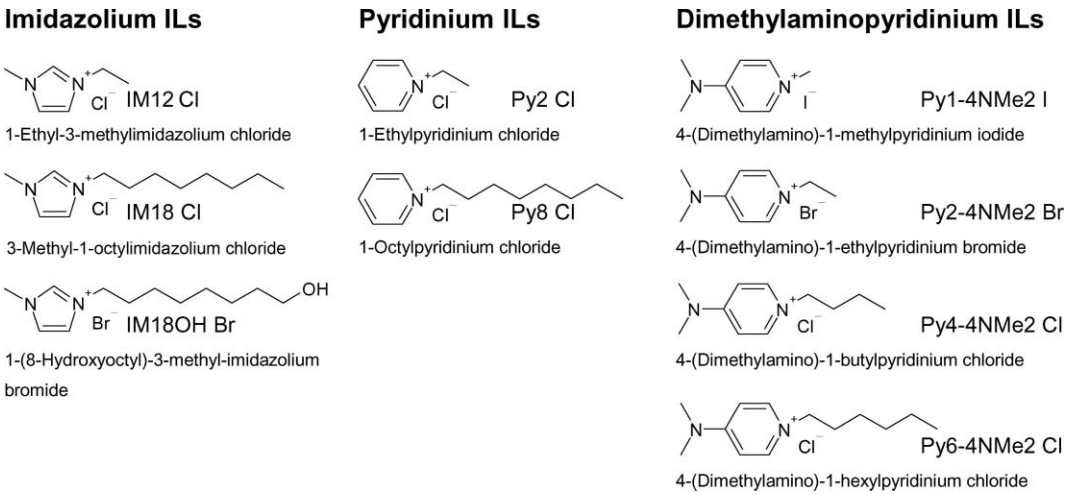


**Fig. 1** Ionic liquid structures, acronyms and names used in this study.



biodegradability testing of ILs in the presence of molecular oxygen.[14] According to these results, the effect of the length of the alkyl side chain, the introduction of functional groups and the effect of different head groups have been systematically determined. Therefore, the following ILs were selected (Fig. 1): (i) Imidazolium ILs: 1-ethyl-3-methylimidazolium chloride (IM12 Cl), 3-methyl-1-octylimidazolium chloride (IM18 Cl) and 1-(8-hydroxyoctyl)-3-methyl-imidazolium bromide (IM18OH Br), (ii) Pyridinium ILs: 1-ethylpyridinium (Py2 Cl) and 1-octylpyridinium (Py8 Cl), (iii) Dimethylaminopyridinium ILs: 4-(dimethylamino)-1-methylpyridinium iodide (Py1-4NMe2 I), 4-(dimethylamino)-1-ethylpyridinium bromide (Py2-4NMe2 Br), 4-(dimethylamino)-1-butylpyridinium chloride (Py4-4NMe2) and 4-(dimethylamino)-1-hexylpyridinium chloride (Py6-4NMe2 Cl). The counter-anions were taken from the group of halides due to their low toxicity and high solubility.

## HPLC systems

The HPLC-UV system used for the specific analysis of ionic liquid cations 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 LC-MS system utilised for the analytical determination of the 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 column (HILIC, Multospher 100 Si - 5 µm, 125 × 4.6 mm) with guard column, both purchased from CS–Chromatographie Service GmbH (Langerwehe, Germany), and a cation exchanger CC 70/4 Nucleosil 100-5SA with guard column from Macherey-Nagel (Düren, Germany) were used. The HILIC column provided a good separation of functional groups, *e.g.* for the determination of metabolites. The cation exchanger showed a better performance and was more robust for samples with a matrix composed of high amounts of organic matter from activated sludge. The latter column was therefore preferred for the huge number of samples from the biodegradation test.

The mobile phase consisted of varying solvent proportions for each substance (Table 1). The solvents were acetonitrile (HPLC grade) and aqueous $K_2HPO_4$ solution. Later the acetonitrile was exchanged with methanol due to a global shortage of acetonitrile, and the analytical methods were adjusted to the new conditions.

The system was operated at a flow rate of 1 mL $min^{-1}$ and 40 °C oven temperature. 10 µL portions of the samples were injected. A detection wavelength of 211 nm was used for quantification of the original compounds based on imidazolium, 254 nm for those based on pyridinium and 288 nm for those based on dimethylaminopyridinium, where they do show maximal absorption.

## Primary biodegradation

The primary biodegradation under denitrifying conditions of the test substances was monitored by specific analysis *via* HPLC-UV for 11 months (328 d). By HPLC-UV measurements, the decrease of concentration of the biodegraded parent compound can be followed. Full mineralisation (generation of $CO_2$, $H_2O$, $N_2$) cannot be determined by this method, but would not occur without the determinable first step of primary biodegradation. Mineral salt medium compositions in anoxic media design are commonly adopted from conventional mineral salt media.[42] Therefore, the used mineral salt media composition was adopted from conventional mineral salt media recipes (*e.g.* OECD guideline 301, DIN EN ISO 14851) as it is typically practised for anoxic media design. Only the phosphate buffer concentration was increased to get a higher buffer capacity for the pH increasing denitrifying process. The final mineral salt medium composition was as follows: phosphate-buffer (3.75 g $L^{-1}$ $KH_2PO_4$ and 8.73 g $L^{-1}$ $Na_2HPO_4 \cdot 2H_2O$) and magnesium and calcium salts (22.5 mg $L^{-1}$ $MgSO_4 \cdot 7H_2O$, 36.4 mg $L^{-1}$ $CaCl_2 \cdot 2H_2O$). As trace element solutions, SL4 and SL6 were used without EDTA. They are listed by the German Collection of Microorganisms and Cell Cultures, *e.g.* in the mineral salt medium N°457. SL4 contains the essential iron ion for microbial growth and SL6 a mixture of essential metals. Ascorbic acid in a non-inhibiting concentration of 0.5 g $L^{-1}$ served as a reducing

 

**Table 1** HPLC methods for selected ILs in the experimental matrix (Flow: 1 mL $min^{-1}$. $T = 40$ °C)

| | CS Multospher 100 Si–5 µm | | | MN CC70/4 NUCLEOSIL 100-5 SA | | |
|---|---|---|---|---|---|---|
| | Acetonitrile | $K_2HPO_{4\,aq}$ | $K_2HPO_{4\,aq}$ Concentration | Methanol | $K_2HPO_{4\,aq}$ | $K_2HPO_{4\,aq}$ Concentration |
| Ionic liquid | in (%) | in (%) | in mmole $L^{-1}$ | in (%) | in (%) | in mmole $L^{-1}$ |
| IM12 Cl | 75 | 25 | 10 | 65 | 35 | 40 |
| IM18 Cl | 80 | 20 | 10 | 60 | 40 | 25 |
| IM18OH Br | 75 | 25 | 10 | 45 | 55 | 25 |
| Py2 Cl | 75 | 25 | 10 | 65 | 35 | 40 |
| Py8 Cl | 80 | 20 | 10 | 60 | 40 | 25 |
| Py1-4NMe2 I | 75 | 25 | 10 | 65 | 35 | 40 |
| Py2-4NMe2 Br | 80 | 20 | 10 | 65 | 35 | 40 |
| Py4-4NMe2 Cl | 80 | 20 | 10 | 65 | 35 | 40 |
| Py6-4NMe2 Cl | 80 | 20 | 10 | 60 | 40 | 25 |



agent for molecular oxygen. The hot autoclaved medium was degassed by a nitrogen stream. Sodium nitrate ($NaNO_3$) was the nitrate source prepared in 775 mmole $L^{-1}$ stock solutions and kept in a refrigerator at 8 °C. The initial concentration of nitrate in the medium was set to 50 mg $L^{-1}$. During the course of the experiment, nitrate was replenished whenever its concentration decreased below 5 mg $L^{-1}$ nitrate nitrogen. When nitrate was replenished, the nitrite concentration had been lower than the detection limit. An accumulation of nitrite would finally be toxic to the inoculum, which was not the case in the conducted experiment. The inoculum used was collected from the activated sludge of the communal wastewater treatment plant in Delmenhorst (Germany) in August 2008 (final concentration of 217 ± 10.5 mg $L^{-1}$ total solids in each sampling vessel). ILs were added as the only carbon source in a final concentration of 200 µmole $L^{-1}$. For the testing of co-metabolism, an equimolar amount of acetate as a second carbon source was added to two further parallel cultures from each IL. The experiment was carried out in 250 mL glass vessels sealed with butyl rubber septa and closed with centre hole caps. The vessels were filled with 200 mL mineral salt medium, 20 mL activated sludge, 2 mL ionic liquid stock solution (20,000 µmole $L^{-1}$) and a nitrogen atmosphere. The samples were kept at room temperature in the dark. 1.3 mL of each sample were taken at each testing day. The pH value was measured for each sample taken with pH indicator strips from Merck in the range of 6.5–10.0 in 0.3 intervals. The sample was centrifuged for 15 min at 14,500 rpm. The nitrite/nitrate concentration was analysed in the centrifugate by nitrite/nitrate testing strips from Merck (Nitrate-Test/Nitrite-Test Merckoquant®). The final specific analysis of the ionic liquid cation was then conducted by HPLC-UV measurements. An internal standard for each substance was run during each measurement HPLC-UV sequence to identify the IL peak and check the consistency of the analytical method. The internal standard was a defined amount of each analyte. The sampling and the addition of nitrate changed the total volume of the samples and thus the concentration of ILs. The measured concentration was therefore corrected to the calculated volume. The correction could reach a change between 2 to 8% of the detected concentration depending on the amount of samples taken and solution added.

The samples were taken on the initial day of the experiment after inoculation and in shorter intervals at the beginning (2-3 d). After two weeks the sampling was reduced to once a week, and after 60 d the samples were taken once a month. After half a year, the vessels were left for five months until the last samples were taken. All in all, two parallel cultures were run for each testing substance and two for each co-metabolism test.

To be able to evaluate the biodegradability of ILs appropriately, additional control samples had been set up without the testing substances: (1) the blank sample (inoculated sample without testing substance) to control the ground noise and additional peaks from contaminations, (2) a positive control to observe the microbial activity, as the positive control served the easily biodegradable acetate. Microbial activity could be measured by pressure increase and pH increase.

## Results and discussion

### Toxicity and adsorption

The decrease of IL concentration was used as an indicator for biological degradation. A toxic effect of the used IL itself could be excluded. In the control vessels with acetate and the testing IL in a concentration range of 200 µmole $L^{-1}$, the microbial activity has been determined by the production of nitrite, depletion of nitrate/nitrite and the increase of the pH-value. No significant toxic effect towards the microbial community has been observed for any of the examined ILs. This means that concentration stability of the tested IL cannot be referred to the toxicity of the IL to the inoculum but to its non-biodegradability under these experimental conditions.

Adsorption of the test substance on activated sludge or the glass vessel surface is another influence on the IL concentration in the sample vessels, which could lead to a false interpretation of the results. If adsorbed, the concentration of the IL would decrease although no microbial activity was involved. Therefore, the adsorption of ILs should have been tested by the addition of $HgCl_2$. The concentration difference between the toxified samples and the samples with microbial activity is seen as the amount of adsorbed IL. However, the bacteria were strongly resistant to the applied concentration of 250 mg $L^{-1}$ $HgCl_2$. A higher concentration of $HgCl_2$ or the addition of $NaN_3$ might increase a toxic effect and might make a study on the adsorption of IL possible. In this study, it was not further examined experimentally, because in this case, the concentration

of the used ILs did not show any significant decrease of concentration during the first three days, which could be related to an adsorption on activated sludge or the glass vessel surface.

Considering these facts, any decrease in concentration of the test compounds after three days was considered as evidence for biological degradation.

### Primary biodegradation and metabolisation

The concentrations of the tested imidazolium, pyridinium and dimethylaminopyridinium cations (Fig. 1) remained stable during the testing period of 328 d, except the concentration of the hydroxylated imidazolium cation (Fig. 2). Therefore, most of the tested ILs were declared as not biodegradable under the experimental conditions. The addition of excess acetate did not help to decrease the measured IL concentration. Thus, the occurrence of a significant co-metabolic process with this substrate is excluded in the conducted experiment (results not shown). A decrease of concentration could only be observed for the 1-(8-hydroxyoctyl)-3-methyl-imidazolium cation (IM18OH). Around 52 to 54% of this substance was already degraded after 9 days. After 34 and 41 days, respectively, no more IM18OH could be detected in the parallel cultures.

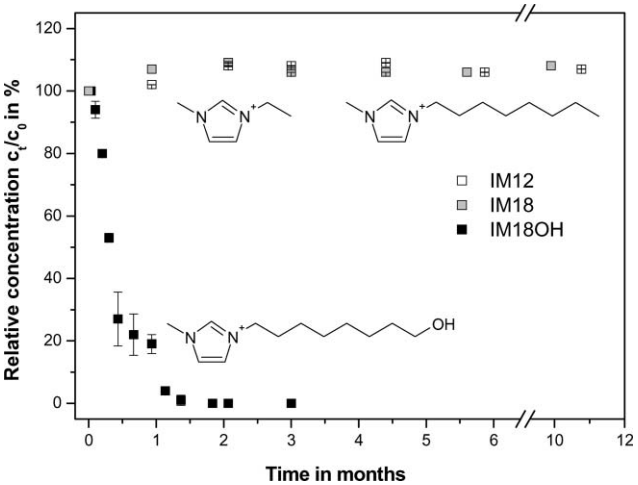


**Fig. 2** Mean values with standard abbreviations of the relative concentrations of IM12, IM18 and IM18OH over time ($n = 2$). They are used as examples for the observed results of the investigated imidazolium, pyridinium and dimethylaminopyridinium ILs. The examined ILs were not biodegradable except IM18OH. 1 month = 30 days.

A closer look at the IM18OH samples by MS measurements revealed the structural changes during the observed degradation period. At the beginning of the experiment, the original IM18OH cation (211 $m/z^+$) could be detected in the corresponding samples (Fig. 3a). In the early phase, a decrease of IM18OH cation has been observed. Substances with a mass-to-charge-ratio of 225 and 197 in the positive mode are now found (Fig. 3b). In the last samples taken, the former observed peaks cannot be detected any more, but a substance with a mass-to-charge-ratio of 169 is found instead (Fig. 3c). The identity of these mass-to-charge ratios was further analysed by MS-MS measurements. For the mass-to-charge-ratio 169, 197 and 225, the 1-methyl-imidazolium fragment (83 $m/z^+$) could be detected. It is characteristic for imidazolium based cations. Therefore, the detected values presumably belong to the biological transformation products 1-(7-carboxyheptyl)-3-methyl-imidazolium cation (IM17COOH), 1-(5-carboxypentyl)-3-methyl-imidazolium cation (IM15COOH) and 3-(3-carboxypropyl)-1-methyl-imidazolium cation (IM13COOH).

The observed metabolites are the expected products when the alkyl side chain is biodegraded *via* β-oxidation (Fig. 4), which has already been observed for the aerobic metabolism of IM18OH.[14]

Comparing the biodegradation of IM18 cation and IM18OH cation (Fig. 4), one can conclude the predominant degradation mechanism. In aerobic biodegradation processes, the initial oxidation of the octyl side chain of the IM18 cation probably involves molecular oxygen as a reactant. The oxygen is inserted by monooxygenase and the alkyl side chain can be further degraded *via* β-oxidation. Under anaerobic conditions, no molecular oxygen can be inserted by monooxygenase and the biodegradation is not initialised. In contrast, the pre-oxygenated IM18OH can be biodegraded under anaerobic conditions (Fig. 4). However, the present experiments denote no full mineralisation of IM18OH or only a very slow biodegradation under the experimental anaerobic conditions. A residual rest of IM13COOH was still detected even after 10 months of the experiment (Fig. 3c). A similar behaviour had been observed for the imidazolium based ILs under aerobic conditions.[14]

All in all, it can be concluded that the head group of the IL cations does not seem to be the target of the initial enzymatic attack under aerobic as well as anaerobic conditions.

These observations of the formation of metabolites during the biodegradation process are to be considered within a proper hazard assessment. In general, a connection between toxicity and lipophilicity of the chemical substances has been discovered in recent studies.[41] Those ILs, which are less lipophilic, are in general less toxic than those with longer alkyl side chains. The majority of detected metabolites during the present biodegradation process already underwent *in vitro* screening toxicity tests on enzyme inhibition (acetylcholinesterase) and cytotoxicity (IPC-81 leukemia cells) for a first rough toxicity assessment (Table 2).

The results show that the transformation of alkyl side chains of the cationic head group to shorter alkyl side chains containing carboxy or hydroxy groups is advantageous in respect to its reduced toxicity towards cells[12] and aquatic organisms.[43] However, it cannot be excluded until now that other reactive species, such as epoxides, might be generated during transformation. In general, for a profound hazard assessment further tests are necessary. The investigated ILs substituted with alkyl side chains

**Table 2** $EC_{50}$-values of ionic liquids: the substrate IM18OH and its detected metabolites in anaerobic biodegradation. The values demonstrate a reduced toxicity of the initial substrate in comparison to its detected metabolites

| | $EC_{50}$-values in μmol $L^{-1}$ | |
|---|---|---|
| Ionic Liquid | Acetylcholinesterase inhibition[44] | IPC-81 leukemia cells cytotoxicity[41] |
| IM18OH Br | 19 | 229 |
| IM17COOH Br | > 1000 | > 3020 |
| IM15COOH Br | No data available | No data available |
| IM13COOH Cl | > 1000 | > 3020 |

 

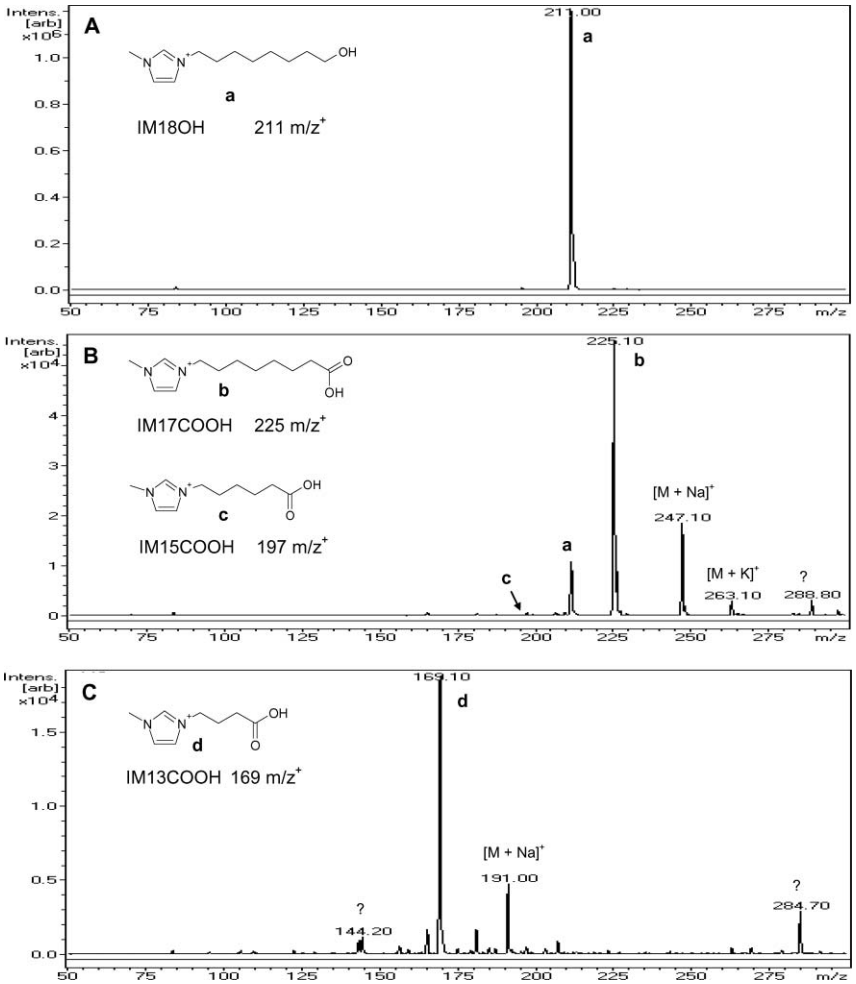


**Fig. 3** Mass spectra of the IM18OH samples from the anaerobic biodegradation process at the beginning (A), in the early phase (B) and after 318 days (C). Substrate: IM18OH Br (211 $m/z^+$). (a) Initial peak with a mass-to-charge ratio 211 $m/z^+$ belonging to the substrate IM18OH Br, (b) 1-(7-carboxyheptyl)-3-methyl-imidazolium cation (IM17COOH; 225 $m/z^+$), (c) 1-(5-carboxypentyl)-3-methyl-imidazolium cation (IM15COOH; 197 $m/z^+$), (d) 3-(3-carboxypropyl)-1-methyl-imidazolium cation (IM13COOH; 169 $m/z$); $[M + Na]^+$ and $[M + K]^+$ are quasi-molecular ions. The sodium and potassium probably come from the mineral salt medium. Other unidentified peaks '?' appear in a lower concentration than the molecular ion.

were not transformed, and therefore, might not be biodegradable during the denitrification in waste water treatment plants, soils *etc.* Nevertheless, anaerobic biodegradability is still possible, if other bacteria exist that are able to degrade ILs under those conditions. For example, the anaerobic biodegradability of hydrocarbons was not discovered until at the end of the last century bacteria were found that are able to degrade these compounds. It depended on the discovery of a new initialisation step, by which the anaerobians can biodegrade the hydrocarbons with the help of fumarate.[39,45-49] A similar initialisation step for the anaerobic biodegradation of ionic liquids is still to be found.

## Conclusion

The biodegradation of ILs under denitrifying conditions has been examined for the first time. It is concluded from the present study that the experimental process does not seem to be a solution to remove the examined alkyl substituted ILs efficiently from the environment. It is assumed that these IL cations cannot be eliminated in the denitrification step of a waste water treatment plant. They might reach the environment if they are not or slowly biodegradable under aerobic conditions or adsorb on activated sludge during water treatment. Therefore, in structural IL design, one cannot rely on anaerobic biodegradation of imidazolium, pyridinium and dimethylpyridinium ILs with an alkyl side chain at the moment. Nevertheless, the hydroxylated imidazolium IL does show activity of primary biodegradation under the experimental denitrifying conditions. Only its headgroup with a short carboxy alkyl side chain remained even after 318 days of the experiment. Although no metabolic pathway that might degrade the imidazolium IL headgroup had been found so far, it might still be advantageous to insert terminal hydroxy groups into long chained ILs either prospectively by structural design or retrospectively through chemical oxidation. Thereby, ILs can be made available to a wider range of microorganisms in oxic and anoxic milieus, but it should also be taken into consideration that such a chemical modification might reduce the industrial applicability. Furthermore, the option to find microorganisms, which are able to degrade ILs, still remains among the huge variety of

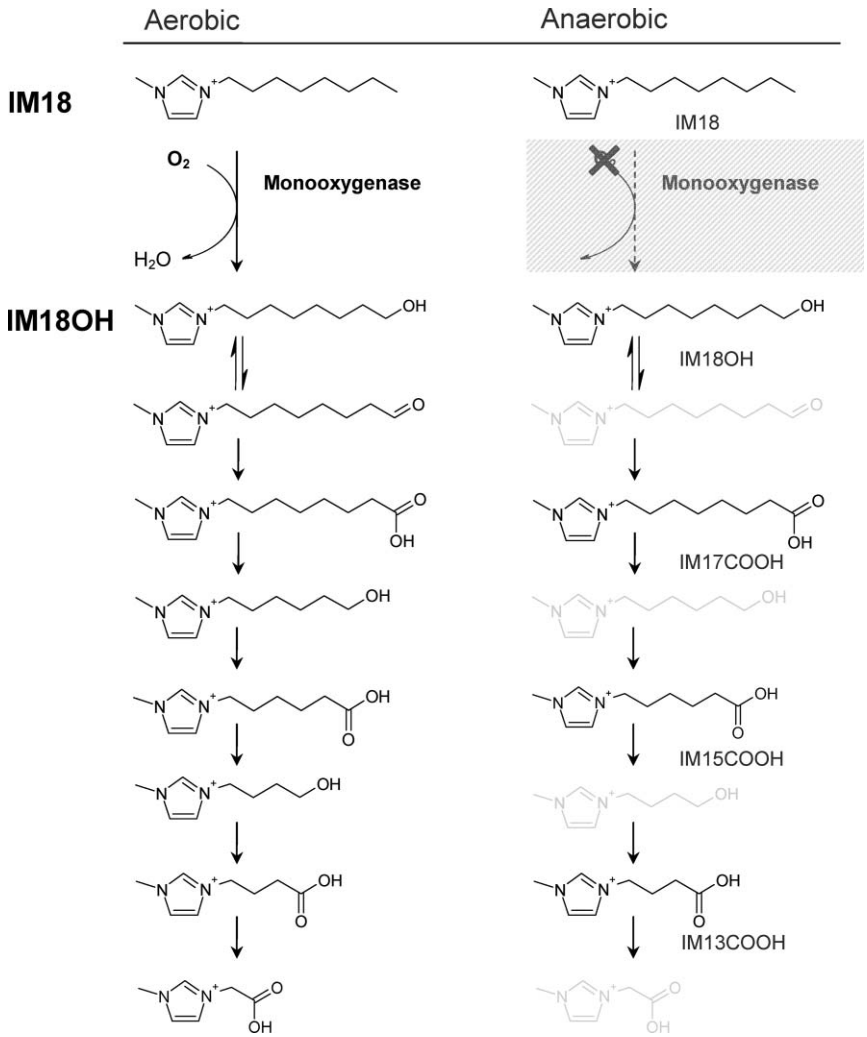


**Fig. 4** Simplified scheme of the proposed aerobic[14] and anaerobic metabolic pathway for 3-methyl-1-octylimidazolium chloride (IM18 Cl) and 1-(8-hydroxyoctyl)-3-methyl-imidazolium bromide (IM18OH Br). Without oxygen the anaerobic biodegradation of the alkyl side chain of IM18 is not initialised. The chemical structures in black were found by MS measurements and are further indicated with their acrynoms. The ones in grey are theoretical intermediates.

microbial life. The biodegradation of ILs by obligate anaerobes, for example, still needs to be investigated.

All in all, we expect the described findings to fill the gap in the hazard assessment of ILs and to contribute to a structural IL design that favours sustainable application of ionic liquids.

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