Chem Soc Rev

REVIEW ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Soc. Rev.*, 2015, 44, 8200

Received 3rd June 2015

DOI: 10.1039/c5cs00444f

www.rsc.org/chemsocrev

Biodegradation of ionic liquids – a critical review†

Andrew Jordan[a] and Nicholas Gathergood*[b]

The importance of biodegradation data as part of the design of safer chemicals is presented using ionic liquids (ILs) as a model study. Structural features that promote/impede IL biodegradation, IL design strategies, methods of biodegradation analysis, properties of IL/surfactant derivatives and computational methods of predicting biodegradation are discussed. The importance of metabolite studies as part of biodegradation assays is highlighted. The relevance of applying the lessons learned developing biodegradable ILs to other chemical classes is proposed. A comprehensive appendix of IL biodegradation data published since 2010 (~300 ILs) has been compiled.

1. Introduction

Are ionic liquids (ILs) green? Do IL based technologies offer an advantage over current best practice? What performance and green chemistry metrics do you use to substantiate these claims? These are questions frequently asked to research groups that promote ILs as a preferred solution to existing problems. Indeed, any new methodology which considers green chemistry principles

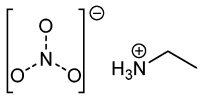
[a] *School of Chemical Sciences, Dublin City University, Collins Avenue, Dublin 9, Ireland*
[b] *Department of Chemistry, Tallinn University of Technology, Academia tee 15, 12618 Tallinn, Estonia. E-mail: nicholas.gathergood@ttu.ee*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5cs00444f

as core values, in our opinion, should undergo the same rigorous critique and debate. This review does not assess the merits of using ILs but rather focuses on one property, biodegradability. The use of persistent or non-biodegradable chemicals, especially as solvents, should be avoided, where suitable biodegradable alternatives exist. This review details the progress made by the IL community in their endeavours to invent biodegradable compounds.

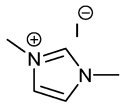
In the field of ILs, substantial effort to evaluate the biodegradation properties of a wide range of derivatives has been expended. This data can be included in the overall evaluation and selection of a specific IL for an application. Over the last 15 years various terms have been used to define ILs (<100 °C m.p.) and their properties; non-volatile, robust, inert, stable to a wide electrochemical window are just a few. Of concern is the apparent 'desirable' stability or resistance to breakdown, especially when initiating a research program to develop biodegradable ILs. However, as we delve more deeply into this fascinating research area, exceptions to these previously widely adopted "classifications" have been found, for example, the negligible vapour pressure has been challenged by Rebelo *et al.* (2005),[1] and Earle and Seddon *et al.* (2006)[2] in which distillation of ILs with minimal decomposition was demonstrated. The non-flammability property has also been questioned as some ILs have been reported by Rogers *et al.* (2006) as being Class IIIB combustible materials.[3] We ask ourselves the question 'Is it possible to design an IL which is stable to a wide range of thermal and chemical conditions yet also biodegrades?' Can this, at first inspection, apparently mutually exclusive conundrum be solved?

Although ILs have been known for over 100 years, it has only been in recent times that chemists have studied their biodegradation in detail. Many papers credit Walden (1914)[4] or some go even further back to Gabriel and Weiner (1888)[5] with their reported synthesis of ethylammonium nitrate, (Fig. 1). However the first conventional example the authors could find of



1

Fig. 1 One of the first ILs, ethylammonium nitrate.

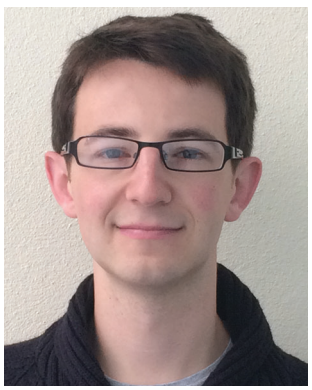


2

Fig. 2 1-Methyl-3-methylimidazolium iodide.

an imidazolium IL goes back even further to 1881 with the synthesis of 1-methyl-3-methylimidazolium iodide by Goldschmidt[6] see Fig. 2. Biodegradation studies as part of the characterisation of these ILs would have been unheard of in those times.

It is our ability to update the definitions, to reclassify and to reinvent design strategy that will underpin continuing success in the field of IL research. Guidelines that have steered the field of IL research over the past two decades have been defined by landmark works such as the creation of the twelve principles of green chemistry by Anastas and Warner.[7] Other examples of concepts that promote cleaner synthesis are atom economy (AE) by Trost,[8] Sheldon's E-factor,[9] and the Andraos reaction mass efficiency (RME).[10] It is by implementing these principles that green chemists strive to develop their target molecules, monitoring their progress using green chemistry metrics. However, clean synthesis is not the only area that needs stringent protocols in the green chemistry research process. What happens to the molecules when they are ultimately released into the environment is also of paramount importance. The parameters of biodegradability, toxicity – and recently mutagenicity[11] – are becoming more significant.



Andrew Jordan

Andrew Jordan completed his undergraduate degree in Chemical and Pharmaceutical Sciences in Dublin City University, receiving a BSc (Honours) in 2008. He is currently in the final year of his PhD in Dublin City University under the supervision of Prof. Nicholas Gathergood from Tallinn University of Technology. His current research focuses include organic chemistry and green chemistry with particular interest in the design of biodegradable ionic liquids and surfactants. Andrew was awarded the best short oral presentation at the Congress of Ionic Liquids in South Korea, June 2015 for his work towards readily biodegradable ionic liquids.

Nicholas Gathergood

Nicholas Gathergood received his PhD in 1999 from the University of Southampton, UK. Postdoctoral research with Prof. K. A. Jørgensen, Aarhus University, Denmark and Prof. P. J. Scammells, Victorian College of Pharmacy, Monash University, Australia, followed. In 2004, he was appointed as a lecturer of organic chemistry at Dublin City University, Ireland. Nicholas moved to Tallinn University of Technology, Estonia in 2015 as ERA Chair of Green Chemistry and is establishing a new team with interests in ionic liquids, surfactants, catalysis, biorenewables, drug discovery, green toxicology and biodegradation. He enjoys the winter in Estonia, especially building snowmen and making snow angels with his family.

Wider adoption of ILs by industry and in academia, *e.g.* BASIL process, biomass dissolution,[12] metal salt dissolution,[13] lubrication,[14] surfactants,[15,16] catalysts,[17–19] reaction solvent,[20] has been observed in the past decade. As the potential for (large scale?) accidental release becomes a possibility, environmental impact studies, including biodegradation are increasingly important.

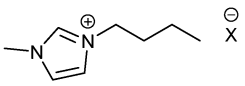
In this review, it is our intention to illustrate the advances, successes and problems encountered in the field of biodegradation of IL research since the preceding publication by Coleman and Gathergood which covered the literature up to 2010.[21]

2. Biodegradation – the 10th Principle of Green Chemistry

Are biodegradation studies important in the context of green chemistry and ILs? Biodegradation is one method of analysis to determine and predict how a molecule (*e.g.* IL) interacts with the environment. The overarching aim is to avoid the introduction of persistent molecules, including persistent organic pollutants (POPs). The issue of environmental persistence of chemicals (including surfactants, antibiotics and solvents), once they have fulfilled their desired application, has been widely studied with detrimental effects on ecosystems observed.[22] Environmental damage ranges from endocrine disruptors against fish[23] to proliferation of bacterial resistance towards important active pharmaceutical ingredients (APIs).[24] Biodegradation is an essential parameter to investigate in ILs, enabling the design of safer analogues where required, reducing environmentally persistent molecules in ecosystems. In 1998 Anastas and Warner highlighted the importance of biodegradation studies for chemists, selecting this as their 10th Principle of Green Chemistry – Design for Degradation.[7]

Design strategies to improve biodegradability of compounds predate Anastas and Warner's green chemistry principles. Seminal work by Boethling *et al.*[25,26] includes the highly cited "rules of thumb" which relates structural features and functional groups to biodegradation properties. These rules have been adopted and applied to IL research over the last decade.[21] Boethling's "rules of thumb" propose that a number of factors affect the percentage of mineralisation of chemical compounds by microbial degradation. Beneficial factors include: the presence of aromatic rings, long non-substituted alkyl chains, and hydrolysable groups such as alcohols, aldehydes and carboxylic acids.[25,26] Factors that are detrimental to biodegradation include: the presence of halides in the molecule, alkyl chain branching, quaternary carbon atoms, tertiary nitrogen atoms, heterocycles and aliphatic ethers. These "rules of thumb" are not absolutes, indeed caution must be exercised when applying these rules in the absence of biodegradation data of closely related structures, and they only suggest general trends in biodegradation pathways, as clearly stated by Boethling in his work.[25,26]

Early generations of ILs, such as the frequently quoted second generation ILs, the butyl-methylimidazolium [bmim] class (Fig. 3), were designed to be robust, liquid at room temperature (RT) and



3, X = BF_4
4, X = PF_6

Fig. 3 [bmim] ILs.

inert to a range of chemical conditions thus enabling a broad applicability. For example, the [bmim] series was envisaged as a replacement for VOC electrolytes due to their inherent electrostability, large electrochemical window,[27] thermal stability,[28] low vapour pressure and low/non-flammability.[29] Applications of these stable and low vapour pressure replacements for electrolytes were demonstrated in batteries and capacitors.[30] However, it was this lack of reactivity that lead to concern that the molecules could be recalcitrant to biodegradation, with limited breakdown in the environment.[21] Consideration of Boethling's "rules of thumb" also suggests poor biodegradability for these classes of ILs and biodegradation data to confirm the suspicions about this class of compounds was lacking as we entered the new millennium. As biodegradation data for [bmim] ILs began to be experimentally determined, it was found to be in agreement with predictions based on Boethling's work. [Bmim][BF_4] (**3**), amongst other early examples of 1-methylimidazolium ILs, did not pass readily biodegradable tests, demonstrating the urgent requirement for further work and advances in this area.[21,31,32]

Recent studies conducted by Mao *et al.* further highlight the importance of environmental studies associated with the second generation of ILs, when [bmim][PF_6], Fig. 3, (**4**) was shown to proliferate and disseminate antibacterial resistance genes in environmental bacteria, emphasising the need for biodegradability to be a key requirement in future IL generations.[33]

3. Biodegradation – standardised tests

The following section provides an overview of the biodegradation tests which are of interest and available to IL researchers. The research area of biodegradation is categorised into the following terms:

(1) Primary biodegradation – the loss of a specific structural moiety, example hydrolysis of an ester bond

(2) Inherently biodegradable – if a compound biodegrades ~20% then the possibility of further degradation is assumed

(3) Readily biodegradable – biodegrades a specific % within a given timeframe

(4) Ultimately biodegradable – complete breakdown of a compound

(5) Mineralisation – decomposition of a compound into molecules available to plants[34]

According to the Organisation of Economic Co-Operation and Development (OECD) and the International Organisation for Standardisation (ISO), there are a number of accepted methods that, if implemented correctly, will provide definitive

biodegradation data.[34] The testing strategy suggested by the OECD is thus:

(1) Examine the aerobic biodegradation to assess if a chemical is readily biodegradable.

(2) If a test for readily biodegradable is failed then the chemical of interest can be examined by other simulation tests (optimised aerobic conditions representing conditions potentially found in a sewage treatment plant) to determine biodegradability. An inherent biodegradability test can also be performed.

(3) Finally, potential biodegradability can be determined under anaerobic conditions.[34]

The tests methods currently supported by the OECD are as follows:[34,35]

3.1 Fresh water

Due to the increased size of the oxygen reservoir in the setup of the ISO 10708 bottle compared to the method described by the OECD 301D, Table 1, a higher test substance concentrations can be used in the ISO 10708 test as the amount of oxygen in the bottle is less of a limiting factor.

3.2 Inherent biodegradation

Unlike the readily biodegradable tests described previously in Table 1, (OECD 301A–D), the inherent biodegradation test has no pass/fail parameters, Table 3. If biodegradation is measured to be above 20% biochemical oxygen demand (BOD) or chemical oxygen demand (COD) or dissolved organic carbon (DOC) then it can be assumed that a chemical can inherently undergo primary biodegradation. If biodegradation of more than 70% is achieved then it is possible that a chemical is inherently capable of undergoing ultimate biodegradation.

3.3 Aerobic sewage – simulation tests

Similar to OECD 302, using a sewage treatment plant simulation test OECD 303, Table 4, no specific limits for pass/fail have been prescribed. This is because the specific operating conditions of every treatment plant are different. The test does however provide an estimate on potential removal of a chemical compound in a sewage treatment plant, thus the quantity of compound that can be released into the environment post treatment can be assessed.

Table 1 OECD testing guidelines no. 301

Test	Pass level after 28 days
Dissolved organic carbon (DOC) die-away test (TG 301A)	70% DOC removal
CO_2 evolution test (TG 301B)	60% $ThCO_2$
Modified MITI test (I) (TG 30C)	60% ThOD
Closed bottle test (CBT) (TG 301D) ISO 10708[a]	60% ThOD
Modified OECD screening test (TG 301E)	70% DOC removal
Manometric respirometry test (TG 301F)	60% ThOD

Note: These pass levels must be achieved within a ten day window within the 28 day test limit. The ten day window begins once 10% DOC (dissolved organic carbon), $ThCO_2$ (theoretical CO_2) or ThOD (theoretical oxygen demand) has been achieved. [a] The ten day window is not applied for ISO 10708.

3.4 Seawater

The biodegradation in seawater test OECD 306, Table 5, differs from the standard 301 tests in that the only microorganisms present are those found naturally in the seawater test media. The flask is not charged with additional inocula, although it is supplemented with nutrients. This test is not intended to represent a marine environment but rather assess biodegradation in seawater media.

3.5 Soil, sediment and water

Due to the inherent complexities of using a solid medium for biodegradation and the use of radio labelled atoms there are a number of parameters suggested by the OECD that can be used to monitor the fate of chemical compounds in a soil or sedimentary environment.

- First order or pseudo-first order rate constant for biodegradation kinetics
- Degradation half-life (DT_{50})
- Half-saturation constant
- Maximum specific growth rate
- Fraction of mineralised ^{14}C, and, if specific analyses are used, the final level of primary degradation
- Identification and concentration of major transformation products – if radio labelled compounds are used

One other prescribed test for inherent biodegradation of chemical compounds in soil exists and this is the OECD TG 304A. The test however requires use of ^{14}C labelled compounds. A sample of ^{14}C treated compound is introduced into the test medium. The evolution of $^{14}CO_2$ is then monitored using alkali absorption and a liquid scintillation counter.

3.6 Anaerobic biodegradation

The TG 311 test, Table 8, outlines a method of measuring biodegradability in an aerobic environment. However, TG 311 is limited as it only assesses biodegradability in anaerobic digesters and does not take into account other anaerobic biodegradation pathways possible in different environmental compartments. The test measures gas evolution with a band of 75–80% of theoretical gas production being used as a sign of complete anaerobic biodegradation.

As can be seen from Tables 1–8, there exist a number of techniques for determining biodegradability with a wide breadth of conditions and scenarios covered. The test methods aforementioned are only approximations of the conditions that exist in the extremely complex and dynamic conditions present in WWTP's and the greater environment and can never truly simulate these conditions. However, if they are treated as tools with which to gain insight into the potential environmental breakdown

Table 2 OECD testing guidelines no. 310

Test	Pass level after 28 days
CO_2 headspace test (OECD 310) ISO 14593[a]	60% $ThCO_2$ evolution

[a] The ten day window is not applied for ISO 14593.

Table 3 OECD testing guidelines no. 302

Test	Pass level
Modified semi-continuous activated sludge (SCAS) test (TG 302A)	BOD, DOC and/or COD; no pass levels assigned
Zahn–Wellens/EMPA[a] test (TG 302B)	BOD, DOC and/or COD; no pass levels assigned
Modified MITI[b] test (II) (TG 302C)	BOD, DOC and/or COD; no pass levels assigned
Concawe test (draft TG 302D)	BOD, DOC and/or COD; no pass levels assigned

[a] EMPA: Swiss Federal Laboratories for Materials Testing and Research. [b] MITI: Ministry of International Trade and Industry, Japan.

Table 4 OECD testing guidelines no. 303

Test	Pass level after 28 days
Aerobic sewage treatment: activated sludge units (TG 303A) and biofilms (TG 303B).	DOC and/or COD no pass levels assigned

Table 5 OECD testing guidelines no. 306

Test	Pass level after 28 days
Biodegradability in seawater (TG 306) Shake flask and closed bottle variants	>70% DOC removal >60% ThOD

of chemicals, then this information can be used as part of an environmental impact assessment as well as assisting in the rational design of future generations of chemicals.

Currently a number of different methods have been employed to induce degradation (biotically and abiotically) in molecules, these include altering the temperature of the degradation vessel, inoculating the test media with a specific bacteria, degradation in an advanced oxidation system,[36] degradation in an electrochemical system[37,38] and ultrasonic degradation.[39] The overall aim is to possibly develop a system for treating wastewater before release into the environment.

3.7 Biodegradation metrics

Currently biodegradation data is categorised by whether a compound passes or fails a test however more recently a visual traffic light metric system has been applied by Gathergood *et al.*[17] to give a colour coded representation of how biodegradation levels compare.[17] The levels of biodegradation are assigned a colour based on the following criteria:

- Green: ≥60% readily biodegradable
- Amber: 20–59%
- Red: 0–19%

This colour coding system allows for libraries of compounds to be compared and categorised visually and with ease. Examples of the system in use will be discussed later in this review.

3.8 Biodegradation in soil, compost and other matrices

3.8.1 Biodegradation in soil. Biodegradation of imidazolium-based ILs in soil has been previously reported by Modelli *et al.*[44] Four ILs [bmim][BF_4] (**3**), [bmim][$N(CN)_2$] (**5**), [$MeOCH_2CH_2$mim][BF_4] (**6**) and [$MeOCH_2CH_2$mim][$N(CN)_2$] (**7**), Fig. 4, were examined for their biodegradability according to the ASTM D 5988-96 protocol. In this protocol a pass/fail mark is not assigned and biodegradation is merely observed. Under the test conditions the test compound is mixed with soil and placed in a large glass vessel with CO_2 evolution regularly measured. Soil can be a species rich mixture but it is expected that the activity will be less than an activated sludge so the test is run over a 6 month period. In this particular study it was found that the linear alkyl [bmim][BF_4]

Table 6 OECD testing guidelines no. 307–309

Test	Test duration and parameters
Aerobic and anaerobic transformation in soil (TG 307)	>120 days testing period – radio labelled and unlabelled compounds can be used
Aerobic and anaerobic transformation in aquatic sediment systems (TG 308)	<100 days testing period – radio labelled and unlabelled compounds can be used
Aerobic mineralisation in surface water – simulation biodegradation test (TG 309)	<60 days testing period – radio labelled and unlabelled compounds can be used

Table 7 Other non-OECD test guidelines for aerobic biodegradability of plastics in soil

ASTM D 5988-96 Standard test method for determining aerobic biodegradation of plastic materials in soil	No pass or fail mark assigned – test method only. Degradation measured by CO_2 present in a trapping solution by titration, over a 6 month period.
ISO 17556 Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved	ISO method biodegradation can be determined by CO_2 evolution or O_2 demand

Table 8 OECD testing guidelines no. 311

Test	Pass level after 60 days
Anaerobic biodegradability of organic compounds in digested sludge/method by measurement of gas production (TG 311).	75–80% theoretical gas evolution = complete anaerobic biodegradation

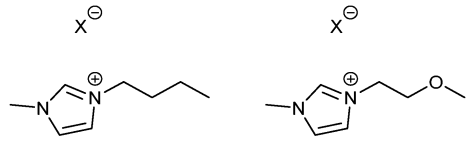


Fig. 4 Imidazolium ILs examined by Modelli *et al.*[44]

example underwent degradation of 52.1 ± 6.6% with the $N(CN)_2$ derivative being less biodegradable than the halide, producing 17.0 ± 4.2% degradation. The oxygenated [$MeOCH_2CH_2$mim] derivatives both failed to degrade, producing negligible biodegradation results of 0.1 ± 4.0%.

3.8.2 ILs – interactions with soil, compost and other matrices. It is of concern that ILs could reach concentrations high enough in industrial waste water treatment plants (WWTPs) to affect their operating conditions. As outlined in a publication by Stolte *et al.* in 2013, it is believed that ILs will never reach a concentration significant enough to inhibit the bacteria in a domestic WWTP, however due to the toxic effects of some ILs, inhibitory effects on a WWTPs efficacy could be foreseen.[40] Some ILs possess the ability to adsorb onto sewage flocs, this can actually be beneficial in a WWTP environment as it can potentially reduce the concentration of ILs in ''solution'' and therefore reduce the toxic inhibitory effects, if indeed the ILs are toxic.[41] The ILs partition coefficients and adsorption coefficients however plays a further role in the ILs interaction with soil, *vide infra*. Whether WWTPs treatments are enough to remove residual ionic compounds is also questionable as demonstrated by Gomez *et al.* in 2011 where ionic and non-ionic surfactant molecules in $\mu g\ L^{-1}$ concentrations were detected in drinking-water treatment plants, including plants with advanced tertiary treatment plans.[42] This observation poses the question of whether ILs will behave similarly due to their comparable structural features to ionic surfactants and further justifies the research employed in designing for degradation. Recent evidence for some 1-methylimidazolium IL derivatives provided by Gendaszewska *et al.*[43] demonstrated, using the OECD 302B test, the degree of adsorption of a number of ILs in an inoculum. It was observed that more lipophilic derivatives adsorbed to the inoculum (~3.5%) whereas the highly polar derivatives did not. SDS was adsorbed at a ~35% level.

The interactions of ILs in soils have been examined in a number of recent publications.[44–48] The ability of ILs to interact with a soil and adsorb to this matrix poses a number of problems. If the ILs are not biodegradable, will they adsorb to the soil indefinitely and if so what are the potential consequences and outcomes of these interactions? Furthermore, if the ILs adsorption profile allows it to eventually elute from the soil can the IL then enter the aquifer?

A number of studies have been conducted on ILs and their interactions with soil, bacteria and plant life with some generalisations reported:

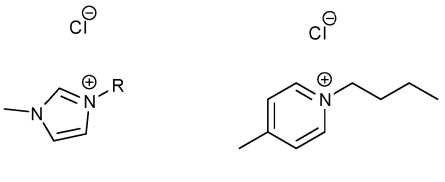
- The lower the lipophilicity the more mobile the IL
- Hydrophobic ILs will only migrate a few centimetres into soil[47]
- IL cations bind more weakly to soils that contain less organic matter[47]
- Mineral content of the soil greatly affects the mechanism of sorption[49]
- The more mobile the IL the greater the ability to pass through soil into ground water
- Sorption of ILs is greatest with strongly exchangeable cations and a high organic content
- The longer the alkyl chain the higher the sorption coefficient and *vice versa*, see Fig. 5[47]
- Desorption can be inversely correlated to sorption (Fig. 6)[47]
- Hydroxylated analogues adsorb more weakly (Fig. 7)[41]
- The higher the hydrophobicity of an IL the greater the phytotoxic effect (Fig. 8)[50]
- Bacteria from a high salt environment or a high hydrocarbon environment are far better able to survive in higher concentrations of ILs[51]

3.9 Biodegradation of ILs under denitrifying conditions

Biodegradation of ILs under denitrifying conditions remains a largely undocumented pathway and has only been reported in one paper.[52] Anaerobic biodegradation conditions are prevalent in nature in soils, aquatic environments and in waste water treatment plants and are considered of potential significance as IL biodegradation pathways.

Ionic liquids	R3		L2		CA-1		CA-2		CA-3	
	K_{d1}	K_{d2}	K_{d1}	K_{d2}	K_{d1}	K_{d2}	K_{d1}	K_{d2}	K_{d1}	K_{d2}
[EMIM][Cl]	3.3	2.3	1.7	0.7	2.5	1.3	2.8	2	1.2	0.5
[EMIMOH][Cl]	2.1	2.5	1.5	0.9	2.6	0.4	2.3	2	1.7	1
[PMIMOH][Cl]	4.1	2.9	1.9	0.7	3.7	2.1	2.6	2	2	1.1
[BMIM][Cl]	8.7	4.6	2.5	1	15.7	6.8	2.7	2.7	4	0.9
[HMIM][Cl]	22	7.5	3.6	2.2	15.5	9	4.6	3.6	9	2
[OMIM][Cl]	34.2	12.1	4.5	1.7	26.4	8.6	8.7	5.1	10	3.7
[MBPy][Cl]	22	6.4	4.1	1.2	16.8	3.1	5.4	1.5	1.6	1.3

Fig. 5 Sorption coefficients (K_d mL g^{-1}) for first layer K_{d1} and final concentration K_{d2} for given soil types.[47] Reprinted from Stepnowski *et al.* (2009) with kind permission from Springer Science and Business Media.



8, R = C_2H_5
9, R = C_4H_9
10, R = C_2H_4OH
11, R = C_3H_6OH
12, R = C_6H_{13}
13, R = C_8H_{17}

14

Fig. 6 Imidazolium compounds (**8–14**) examined for soil adsorption.

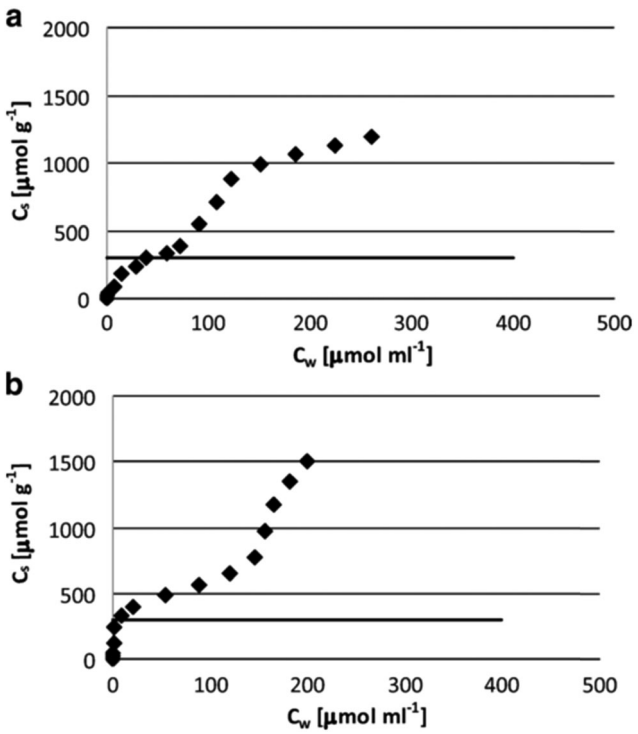


Fig. 7 Sorption isotherms for (a) [bmim][Cl] and (b) [hmim][Cl] on soil type R3.[47] Reprinted from Stepnowski *et al.* (2009) with kind permission from Springer Science and Business Media.

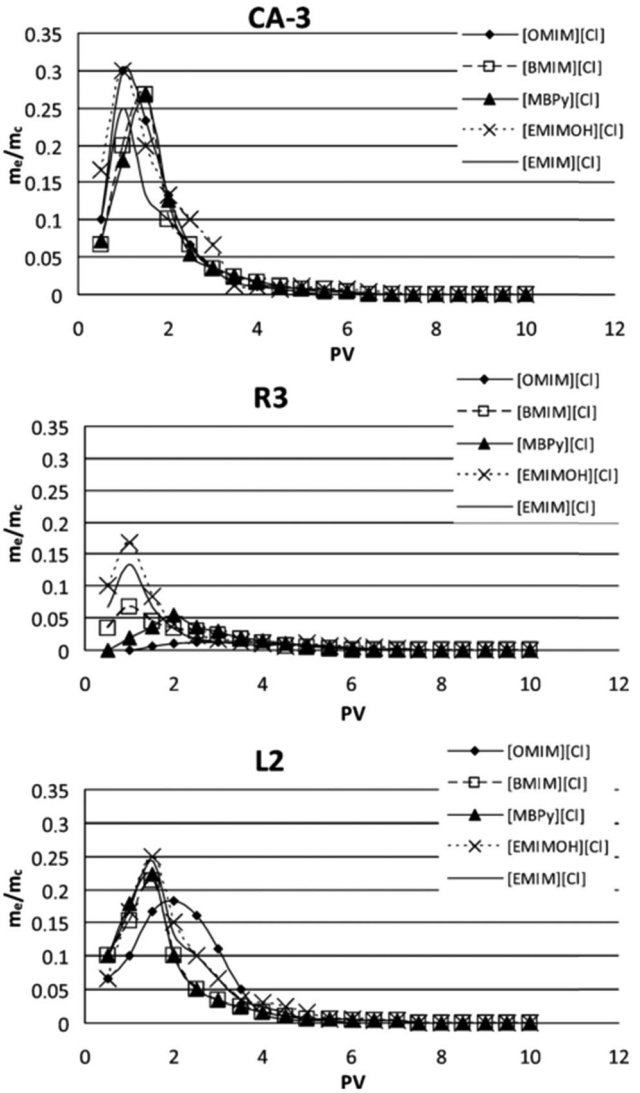


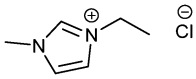
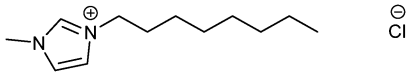
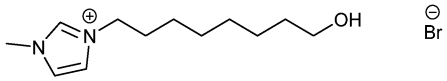
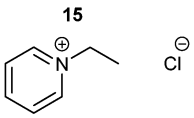
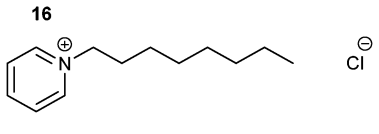
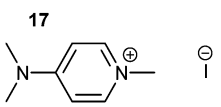
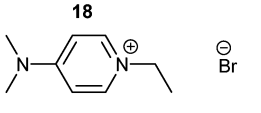
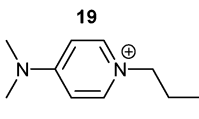
Fig. 8 Soil elution profiles (breakthrough curves) of ILs [omim][Cl], [bmim][Cl], [mbpy][Cl], [emimOH][Cl] and [emim][Cl] in the three different soil types, CA3, R3 and L2.[47] Reprinted from Stepnowski *et al.* (2009) with kind permission from Springer Science and Business Media.

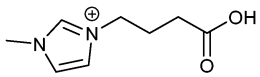
3.9.1 Anaerobic biodegradation. In the study carried out by Stolte *et al.* in 2010, nine ILs (**8, 13, 15–20**) were examined for their anaerobic biodegradability including a metabolite study with compound identification by HPLC-UV.[52] The ILs were subjected to the denitrifying conditions for 11 months.

The results of the anaerobic study, Table 9, showed no reduction in concentration for the ILs studied except for the octyl alcohol derivative (**15**) which broke down completely, within 30–40 days, to the carboxylic acid metabolite (**21**), Fig. 9. The biodegradation pathway and associated metabolite study is further discussed later in this review in Section 5.4, *vide infra*. Attempts to induce biodegradation by co-metabolism through the addition of an acetate salt failed.[51] Alkyl substituted imidazolium (**8, 13**), pyridinium (**16, 17**) and dimethylamino-pyridinum (**18–20**) ILs were not found to be readily anaerobically biodegradable compounds under the studies test conditions (Table 9).

3.9.2 Anaerobic dehalogenation. Another benefit of investigating the anaerobic biodegradation of ILs is the potential for biodegradation of halogenated compounds. Organohalides make up a large proportion of compounds used as solvents, biocides, pesticides, refrigerants *etc.* and many can be classed as persistent organic pollutants.[22,53] With the introduction of halogenated ILs into industrial applications, the question of how these compounds will respond to release into the environment must be asked. Under the Stockholm Convention on Persistent Organic Pollutants 2004, bans and reductions on some of the world's most hazardous organic pollutants were put into effect.[54] With a legal and political framework established for the reduction of halogenated and persistent organic pollutants in place, the need to provide readily biodegradable organohalide compounds is imperative. Halorespiring bacteria are known and the reductive dechlorination of common chlorinated solvents has been

Table 9 Anaerobic degradation of imidazolium and pyridinium ILs reported by Stolte *et al.*[52]

IL screened	Primary biodegradation as determined by HPLC/MS
 **8**	0%
 **13**	0%
 **15**	100% metabolite detected – see Fig. 9
 **16**	0%
 **17**	0%
 **18**	0%
 **19**	0%
 **20**	0%



21

Fig. 9 Metabolite detected at the end of the study.

reported including trichloroethane, tetrachlorethene and aromatic halides.[55–57] Examination of ILs for anaerobic biodegradation therefore is of importance, especially if the IL is not readily biodegradable under aerobic conditions or if a metabolite study shows that a halogenated moiety remains after degradation has plateaued. Inorganic halide counterions can breakdown with no release of CO_2, for example BF_4 hydrolysis.[58] In a 2012 study conducted by Stolte *et al.* the biodegradability of fluoro and cyano derived anions was assessed.[59] Under the aerobic and anaerobic degradation test conditions a number of metal salts of the anions were examined:

- $NaN(CN)_2$ – sodium *N*-cyanocyanamide,
- $LiNTf_2$ – lithium bis(trifluoromethylsulfonyl)amide
- $KC(CN)_3$ – potassium tricyanomethanide
- $KB(CN)_4$ – potassium tetracyanoborate
- $K(C_2F_5)_3PF_3$ – potassium trifluoridotris(pentafluoroethyl)-phosphate

Under both aerobic and anaerobic test conditions it was noted that the metal salts of these anions did not possess any biodegradation potential and were not toxic to the microorganisms in the test sludge (at the concentrations used in the test). It was suggested that the ability for the anions to undergo de-fluorination was also unlikely.[59]

3.10 Biotreating ILs for enhanced biodegradation – axenic cultures

An investigation into the use of axenic bacterial cultures – cultures of bacteria containing only one organism/strain – in enhancing biodegradation was performed by Catalina *et al.* in 2011.[60] The use of judicially selected bacteria, specifically a *S. paucimobilis* strain, has demonstrated that modifying the test conditions from the prescribed OECD guidelines can heighten biodegradation. The results of these tests are not recognised under the OECD or ISO guidelines; however they do give insight into alternate bio-based treatment options for ILs. Previously recalcitrant compounds were observed undergoing biodegradation under optimised test conditions of 45 °C and IL concentrations of 0.5–3.0 g L^{-1}. Out of 37 commercially available ILs tested, 20 were found to undergo $\geq$60% biodegradation in less than 28 days, (**3–5**, **22**, **23**, **27–32**, **36–41**, **45**, **46**, **49**, **50**) see Table 10, with the choice of anion playing a significant factor in % degradation. Shortening the length of substituted alkyl chains increased biodegradability, *e.g.* for the chloride series of 1-methylimidazole series (**8**, **9**, **12**, **13**, **35**). The successful application of a specific bacterial strain capable of adapting to higher concentrations of ILs shows that a potential wastewater pre-treatment of non-biodegradable ILs is possible. It is also important that the concentration of IL used in each test played a major role in % biodegradation values, with $\sim$2 mmol L^{-1} giving optimum results. It is stated by Catalina *et al.*[60] that no clear relationship between toxicity and biodegradation results were observed for the compounds tested.

Similar success in the use of axenic cultures was observed in the work by Zhang *et al.* in 2010, where it was demonstrated that a *Corynebacteria* species could effectively metabolise the butylpyridinum ILs (**52** and **53**), Fig. 10 but not [bmim] $[PF_6]$ (**4**).[63]

A proposed degradation pathway determined by ESI/MS/MS is presented in Section 5.2, *vide infra*. The potential capabilities of judiciously selected bacterial cultures has shown that biodegradation of previously determined recalcitrant IL compounds is possible, subject to optimised conditions.

Jungnickel *et al.* examined the use of axenic cultures of bacteria in the biodegradation of [omim][Cl] (**13**) in 2014 using the manometric respirometry method (OECD 301F).[64] Under the biodegradation tests 9 bacteria were isolated from an activated

Table 10 Biodegradation results for *S. paucimobilis* at 45 °C

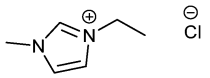
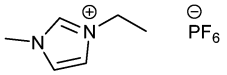
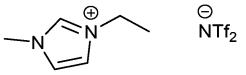
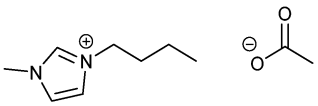
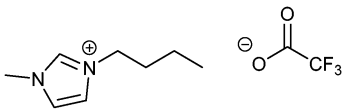
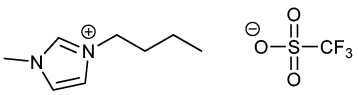
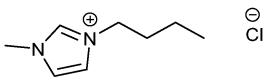
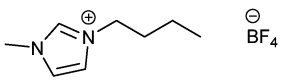
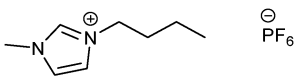
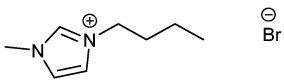
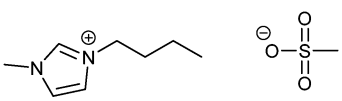
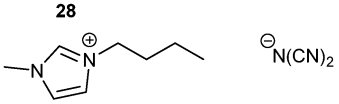
Name	Structure	Biodegradation[a]
[emim][Cl]	 **8**	53[b]
[emim][PF_6]	 **22**	87
[emim][NTf_2]	 **23**	67
[bmim][CH_3CO_2]	 **24**	51
[bmim][CF_3CO_2]	 **25**	20
[bmim][CF_3SO_3]	 **26**	34
[bmim][Cl]	 **9**	39[b]
[bmim][BF_4]	 **3**	80[b]
[bmim][PF_6]	 **4**	65[b]
[bmim][Br]	 **27**	75[b]
[bmim][CH_3SO_3]	 **28**	75[b]
[bmim][DCN]	 **5**	77

Table 10 (*continued*)

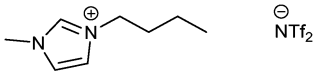
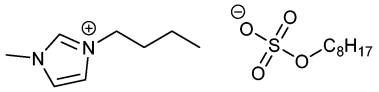
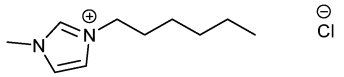
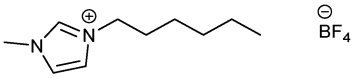
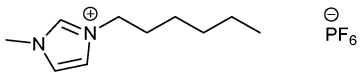
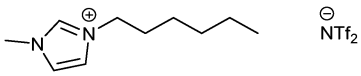
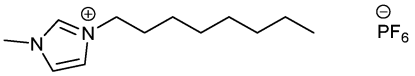
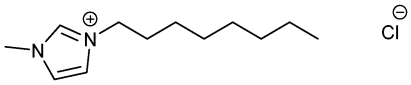
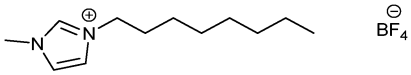
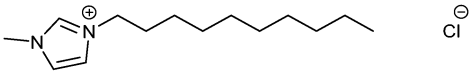
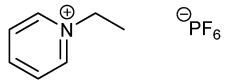
Name	Structure	Biodegradation[a]
[bmim][NTf_2]		90[b]
[bmim][$C_8H_{17}SO_4$]	**29** 	>95[b]
[hxmim][Cl]	**30** 	37
[hxmim][BF_4]	**12** 	40
[hxmim][PF_6]	**31** 	70
[hxmim][NTf_2]	**32** 	73
[omim][PF_6]	**33** 	54
[omim][Cl]	**34** 	32
[omim][BF_4]	**13** 	33
[dcmim][Cl]	**35** 	12
[1EtPy][PF_6]	**36** 	>95
	37	

Table 10 (*continued*)

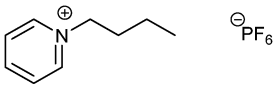
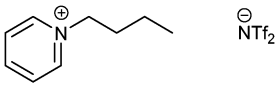
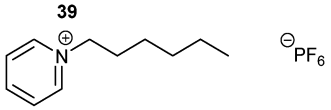
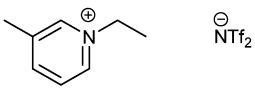
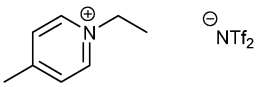
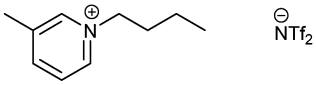
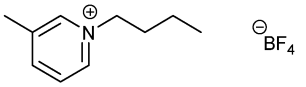
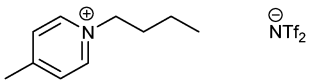
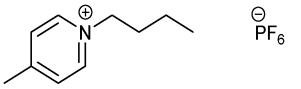
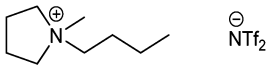
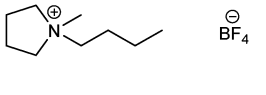
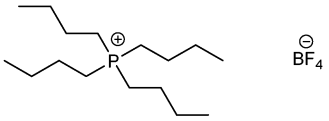
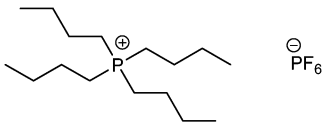
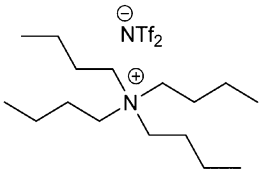
Name	Structure	Biodegradation[a]
[1BuPy][PF_6]		80
[1BuPy][NTf_2]	38 	86
[1HxPy][PF_6]	39 	62
[1Et3MePy][NTf_2]	40 	>95
[1Et4MePy][NTf_2]	41 	>95
[1Bu3MePy][NTf_2]	42 	17
[1Bu3MePy][BF_4]	43 	35
[1Bu4MePy][NTf_2]	44 	30
[1Bu4MePy][PF_6]	45 	78
[1Bu1MePyrr][NTf_2]	46 	>95
[1Bu1MePyrr][BF_4]	47 	49
	48	

Table 10 (*continued*)

Name	Structure	Biodegradation[a]
$[PBu_4][BF_4]$	 **49**	41
$[PBu_4][PF6]$	 **50**	66
$[NBu_4][NTf_2]$	 **51**	64

[a] Colour coded to TSL, (see green chemistry metrics section). [b] Compounds previously reported as recalcitrant.[61,62]

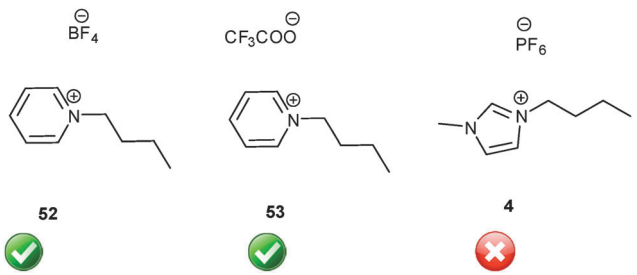


Fig. 10 [PyBu] derivatives (**52** and **53**) found to degrade and recalcitrant [bmim][PF_6] derivative (**4**).

sludge and identified by 16S rDNA PCR (polymerase chain reaction) and compared to genetic data available on Genbank. The bacteria were then examined for their biodegradation potential. The bacteria isolated were as follows:

- *Flavobacterium* sp. WB3.2-27
- *Shewanella putrefaciens* CN-32
- *Moraxellaceae bacterium* MAG
- *Flavobacterium* sp. FB7
- *Microbacterium keratanolyticum* AO17b
- *Flavobacterium* sp. WB 4.4-116
- *Arthrobacter* sp. SPC 26
- *Rhodococcus* sp. PN8
- *Arthrobacter protophormiae* strain DSM 20168

It was found that the individual axenic cultures were inefficient at biodegrading the [omim][Cl] exhibiting only $\leq$8% biodegradation. When all 9 bacteria strains were combined into a mixed culture biodegradation experiment, only 30% biodegradation could be achieved. Under the same experimental conditions, using an activated sludge, degradation of $\sim$60% was observed for [omim][Cl] (determined by the manometric respirometry method (OECD 301F)).[64] Thus it was proposed by Jungnickel *et al.* that isolated organisms may not facilitate biodegradation as efficiently as they would in an activated sludge of far greater complexity where the possibility of symbiotic relationships of multiple strains of bacteria is present. Another possibility suggested was that not all of the bacteria required for IL metabolism were isolated for the individual and combined culture experiments.[64]

3.11 Suitability of biodegradation tests to IL classes – toxicity *vs.* biodegradability

Not every IL tested for biodegradation by one of the standard OECD methods will be compatible with the test procedure and it has been previously reported that a number of issues exist including sorption and kinetic parameters of ILs in activated sludge as previously described and toxicity of IL to the inoculum.[65] Other issues then arise such as concentration effects of toxic ILs and biodegradation inhibition that may ensue. See Table 11 for a general compound suitability to biodegradation test methods.

According to current trends from reported biodegradation data, the increase of alkyl side chains has shown to directly lead to an increase in biodegradation due to the extra oxidisable carbons in the chain. However a design conflict is present and an increase in alkyl chain length has been shown to increase antimicrobial toxicity especially around the C12–C14 chain length. Chains of C16 and C18 exhibit very poor water solubility and hence have a lower bioavailability.[66] A design optimisation in the addition of ether oxygen in alkyl chains has shown to reduce toxicity but maintain levels of biodegradability.[67]

Table 11 Suitability of organic compounds to biodegradation screening method[21,34]. Reproduced from ref. 21 with permission from the Royal Society of Chemistry

Test	Analytical method	Suitability[a] for compounds which are		
		Poorly soluble	Volatile	Adsorbing
DOC die-away (301A)	Dissolved organic carbon	−	−	±
CO_2 evolution (301B)	Respirometry: CO_2 evolution	+	−	+
MITI (I) (301C)	Respirometry: oxygen consumption	−	±	+
Closed bottle (301D)	Respirometry: dissolved oxygen	±	+	+
Modified OECD screening (301E)	Dissolved organic carbon	−	−	±
Manometric respirometry (301F)	Oxygen consumption	+	±	+
CO_2-headspace test (ISO 14593)	CO_2 evolution	+	+	+
OECD 309	^{14}C labelling	±	+	+
ASTM 5988	CO_2 production/BOD	−	−	±

[a] Suitable method to screen compound: +; unsuitable method to screen compound: −.

Validation of biodegradation results is of the utmost importance. For example, when carrying out the CBT, sodium acetate is often used as a control. The high biodegradability of sodium acetate is known and by comparing biodegradation results to the control it can be determined whether the inoculum chosen for the test is effective.

Further validation is required regarding the toxicity of ILs and the sorption characteristics. Removal of ILs by sorption can be calculated using the guidelines in OECD 302B Zahn–Wellens test.[34] Toxicity of ILs to the inoculum can be assessed by including a toxicity control series in tandem to the biodegradation test series and a blank sodium acetate + inoculum series. The toxicity reference vessel contains IL + sodium acetate + inoculum, the sodium acetate control contains sodium acetate + inoculum. Any significant differences in oxygen demand between the toxicity control, when compared to the sodium acetate control, can be attributed to toxic effects.

While different research groups may prefer to perform one biodegradation test over another, in particular with the CO_2 headspace test (OECD 310 or ISO 14593), or even primary *vs.* readily biodegradation tests, it is key to note that this data is just a starting point for a more detailed investigation about chemical fate in the environment. Regardless of where you start the assessment program, the aim is to ultimately complete a thorough (eco)toxicity and biodegradation study of chemicals likely to be used on a large scale and where contamination of the environment is a possibility.

4. Biodegradation data on classes

4.1 Ionic liquids: anion and cation effects with regards to biodegradation

There are a number of different perspectives to consider when interpreting biodegradation data of ILs. All ILs consist of an anion and cation thus two parts must be considered, albeit as a pair. Another consideration is whether the anion or cation is organic or inorganic. For instance if an inorganic ion is used, such as a halide or pseudohalide (or positively charged metal halide cation), then it does not contribute directly as a carbon source for biodegradation determination. Conversely, if an IL contains an anion of high carbon content which is known to have excellent biodegradation characteristics (*e.g.* octylsulphate anion, Fig. 12), then one must be careful not to assume high biodegradability for the ion pair, and thus infer good biodegradability for the other less degradable ion present in the IL.

Within the OECD test regulations a biodegradation level of 60% is usually required within 28 days. The test treats the IL as a single chemical compound and therefore the outcome is that the IL ion pair either passes or fails the biodegradation test. While caution must be exercised when utilising this pass or fail result for biodegradation tests to design future biodegradable ILs, the pass or fail result for each IL is valid as defined by the regulations of the OECD test criteria. It is our expectation that this review will assist researchers to design and develop ILs which pass ultimate biodegradation and mineralisation assays, which is only possible if both ions biodegrade or are mineralised.

4.2 Anions

A popular and widespread class of anions are the halides, Fig. 11. The halide anions and pseudohalides (*e.g.* BF_4 and PF_6) are not a carbon source for biodegradation and thus biodegradation values are dependent on the organic cation. IL [bmim] derivatives have been reported to give poor biodegradability, with values in the, 0–5% range.[21] Anion choice is usually directed by function and application (*e.g.* in many cases, NTf_2 for hydrophobic ILs). In this section we review the study of organic anions in ILs and their progress towards the development of biodegradable ILs.

Organic anions offer a myriad of possibilities when selecting suitable structures to incorporate into ILs. Many natural products (*e.g.* carboxylic acids, amino acids, and carbohydrates) have been tested as feedstocks for anions. Other examples have

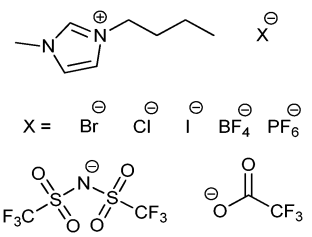


Fig. 11 Some of the most commonly employed halide/pseudohalide anions as their [bmim] ILs.

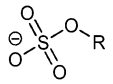
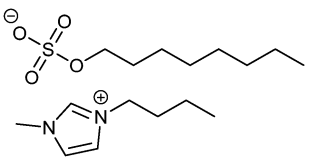


Fig. 12 Sulphate anion core, R = C_nH_{2n+1}, n = 8–12.



30

Fig. 13 [bmim] paired with the biodegradable, octyl sulphate anion failed the CBT and CO_2 headspace test.[62]

been adopted from the surfactant industry, such as the sulphate anions, Fig. 12. The alkylsulphate anions exhibit good rates of biodegradation. Of note, sodium dodecylsulphate is used frequently as the positive test control for activated sludge activity.[17] Studies by Scammells *et al.* showed that [bmim][$C_8H_{17}SO_4$], (**30**), Fig. 13, biodegrades 25% in the CBT (OECD 301D),[62] or up to 40% in the CO_2 headspace test (ISO 14593).[68] A reasonable assumption is that the anion is converted to CO_2 with high efficiency and the [bmim] cation is not transformed, however this was not confirmed at the time due to the absence of LCMS data and metabolite identification.

Formates, acetates and other simple carboxylates, Fig. 14, have been employed as IL anions with very good biodegradation results found when paired with the cholinium cation, Fig. 15. Cation modification can lead to significant changes in biodegradability which is discussed later in Section 4.3, *vide infra*.

Similar success has been demonstrated in the use of malonates, succinates, tartrates and other natural organic acids and

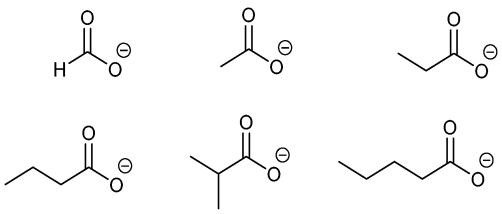
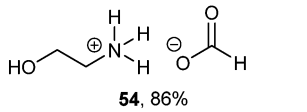
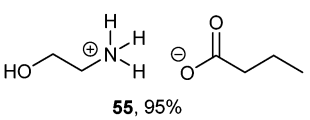


Fig. 14 Formate and carboxylate anions.



54, 86%



55, 95%

Fig. 15 Two readily biodegradable cholinium protic carboxylate anion ILs.[69] Biodegradation values assessed by manometric respirometry (OECD 301F).

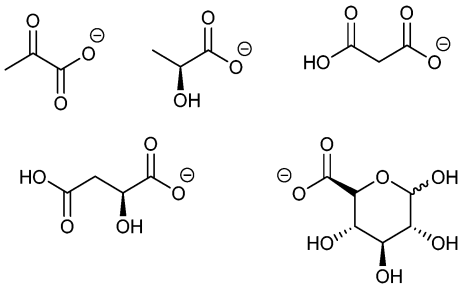
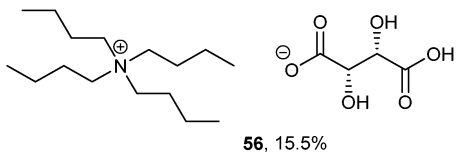
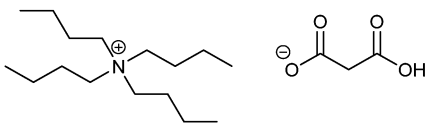


Fig. 16 Biodegradable organic acids that have been employed as anions in ILs.[71]

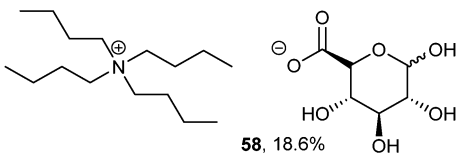
sugars, Fig. 16. However, pairing with an appropriate cation is crucial. In the following examples tetrabutyl ammonium (TBA) cations have been employed where only the organic anion is degraded, Fig. 17. Replacing the TBA cation with a dibutyldimethyl ammonium cation (DMDBA), Fig. 18, promotes biodegradation for the lactate derivative (**59**). The biodegradation values for the TBA derivatives are in good agreement with the percentage carbon equivalent of the anion and it is assumed that the cation is



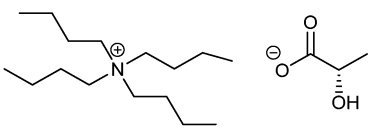
56, 15.5%



57, 9.9%

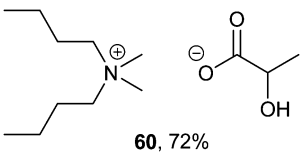


58, 18.6%



59, 15.5%

Fig. 17 TBA carboxylate ILs.[71] Biodegradation values assessed by CBT (OECD 301D).



60, 72%

Fig. 18 A readily biodegradable lactate DMDBA IL.[72] Biodegradation values assessed by manometric respirometry (OECD 301F).

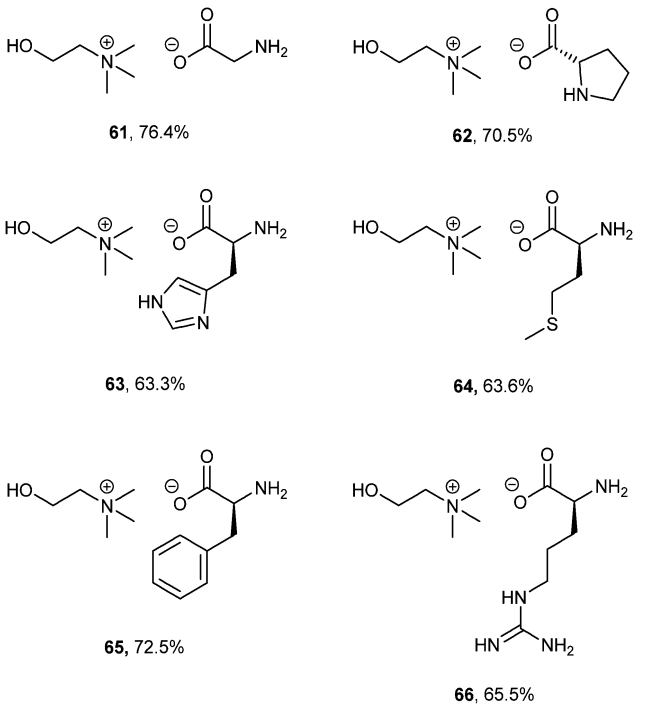


Fig. 19 Biodegradable cholinium amino carboxylate ILs.[73] Biodegradation values assessed by CO_2 headspace test (OECD 310).

persisting with only the anion undergoing biodegradation, CBT (OECD 301D).[70] See Section 4.3.2.5 on tetraalkyl ammonium ILs for more information on TBA and DMDBA ILs.

In addition to organic acids and sugar acids, amino acids have been investigated as the anion in ILs, Fig. 19. There are 23 proteinogenic amino acids for prokaryotic organisms and 21 for eukaryotes. A recent publication by Zong *et al.*[73] showed the successful combination of 18 amino acids (Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asp, Glu, Asn, Gln, Lys, His, Arg, Pro, Phe, Trp) with a cholinium cation. All of the ILs passed the CBT (OECD 301D) (65.3–87.1%) and the CO_2 headspace test (OECD 310), (62.1–85.2%) and can be classed as readily biodegradable. Selected examples are shown in Fig. 19, for full list see appendix compounds (**310–322**).

A common structural feature of all these anions is the free carboxylate which, as suggested by Boethling, can promote biodegradability.[74] It was also noted that the amino acids with branched side chains were more resistant to biodegradability 69–72% (OECD 301D) and (OECD 310) compared to >80% biodegradation for unbranched sidechains. The choice of cation will inevitably have a determining factor on the overall biodegradability of the organic anions as can be seen when comparing cholinium prolinate (**62**) to the TBA derivative (**67**), Fig. 20. TBA L-prolinate (**67**) undergoes levels of 15–20% biodegradation whereas >70% biodegradation was observed for the cholinium analogue (**62**).

As an overarching design principal, the authors suggest that a halide anion such as chloride or bromide can provide the foundation for potentially biodegradable ILs as the halide counterion cannot undergo further degradation as it is already

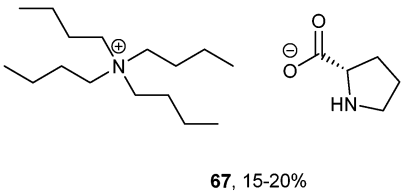


Fig. 20 TBA L-prolinate IL.[70] Biodegradation values assessed by CO_2 headspace test (OECD 310).

in a mineralised form. Thus, an IL formed from a Br or Cl anion will only biodegrade according to the carbon content of the cation. Perfluorinated anions should be avoided as they have been shown to be recalcitrant or their degradation products are more harmful than their parents, *e.g.* BF_4 degrading to HF.[75]

Overall the organic acid anions appear to be the best selection for promoting biodegradability. Biodegradation information provided by the choices of amino acids screened by Zong *et al.*[73] ILs (**61–66, 310–322** appendix) suggest that the amino acids all undergo high levels of biodegradation in both the CBT (OECD 301D) and the CO_2 headspace test (OECD 310) and will not persist under the test conditions.

The natural organic acids (succinic, tartaric, lactic, malonic, malic, pyruvic, glucoronic, galacturonic *etc.*) such as those employed by Ferlin *et al.*[71] and Jérôme *et al.*[72] can be shown to promote biodegradability. In these examples, the tetrabutly ammonium (TBA) ILs (**56–59**) and (**168–172**), it is assumed that the TBA cation is recalcitrant to biodegradation (OECD 301D), and that the organic acid anion is solely breaking down, this is discussed in more detail under Section 4.3.2.5 on tetra alkyl ammonium cations.

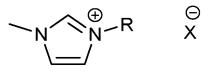
The DMDBA examples (**60, 175–180**) also displayed great success in the choice of organic acid anions.[72] Finally the amino diol and triol examples (**161–167**)[69] also have high levels of biodegradability (OECD 301F) when simple carboxylic acid anions such as acetate, propanoate, isopropanoate, butanoate and pentanoate feature.

4.3 Cation

The choice of cation is as large and developed as the choice of anion, with the most common cations including the heterocycles 1-methylimidazolium, pyridinium and the non-aromatic cholinium and tetrabutyl ammonium. For the majority of ILs synthesised to date the cation has had a higher carbon content than the anion. This higher carbon content can equate to a larger contribution towards the overall CO_2 evolution/O_2 demand of the IL within biodegradation screening methods and potentially higher levels of biodegradation. While a shift from inorganic anions to organic anions has been observed, the research conducted towards cation biodegradability has seen many investigations carried out on different organic scaffolds and the effect of various functional groups that may facilitate biodegradation.

4.3.1 Aromatic cations – imidazolium, thiazolium, pyridinium

4.3.1.1 Imidazolium. Derivatives of 1-methylimidazole have long been a choice of cation in IL based technologies with considerable research in the past two decades focussed on 2nd and



4, R = C_4H_9, X = PF_6, 0% *
8, R = C_2H_5, X = Cl, 0% **
9, R = C_4H_9, X = Cl, 0% *
12, R = C_6H_{13}, X = Cl, 2%***
29, R = C_4H_9, X = NTf_2, <5%**
68, R = C_2H_5, X = CH_3SO_4, 10%***

Fig. 21 Biodegradation data for some 2nd and 3rd generation ILs.[61,62,77] *Biodegradation values assessed by manometric respirometry (OECD 301F). **Biodegradation values assessed by CBT (OECD 301D). ***Biodegradation values assessed by BOD measurement using an OxiDirect kit.

3rd generation ILs (**4**, **8**, **9**, **12**, **29**, **68**) see Fig. 21.[76] The commercial availability of [bmim] and other alkyl derivatives has facilitated the popularity of ILs as replacement solvents but as previously discussed, identifying readily biodegradable examples has proven difficult.

Since the previous IL biodegradation reviews published by Gathergood *et al.* 2010[21] and Stolte *et al.* 2011[78] a number of substituted imidazolium derivatives have been screened for their biodegradability. The imidazolium cation has been functionalised in two ways in efforts to improve biodegradation:

(1) modification of the *N* substituents

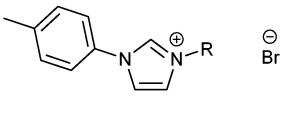
and/or

(2) substitution at the C2 and C4/5 positions of the imidazolium ring, Fig. 22.

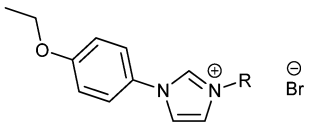
Aryl derivatives (**69–76**) screened by Stolte *et al.* 2013[79] (Fig. 23) show that aryl imidazolium derivatives are recalcitrant to biodegradation (OECD 301F) with no greater than 8% biodegradation achieved for (**75**) and (**76**), most likely undergoing primary biodegradation *via* ethyl ester hydrolysis to the carboxylic acid (**77**), Fig. 24. The introduction of an oxygen atom in the form of the ethyl ether in (**71**) and (**72**) did not initiate biodegradation.

Similar poor levels of biodegradation (0–2% in the CO_2 headspace test ISO 14593) for ILs containing phenyl rings were obtained for the *N*-benzyl derivatives (**78**, **79**) screened by Gathergood *et al.* 2013[17] Fig. 25.

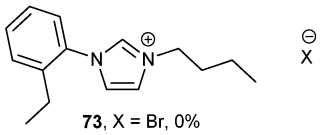
Modifications by Gathergood *et al.* 2013[17] of the imidazolium cation to introduce ester groups and amide groups at the C2, C4 and C5 positions ILs (**80–96**) did not succeed in raising biodegradation levels (ISO 14593) beyond that which could be explained by ester degradation, Fig. 26 and 27. The imidazolium ILs were categorised by the authors according to the aforementioned traffic light metric and are organised according to Amber classified ILs, Fig. 26 and Red classified ILs, Fig. 27.[17]



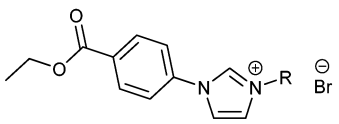
69, R = C_3H_7, 0%
70, R = C_4H_9, 0%



71, R = C_4H_9, 0%
72, R = C_5H_{11}, 0%



73, X = Br, 0%
74, X = I, 0%



75, R = C_6H_{13}, 8%
76, R = C_4H_9, 7%

Fig. 23 Biodegradation data for imidazole ILs synthesised by Stolte *et al.*[79] Biodegradation values assessed by manometric respirometry (OECD 301F).

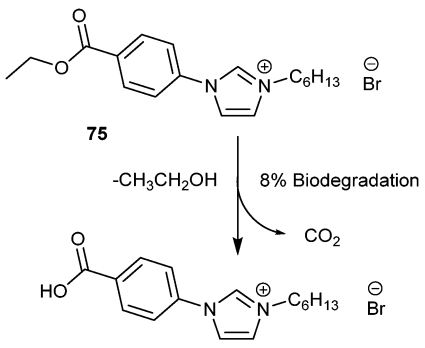


Fig. 24 Aryl imidazolium IL and potential degradation product.[79]

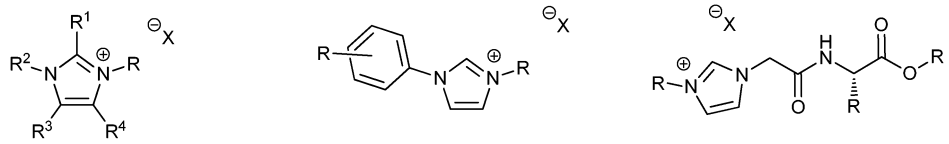


Fig. 22 The imidazolium cation and potential sites for derivatisation.

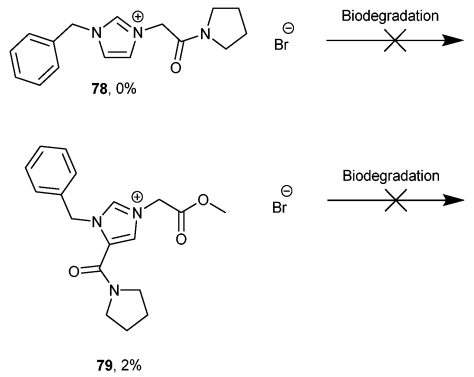


Fig. 25 *N*-Benzyl imidazolium ILs.[17] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

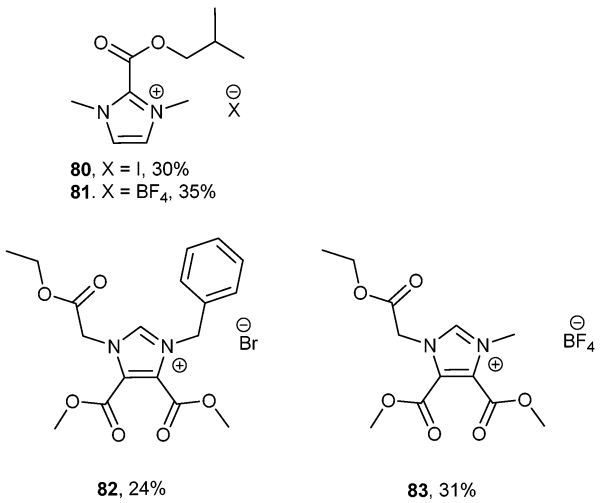


Fig. 26 Imidazolium ILs classified as Amber.[17–19] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

Examples of ester modifications at the imidazolium C2 position, ILs (**80**, **81**), Fig. 26 show a moderate amount of biodegradation (30–35%), however this can be attributed to only the degradation of the ester, with the imidazolium ring presumably remaining intact. Imidazolium ILs disubstituted at C4,5 (**82**, **83**, **86–88**, **92**), Fig. 26 and 27 with ester groups lead to observed biodegradation in the CBT in the range of 2–31% and did not pass the test. Low biodegradation (5–10%) for the C4 substituted analogues (**84**, **85**), Fig. 27, was also observed. There is no evidence that the introduction of an ester group to the imidazolium ring leads to breakdown of the charged heterocycle. This observation is in contrast to the increase in biodegradability observed when modifying some pyridinium ILs to nicotinium ILs, see Section 4.3.1.3. Possible reasons for this lack of reactivity for the C4 and C4,5 is that after hydrolysis the carboxylic acid or carboxylate salt stabilises the ring making breakdown difficult. For C2 ester derivatives, hydrolysis forms an unstable intermediate, with facile evolution of CO_2, and the imidazolium ring intact.[17] Thus the more stable to hydrolysis amide group was examined to investigate the effect of a stable EWG on the imidazolium ring to promote breakdown. However, the authors report that with the amide in the C2 (**89**, **90**, **91**), C4 (**79**) or C4,5 (**92**) low biodegradation in the range 2–17% was observed.

Amide functionalisation at the nitrogen position to give ILs (**78**, **95**, **96**, **90**, **91**), Fig. 25 and 27, also show a resistance to biodegradation. This is best exemplified by (**95**) and (**96**) with levels of 3% biodegradation recorded.

Ester functionalisation at the nitrogen positions (**82–86**, **89**, **92–94**,) again followed the trend of poor biodegradability with levels attributed solely to ester hydrolysis, derivatives (**82**) and (**83**) underwent ester hydrolysis at all three ester positions to give Amber classified ILs, Fig. 26, while the remaining ILs were classified as Red with $<20\%$ biodegradation. The monoester derivatives (**93**) and (**94**) only attained 10–14% biodegradation in the CBT.

The effect of methylation of the C2 position of the imidazolium ring can also be determined from compounds (**95**) and (**96**), with no difference in biodegradation (3%) observed. This is in agreement with previous studies by Scammells *et al.*[80]

The poor biodegradability (OECD 301D) of C2, C4 and C4,5 modified imidazolium ILs was further demonstrated by the work carried out by Gendaszewska *et al.* in 2014 which established the poor biodegradability of four peralkylated imidazolium ILs (**97–100**), Fig. 28 and 29.

To tackle the problem of poor biodegradability of imidazolium based ionic liquids containing amides (Fig. 25–27), Gathergood *et al.* designed examples in which the amide bond was more 'peptide like' than the 'synthetic' examples previously screened.[81] The rational was that an amidase enzyme would be more likely to accept the former substrate than the latter. Thus, modification of the imidazolium core has been by the incorporation of an amino acid residue into the side chain of the cation. In 2012 Gathergood *et al.* disclosed two readily biodegradable amino acid derived imidazolium ILs bromides, Fig. 30. The two ILs, (**101**, **102**) were screened in the CO_2 headspace test and achieved $>60\%$ biodegradation after 28 days and can hence be classed as readily biodegradable. Compound (**101**) an IL based on a butyl ester of L-phenylalanine provided biodegradation of 61% and compound (**102**) a dipeptidyl IL based on L-phenylalanine-L-leucine methyl ester biodegraded 64% under the test conditions. The high levels of biodegradation are beyond that which can be explained by ester hydrolysis and alcohol oxidation.

The selection of compounds screened has shown that the majority of imidazolium based ILs screened are not readily biodegradable with $\leq 35\%$ biodegradation observed. The rule of thumb regarding imidazolium compounds still stands that *N*-substitution leads to a significant reduction on biodegradability, whereas imidazole and neutral *C*-substituted imidazole derivatives (*i.e.* not charged ILs) are readily biodegradable under the OECD 301D (100%)[61] and 302B (83%) test conditions.[82] While progress has been made to develop readily biodegradable imidazolium halide ILs which contain amide groups in the side chain,[81] no evidence of breakdown of the charged core has been presented in a standardized test.

4.3.1.2 Thiazolium. A heterocyclic thiazole derivative of the methyl imidazole core has also been screened by Scammells *et al.* in 2010,[68]

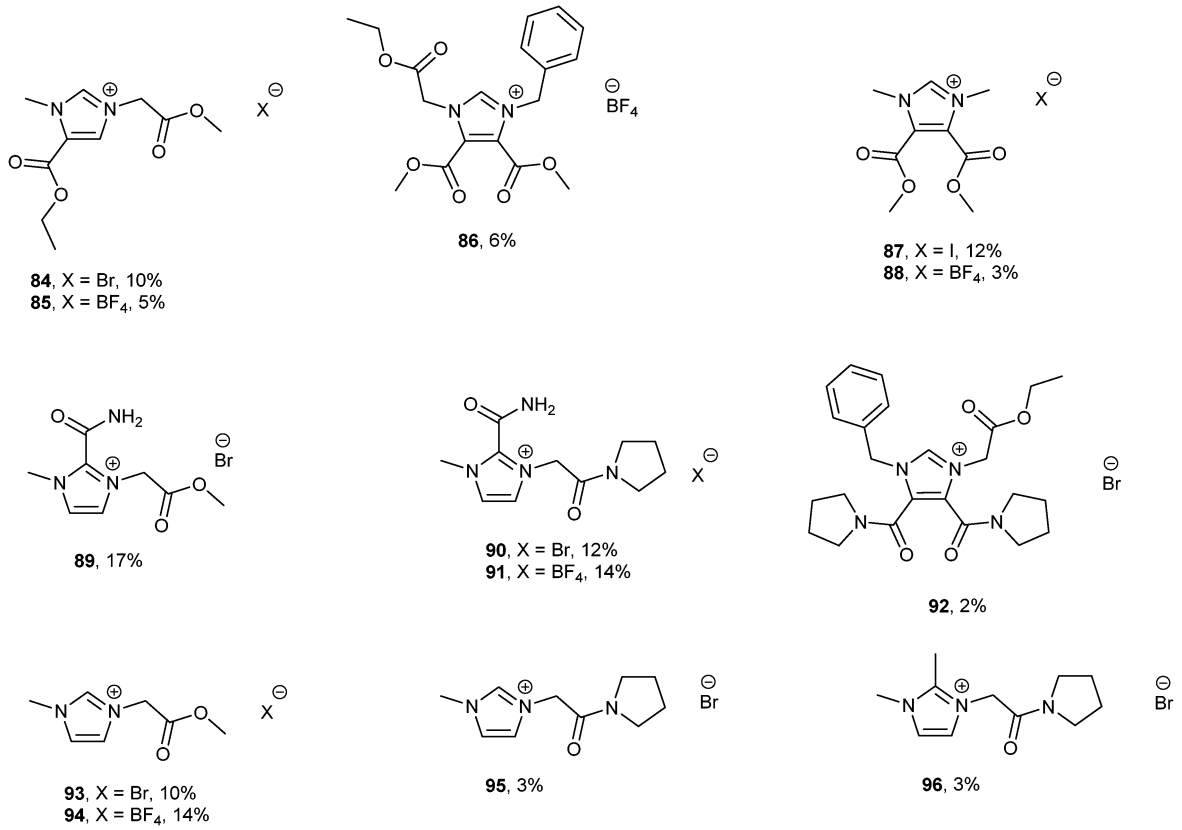


Fig. 27 Imidazolium ILs classified as Red by Gathergood *et al.*[17–19] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

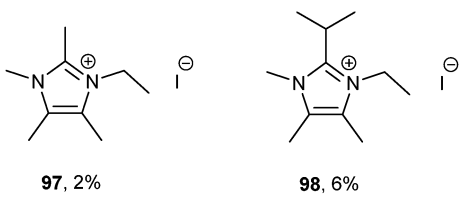


Fig. 28 Biodegradation data for C-2,4/5 modified imidazolium ILs.[43] Biodegradation values assessed by CBT (OECD 301D).

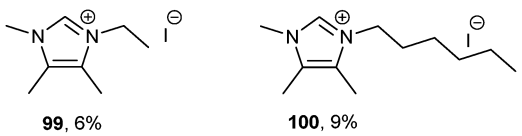


Fig. 29 Biodegradation data for C-4/5 modified imidazolium ILs.[43] Biodegradation values assessed by CBT (OECD 301D).

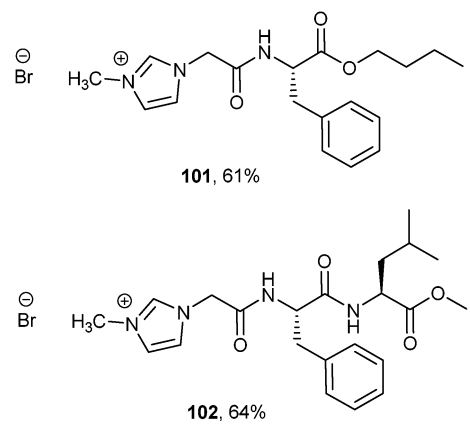


Fig. 30 Biodegradation data for amino acid derived imidazolium ILs.[81] Biodegradation values assessed by the CO_2 headspace test ISO 14593.

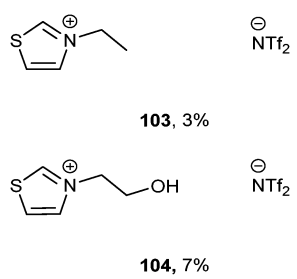


Fig. 31 Biodegradation data for thiazolium ILs synthesised by Scammells *et al.*[68] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

however the inclusion of a sulphur atom in the aromatic ring did not give any promising biodegradation results with $\leq$7% biodegradation being achieved in the CO_2 headspace test (ISO 14593), Fig. 31.

4.3.1.3 Pyridinium. The popularity of pyridinium and pyridinium based cations from methoxy-pyridinium and nicotinic acid/esters is high with a larger number screened for their biodegradability. Early generations of pyridinium ILs showed similar inertness to

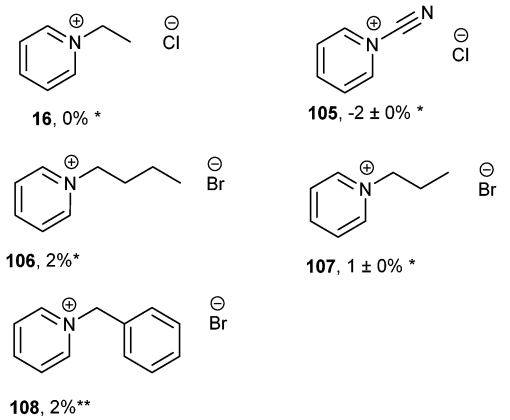


Fig. 32 Alkyl pyridinium ILs classified as not readily biodegradable.[17,83] *Biodegradation values assessed by manometric respirometry (OECD 301F). **Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

biodegradation (OECD 301F) as imidazolium ILs, with several compounds classified as not readily biodegradable (*e.g.* **16, 105–108**), Fig. 32.[61,83]

However, seminal work by Scammells *et al.* 2010[68] and Stolte *et al.* 2014[83] demonstrated that pyridinium derivatives can pass the CO_2 headspace test (OECD 310) and manometric respirometry test (OECD 301F). The primary ethyl alcohol pyridinium derivatives, compound (**109–111**), Fig. 33, have biodegradation levels of 65% and 62%, respectively, in the CO_2 headspace test whilst the iodide (**111**) example screened in the manometric respirometry test gave 65%. Increasing the length of the alcohol substituent from an ethyl alcohol to a propyl alcohol giving derivative (**112**) displayed a reduction in the biodegradability to 51%.

The elevated biodegradation levels can be attributed to the primary alcohol on the alkyl chain promoting biodegradation as a potential site for oxidation. The carbon count of compound (**109**) is 7 × C. The biodegradation levels of 65% would therefore suggest that approximately between 4 and 5 carbon atoms are being mineralised from this compound. With the side chain only supplying 2 × C and the inorganic anion not contributing to CO_2 values, it may be inferred that the pyridinium core is undergoing breakdown under the CO_2 headspace test conditions.

Derivatising the pyridinium core can have dramatic effects on the biodegradability of the ILs produced. Addition of a methoxymethyl or a methoxyethyl group at the 3-position to give compounds (**113–119**), Fig. 34, showed a reduction of biodegradability.

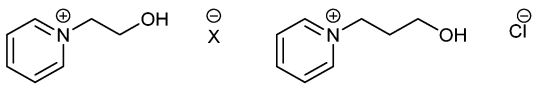


Fig. 33 Pyridinium ILs (**109–112**).[68,83] *Biodegradation values assessed by the CO_2 headspace test (ISO 14593). **Biodegradation values assessed by manometric respirometry (OECD 301F).

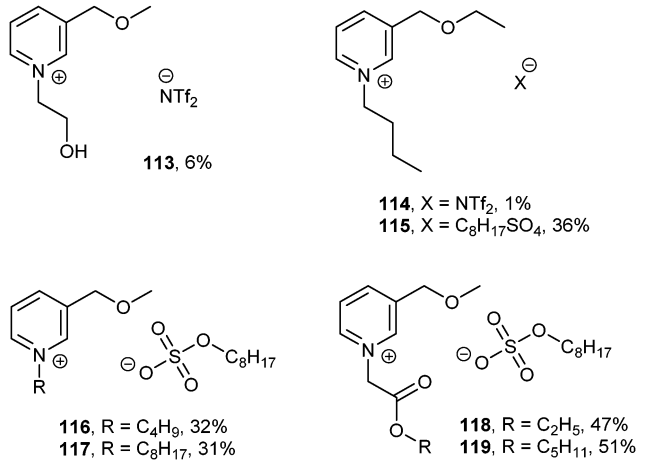


Fig. 34 (**113–119**) pyridinium ILs ether substituted at the 3-position.[68] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

For the NTf_2 derivative (**113**) a substantial reduction in biodegradation was observed to 6%.

The 3-position on the pyridinium core appears to be particularly sensitive to its substituent. When comparing methoxymethyl IL (**113**), Fig. 34, to its pyridinium counterpart (**110**), Fig. 33, a reduction in biodegradation (ISO 14593) from 62% to just 6% is observed, directly attributable to the addition of a methoxymethyl group on the 3-position. The ethoxymethyl derivative of *N*-butyl pyridinium again highlights the recalcitrance of 3-substituted ILs, with biodegradation of 1% observed for the NTf_2 derivative, IL (**114**) and 36% for the octylsulphate derivative (**115**), the elevated levels of biodegradation for the latter example are attributable to biodegradation of the anion. For derivatives (**116, 117**) there is no evidence of biodegradation of the *N*-butyl or methoxymethyl sidechain as levels of biodegradation of 32% and 31%, respectively, once again implies biodegradation of the anion solely. With the addition of an ester on the *N*-position to give ILs (**118, 119**) the elevated levels of biodegradation (47–51%) can be attributed to ester hydrolysis plus the degradation of the octylsulphate anion with the methoxymethyl pyridinium core remaining intact.

With 3-position ethers giving such poor results, a progressive examination of sidechain functionalities logically leads to inspection of ester and amide functionalities at the 3-positon, Fig. 35.

Ester functionalities on the three position, Fig. 35, to give nicotinium derivatives, appear to have moderate to good biodegradability with derivative (**120**) being considered readily biodegradable (ISO 14593). Changing the ester to an amide (**121**) produced a considerable decrease in biodegradation to almost nothing that could be attributed to the cation, as derivative (**121**) is mostly likely undergoing anion degradation. Further substitution of the pyridinium core, compound (**122**), to give an ester at the 3-position and a primary alcohol on the *N*-position has shown to elevate the biodegradability back up to 71%. Modification of the ester derived pyridinium ILs with a group other than a terminal alcohol at the *N*-position, compound (**123**), leads to a reduction in biodegradation to 25%.

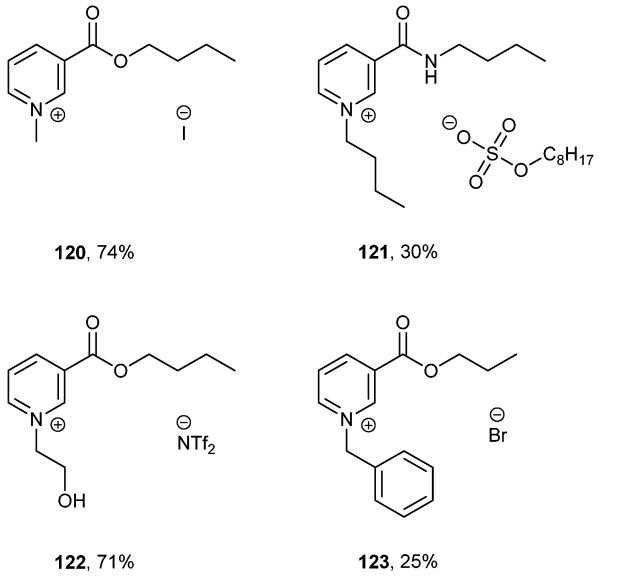


Fig. 35 Nicotinium derived ILs.[17,84] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

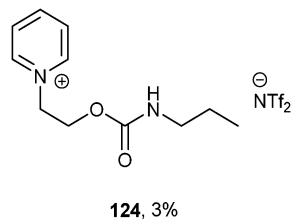


Fig. 36 A carbamate functionalised pyridinium IL.[68] Biodegradation values assessed by the CO_2 headspace test (ISO 14593).

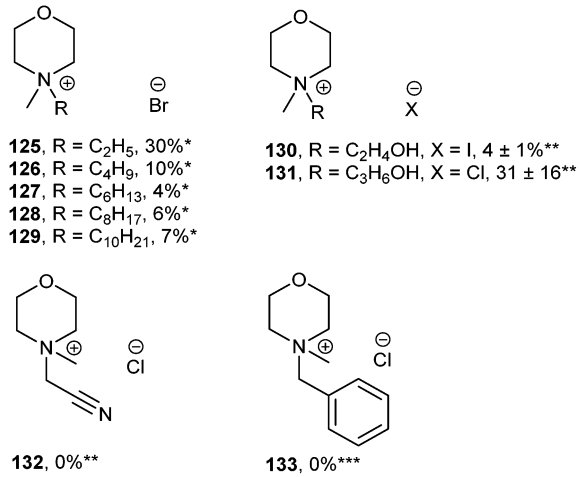


Fig. 37 Biodegradation data for morpholinium ILs.[83,85,86] *Biodegradation values assessed by CO_2 headspace test (ISO 14593). **Biodegradation values assessed by manometric respirometry (OECD 301F). ***Biodegradation values assessed by CBT (OECD 301D).

The rule of thumb observable for increased pyridinium biodegradation appears to be the presence of a primary alcohol attached at the *N*-position with careful introduction of other functionality on the aromatic ring, as required by application.

The last functional transformation attempted was the introduction of a carbamate group with the aim that the carbamate would undergo hydrolysis at this site. This synthetic transformation lead to compound (**124**), Fig. 36, which proved to be recalcitrant to biodegradation at 3%.[68]

4.3.2 Non aromatic cations – morpholinium, DABCO, piperidinium, cholinium, quaternary ammonium – quaternary ammonium cations (QAC's)

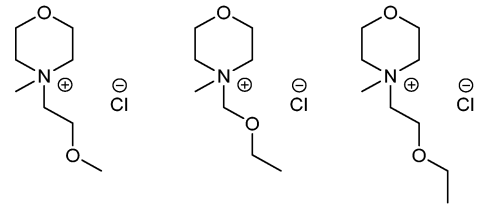
4.3.2.1 Morpholinium, DABCO. The non-aromatic headgroups can be further broken down into two subsets, cyclic and acyclic. The former set includes data from the morpholinium and DABCO (1,4-diazabicyclo[2.2.2]octane) series of ILs presented by Pretti *et al.* 2011, Stepnowski *et al.* 2011 and Neumann *et al.* 2014, see Fig. 37.[83,85,86] A series of ILs were prepared using morpholine and DABCO as headgroups with various extensions of alkyl chains substituted at the *N*-position, Fig. 37, (**125–133**). The shortest alkyl chain morpholinium IL prepared by Pretti *et al.* (**125**) displayed biodegradation (ISO 14593) of 30% after 28 days. If the carbon content of the alkyl chain is taken into account, 2 × C, and the overall carbon count of the molecule is 7 then the 30% biodegradation infers mineralisation of just two carbon atoms, perhaps the alkyl chain, however a metabolite study will only tell if any other portion of the molecule is being metabolised. The original postulation that with an increase in alkyl chain length there is an increase in biodegradation is not observed for this series of ILs. This is an example of how one of Boethling's rules of thumb did not apply to this subset of ILs, as biodegradation is seen to decrease with increasing alkyl chain length, *N*-butyl (**126**), *N*-hexyl (**127**), *N*-octyl (**128**) and *N*-decyl (**129**), Fig. 37. The decreasing biodegradability is in direct correlation with the toxicity results published by the same author demonstrating the increase in toxicity with increase in alkyl chain.[85]

Furthermore, addition of primary ethyl alcohol to the morpholinium headgroup, compound (**130**), gave very poor biodegradation results (OECD 301F).[83] This compound, though analogous to the readily biodegradable pyridinium derivative, Fig. 33, (**109**), only provided ~1% biodegradation. Increasing the length of the alcohol from C2 to C3 increased the biodegradability by up to 30% (**131**). This suggests that the rule of thumb of including primary alcohol groups on the head group to increase biodegradation is questionable for morpholinium derivatives and may be sensitive to chain length.

For the cyano (**132**) and benzyl (**133**) derivatives examined 0% biodegradability (OECD 301F) was observed, however for (**132**) biotic hydrolysis of the cyano group was detected.[83]

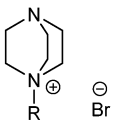
The *N*-ether derivatives (**134–136**) Fig. 38, did not undergo primary biodegradation when examined and agree with the rule of thumb that introducing ether groups can reduce biodegradation. Readily biodegradable screening was not carried out on these derivatives.

Similar results to those observed for the morpholinium ILs were found for the DABCO series of ILs synthesised by Pretti *et al.*, Fig. 39. Decreasing levels of biodegradation (OECD 301F) were observed when alkyl chain lengths longer than ethyl (**137**) were studied for the series (**137–141**), Fig. 39. Similarly,



134, -1 ± 4% **135**, -3 ± 4% **136**, 0 ± 8%

Fig. 38 Morpholinium ILs screened for primary degradation only.[83]



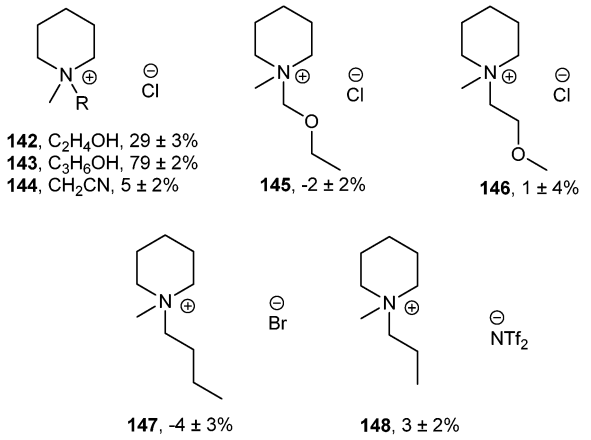
137, R = C_2H_5, 40%
138, R = C_4H_9, 36%
139, R = C_6H_{13}, 23%
140, R = C_8H_{17}, 34%
141, R = $C_{10}H_{21}$, 34%

Fig. 39 Biodegradation data for DABCO ILs (**137–141**).[83,85] Biodegradation values assessed by manometric respirometry (OECD 301F).

toxicity increase as chain length increased was attributed to the reduced levels of biodegradability for these DABCO ILs. As with the ethyl morpholinium IL (**125**), the ethyl substituted DABCO IL, compound (**137**), showed the highest level of biodegradation within its class, with biodegradation of 40% observed for (**137**) after 28 days. However, unlike the morpholinium ILs, in this case there exists a possibility that a portion of the DABCO ring is undergoing biodegradation. The ethyl side chain of (**137**) can only contribute 25% of the theoretical carbon for biodegradation, and 40% is observed, thus some portion of the ring must be undergoing biodegradation. In general the DABCO derivatives biodegraded to a greater extent than the morpholinium derivatives. However the lowest value observed for DABCO IL biodegradation was 23% for the hexyl derivative (**139**). In general the range of values for the analogues (**137–141**) is narrow (23–40%).

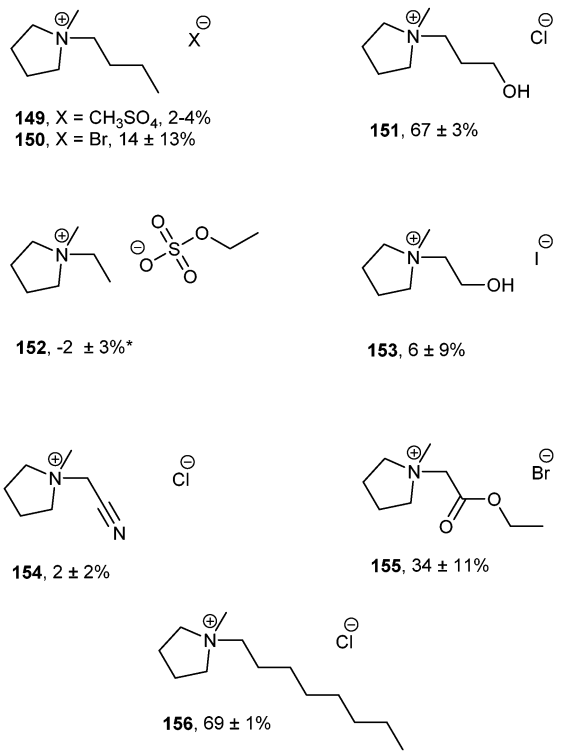
4.3.2.2 Piperidinium. The piperidinium ILs (**142–148**) screened for biodegradability (OECD 301F) by Stolte *et al.* 2014, Fig. 40, show a similar recalcitrance to biodegradation.[83,85] The *n*-propyl alcohol substituted IL, (**143**), was the only derivative from this group to be classified as readily biodegradable. The shorter ethanol substituted piperidinium derivative, (**142**) proved to biodegrade to a lower extent and cannot be classed as readily biodegradable. Although when the test time was extended to 60 days (**142**) had undergone ~85% biodegradation.[83,85] As previously observed, ILs substituted with a single ether group can reduce biodegradability and this was observed for ILs (**145**) and (**146**). Removing the oxygen atom from the *N*-substituted alkyl chains, (**147**) and (**148**) also gave ILs which exhibited poor biodegradation (<5%) in the manometric respirometry test.

4.3.2.3 Pyrrolidinium. Pyrrolidinium IL biodegradation has rarely been examined except for the work carried out by Stolte *et al.* in



142, C_2H_4OH, 29 ± 3%
143, C_3H_6OH, 79 ± 2%
144, CH_2CN, 5 ± 2%
145, -2 ± 2%
146, 1 ± 4%
147, -4 ± 3%
148, 3 ± 2%

Fig. 40 Biodegradation data for piperidinium ILs (**142–148**).[83,85] Biodegradation values assessed by manometric respirometry (OECD 301F).



149, X = CH_3SO_4, 2-4%
150, X = Br, 14 ± 13%
151, 67 ± 3%
152, -2 ± 3%*
153, 6 ± 9%
154, 2 ± 2%
155, 34 ± 11%
156, 69 ± 1%

Fig. 41 Biodegradation data for pyrrolidinium ILs.[83,87] Biodegradation values assessed by manometric respirometry (OECD 301F). *Examined for primary biodegradation only.

2012 and 2014, Fig. 41.[83,87] *N*-Alkyl substituted pyrrolidinium ILs (**149**, **150**, **152**) were observed to be recalcitrant to biodegradation (OECD 301F). Cyano derivative (**154**) also did not undergo biodegradation >2%, though the cyano group was observed to undergo biotic hydrolysis.[83] An *N*-substituted propyl alcohol IL (**151**) was observed to be readily biodegradable (67 ± 3%) whereas the ethyl alcohol IL (**153**) biodegraded <10%. The continuing trend of much greater levels of biodegradation for *N*-substituted C3 alcohols over C2 alcohols is observed here. Ethyl ester derivative (**155**) only provided 34 ± 11% biodegradation.

Upon extension of the alkyl substituent to C8, IL (**156**), a readily biodegradable IL was discovered, with 69% biodegradation observed. This ILs structural features are in agreement with the rules of thumb regarding longer chain lengths being able to provide greater levels of biodegradability, in this case the IL did not inhibit the bacteria of the test medium. The study shows that several of the rules of thumb can be accurately applied for the pyrrolidinium derivatives and is a significant success for the design for degradation concept.

4.3.2.4 Linear ammonium cations. Among the acyclic ammonium cation class are the quaternary ammonium compounds (QAC), such as cholinium and tetrabutyl ammonium and ILs.

The cholinium cation has been selected as a promising choice for the synthesis of biodegradable ILs, due to the high biodegradability (OECD 301D) and low toxicity of cholinium halide salts.[88,89] Thus, providing the cation is appropriately paired with an anion that is biodegradable (**157**, Fig. 42), or an inorganic anion that does not inhibit metabolism, readily biodegradable ILs are a reasonable outcome. Anions derived from organic acids, (see Fig. 15) and the amino acids, (see Fig. 19) have all proven successful. See Section 4.2 on anions for more cholinium IL biodegradation data and appendix structures (**310–322**). Similar protic derivatives synthesised from aminoethanol, to give protic ILs (**54**, **55**) Fig. 15, can also be classified as readily biodegradable, see Section 4.2 for anion choices.

Biodegradation of the cholinium cation however is very sensitive to structural modification. The linear alkyl chain derivative trimethylbutylammonium (TMBA) (**158**), Fig. 43, maintains high levels of biodegradability (OECD 301F) at ~87–88%. However, once the free alcohol is converted to a methyl ether (**159**) a large decrease in biodegradation is observed from 88–90% to 27–29%. No inhibitory toxic effects for ILs (**157–159**) were observed.

Protic IL analogues of cholinium salts, with two ethyl alcohol groups (**160–165**), Fig. 44, or three ethyl alcohol groups (**166–167**) Fig. 45, gave favourable biodegradation results (see compounds (**160–167**)), except when the anion employed was a formate anion (**160**). Carboxylate examples (acetate **161** to pentanoate **165**) pass the manometric respirometry test, and (**166–167**) the triol derivatives (despite failing the test) achieve biodegradation levels very close to a pass (57–59%). In the study carried out by Peric *et al.* 2013 it was shown by LC analysis that the cations had entirely degraded after the 28 day period and anion choice was of more concern, with 7 out of 10 compounds (**54–55**, **161–165**) being classed as readily biodegradable.[69]

Overall the cholinium and the protic quaternary aminoethanol based ILs with methylsulphonates, methylsulphates,

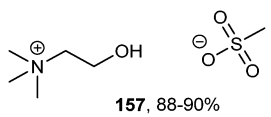


Fig. 42 Readily biodegradable cholinium mesylate (**157**).[87] Biodegradation values assessed by manometric respirometry (OECD 301F).

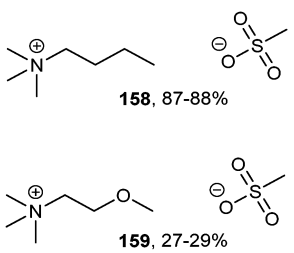


Fig. 43 Quaternary ammonium ILs (**158**, **159**).[87] Biodegradation values assessed by manometric respirometry (OECD 301F).

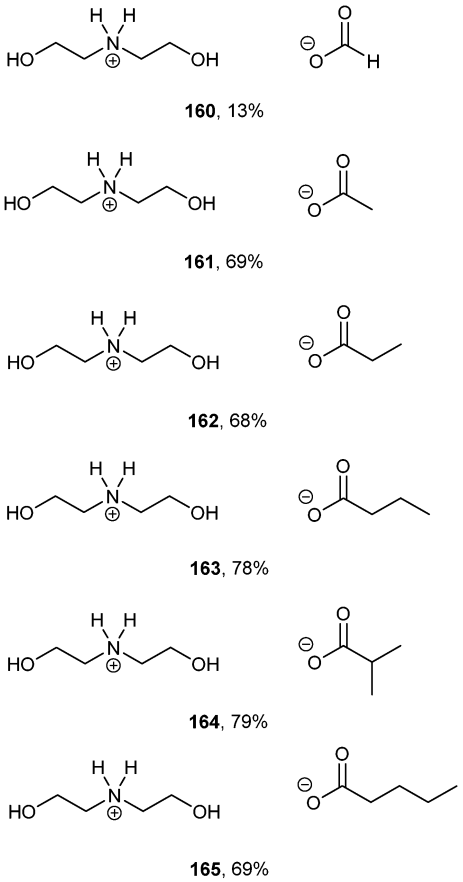


Fig. 44 Biodegradation data for protic aminodiol derived ILs (**160–165**).[69] Biodegradation values assessed by manometric respirometry (OECD 301F).

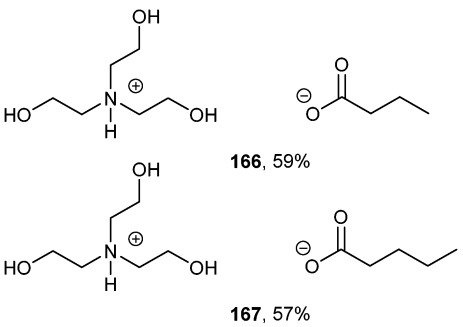


Fig. 45 Biodegradation data for protic aminotriol derived ILs (**166–167**).[69] Biodegradation values assessed by manometric respirometry (OECD 301F).

amino acid carboxylates and some organic carboxylate anions have high biodegradability (OECD 301F).

Choline ILs have also been successfully employed as cosubstrates in the biodegradation of azo dyes.[90] A number of choline salts (lactate, tartrate, saccharinate, dihydrogen phosphate, citrate), were used in conjunction with the bacteria *S. lentus* under optimised conditions to efficiently biodegrade acid blue 113 (92% degradation in 72 h), with choline lactate being the most effective. The degradation products were found to be less toxic than glucose mediated co-degradation.[90]

4.3.2.5 Tetraalkylammonium. One of the popular alkyl ammonium cations is the tetraalkylammonium cation, Fig. 46, with widespread use in the surfactant industry,[91] as antimicrobials,[92] and other applications including phase transfer catalysis.[93] Amongst the QAC's employed in IL research the tetrabutylammonium (TBA) cation stands out as being one of the most widely used. Recently investigations have also been carried out with the dimethyldibutylammonium (DMDBA) cation, Fig. 46.

Biodegradation data (OECD 301D and OECD 310) of TBA ILs has provided no evidence that the TBA biodegrades, Fig. 17. (**56–59**) and Fig. 47, (**168–172**). As can be seen in Fig. 47, TBA ILs with anions synthesised from organic acids and amino acids achieve levels of biodegradation that can be attributed to anion degradation only, with the TBA cation remaining intact. A more detailed metabolite analysis of the breakdown products would be required to confirm this assumption. Similarly, the triethylmethylammonium IL (**173**) examined by Stolte *et al.*[87] Fig. 48, did not undergo levels of biodegradation $>5\%$ in the manometric respirometry test.

A slight modification to the TBA cation produces the DMDBA family of QAC's. This modification, in stark contrast to other tetraalkyl ammonium salts, significantly increases the biodegradability (OECD 301F) of the QAC's, but only when paired with a biodegradable organic anion. In the study conducted by Jérôme *et al.* 2014 it was shown that the [DMDBA][Cl] (**174**) Fig. 49, gave 5% biodegradation after 28 days, however changing to the acetate (**175**) lead to 77% biodegradation after 28 days, a dramatic overall increase. It is also important to note that the acetate anion can contribute only 28% to the overall carbon content of the IL. This strongly suggests that the anion has a crucial effect on the cations biodegradation and could be occurring through a co-metabolic pathway; the cation and anion biodegrading concomitantly.[72] The presence or absence of an organic anion appears to promote (**60**, **175–180**) or prohibit (**173**), respectively, biodegradability for this series of compounds. The ILs studied in this work also showed excellent ability at dissolving cellulose with high recyclability.[72]

Unfortunately the same effect of promoting biodegradation (OECD 301D) through the use of an organic anion is not apparent

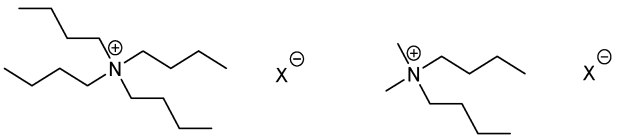


Fig. 46 Tetrabutyl ammonium cation and [DMDBA] cation.

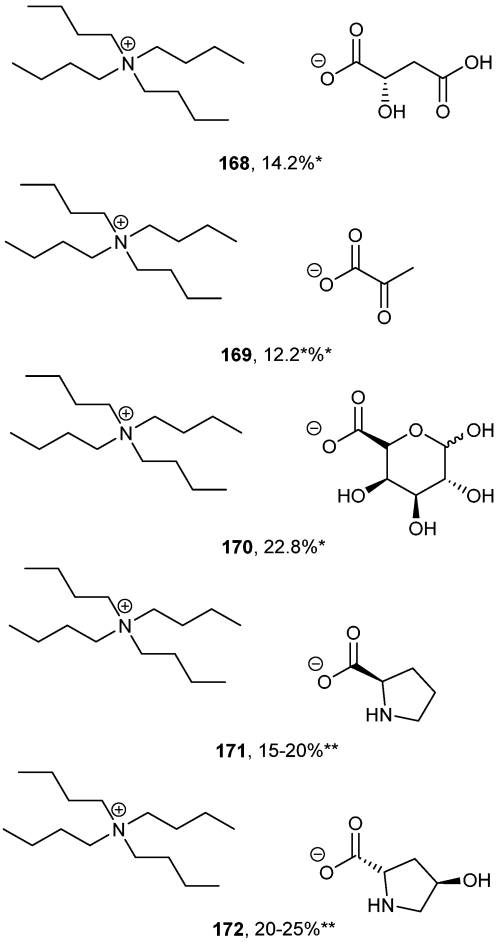


Fig. 47 Biodegradation data for selected tetrabutylammonium ILs.[70,71] *Biodegradation values assessed by CBT (OECD 301D). **Biodegradation values assessed by CO_2 headspace test (OECD 310).

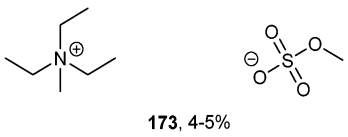


Fig. 48 Triethylmethylammonium IL.[87] Biodegradation values assessed by manometric respirometry (OECD 301F).

for the symmetrical tetraalkylammonium cations (**56–59**) Fig. 17, (**168–172**) Fig. 46, as the results published by Bouquillon *et al.* demonstrates the robustness of the tetrabutyl ammonium cation, even when paired with a biodegradable carboxylate.[70,71]

4.3.2.6 Phosphonium ILs. In 2009 Scammells *et al.*[94] screened a range of phosphonium ILs for their biodegradability (ISO 14593) and, to the authors knowledge, represents the only phosphonium ILs to be examined for their biodegradability to date. The two cations screened were tricyclohexyl- and trihexyl-phosphonium, Fig. 50.[94]

The general trend observed from the tricyclohexyl derived ILs, Fig. 51, was a high resistance to biodegradation even though hydrolysable esters were included as one of the side chains.

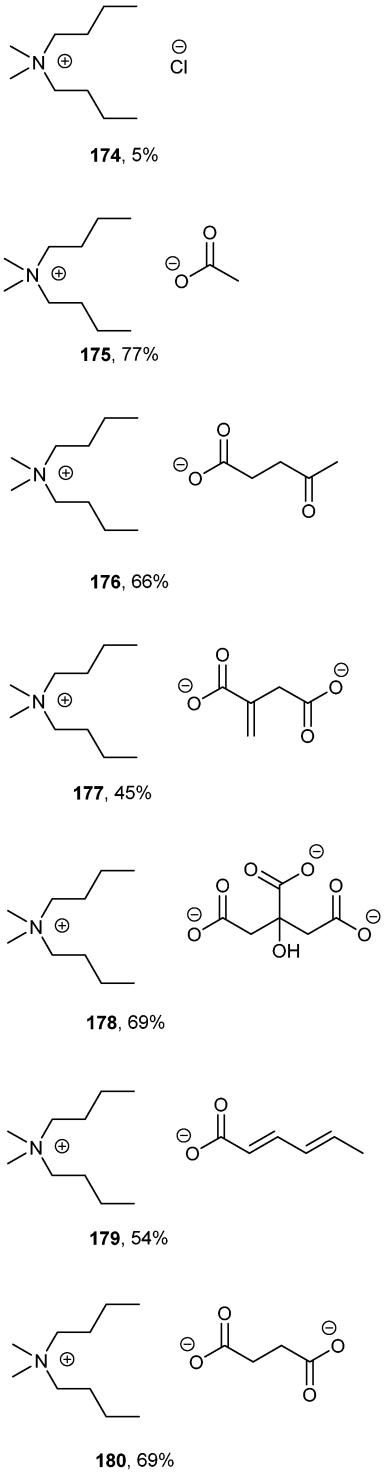


Fig. 49 Biodegradation data for selected DMDBA ILs.[72] Biodegradation values assessed by manometric respirometry (OECD 301F).

For example, compounds (**181–183**) gave between 2–4% biodegradation. Similarly with the ester bond in a different orientation (**190**), only a slight improvement in biodegradation to 9% was observed. It is believed that the steric bulk of the tricyclohexyl rings inhibits access of esterase enzymes to the labile ester bond. Further altering the cation to NTf_2 did not improve

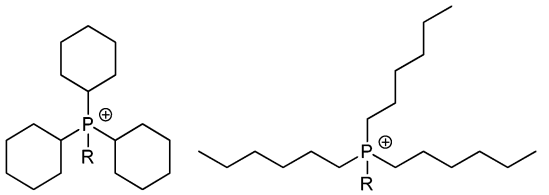


Fig. 50 General structure of phosphonium cations, studied by Scammells.[94]

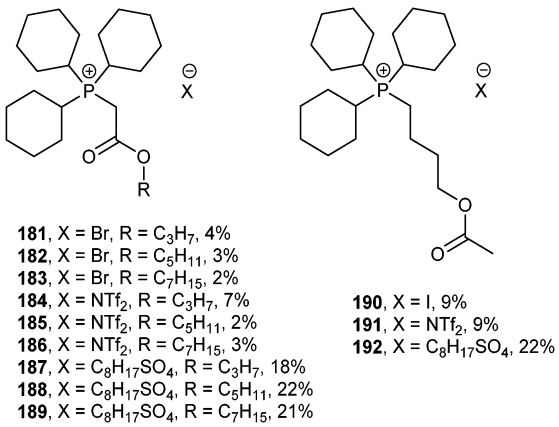


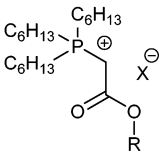
Fig. 51 Biodegradation data for tricyclohexylphosphonium ILs.[94] Biodegradation values assessed by CO_2 headspace test (ISO 14593).

on biodegradability 2–9%, (**184–186, 191**). When the anion was exchanged to octyl sulphate, (**187–189, 192**), the expected increase in biodegradation (up to 22% was observed), but can be solely attributed to the biodegradation of the anion and not the cation.

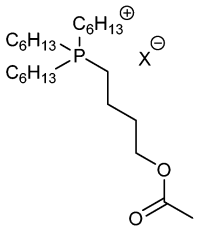
When examining the analogous trihexyl phosphonium derivatives, Fig. 52, a similar recalcitrance to biodegradation (ISO 14593) was observed. Long alkyl chain ester bromide derivatives, (**193–195**), exhibited poor biodegradation of 4–12%. The reverse orientated ester (**202**), allyl (**205**), ether (**208**) and primary alcohol (**211**) halide analogues did not biodegrade (0–2%). The NTf_2 derivatives (**196–198, 203, 206, 209, 212**) and octyl sulphate derivatives (**199–201, 204, 207, 210, 213**) were also screened. In agreement with the tricyclohexyl derivatives results, exchanging the halide for NTf_2 lead to no improvement while the octyl sulphate derivatives gave a range of biodegradation values between 5 and 30%.

4.3.3 C_2 symmetric and dicationic ILs – a new class of ILs. Biodegradation studies of pyridinium cations tethered together using acetal linkers was initially examined by Scammells *et al.*,[68] Fig. 53 (**214–216**). The design rational behind this series of bispyridinium compounds was based on the hypothesis that under biodegradation test conditions the acetal could be hydrolysed to a free alcohol, generating the biodegradable compound (**109**), Fig. 33. Upon investigation, however poor biodegradation was observed, $<5\%$ in the CO_2 headspace test, for the chloride, bistriflimide and PF_6 ILs (**214–216**).

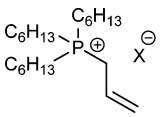
Other bis or dicationic ILs that have been tested for biodegradability include those synthesised and reported by Stolte *et al.* 2014, Fig. 54. The C_2 symmetrical imidazolium structures



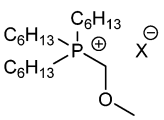
193, X = Br, R = C_3H_7, 12%
194, X = Br, R = C_5H_{11}, 4%
195, X = Br, R = C_7H_{15}, 10%
196, X = NTf_2, R = C_3H_7, 0%
197, X = NTf_2, R = C_5H_{11}, 2%
198, X = NTf_2, R = C_7H_{15}, 0%
199, X = $C_8H_{17}SO_4$, R = C_3H_7, 15%
200, X = $C_8H_{17}SO_4$, R = C_5H_{11}, 20%
201, X = $C_8H_{17}SO_4$, R = C_7H_{15}, 30%



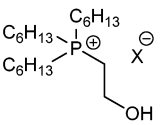
202, X = I, 0%
203, X = NTf_2, 0%
204, X = $C_8H_{17}SO_4$, 5%



205, X = Br, 0%
206, X = NTf_2, 0%
207, X = $C_8H_{17}SO_4$, 18%

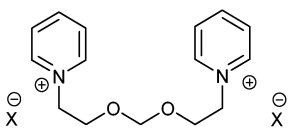


208, X = Cl, 2%
209, X = NTf_2, 0%
210, X = $C_8H_{17}SO_4$, 11%



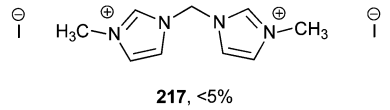
211, X = Br, 0%
212, X = NTf_2, 0%
213, X = $C_8H_{17}SO_4$, 9%

Fig. 52 Biodegradation data for trihexylphosphonium ILs.[94] Biodegradation values assessed by CO_2 headspace test (ISO 14593).

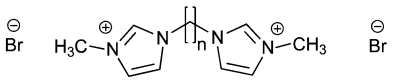


214, X = Cl, 5%
215, X = NTf_2, 4%
216, X = PF_6, 3%

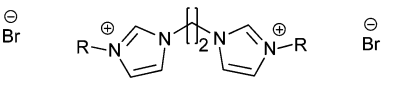
Fig. 53 Bis-pyridinium ILs (**214–216**).[68] Biodegradation values assessed by CO_2 headspace test (ISO 14593).



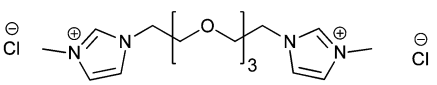
217, <5%



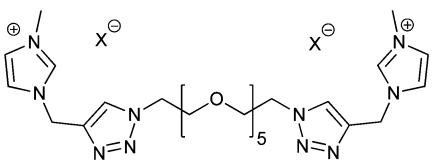
218, n =2, <5%
219, n =3, <5%
220, n =6, 0 ± 0.5%



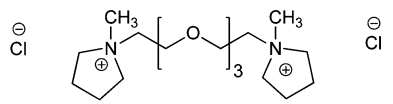
221, R = C_4H_9, <5%
222, R = C_6H_{13}, 2 ± 2.5%



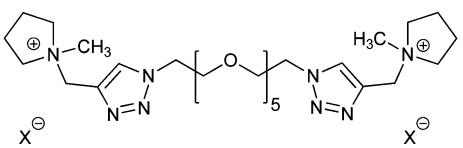
223, <5%



224, X = CH_3SO_4 <5%



225, <5%



226, X = CH_3SO_4, 2 ± 2.5%

Fig. 54 Biodegradation data for dicationic imidazolium and pyrrolidinium ILs (**217–226**) examined by Stolte *et al.*[95] Biodegradation values assessed by manometric respirometry (OECD 301F).

(**217–224**) and the pyrrolidinium structures (**225, 226**) encompass great diversity and complexity in structure; from bis-ILs linked by an alkyl chain (**217–222**) to less lipophilic poly-ether moieties bridging the two cations (**223–226**). Unfortunately, the trend for biodegradability (OECD 301F) for these dicationic ILs is the same as for the pyridinium species (**214–216**). A characteristic strong resistance to biodegradation is observed for these species, including examples containing butyl or hexyl alkyl chains (**221–222**).[95] Biodegradation for this series of compounds by Stolte *et al.* was observed to be $<5\%$.

A number of trends have been observed when reviewing the biodegradation results for all of the ILs encompassed by this review. The trends can be summarised as follows:

- Terminal cyano groups are readily hydrolysable
- Terminal alcohol groups can lead to an increase in biodegradation and are necessary for anaerobic degradation of alkyl chains
- Length of alcohol substituent is important, some ethanol substituted compounds won't degrade yet the propyl derivative undergoes a disproportionate increase in biodegradability.
- Ester bonds are readily hydrolysable but can slow down rate of biodegradation
- Amide bonds are far more robust than esters
- Long alkyl chains help to increase biodegradation except for morpholinium derivatives where it actually decreases

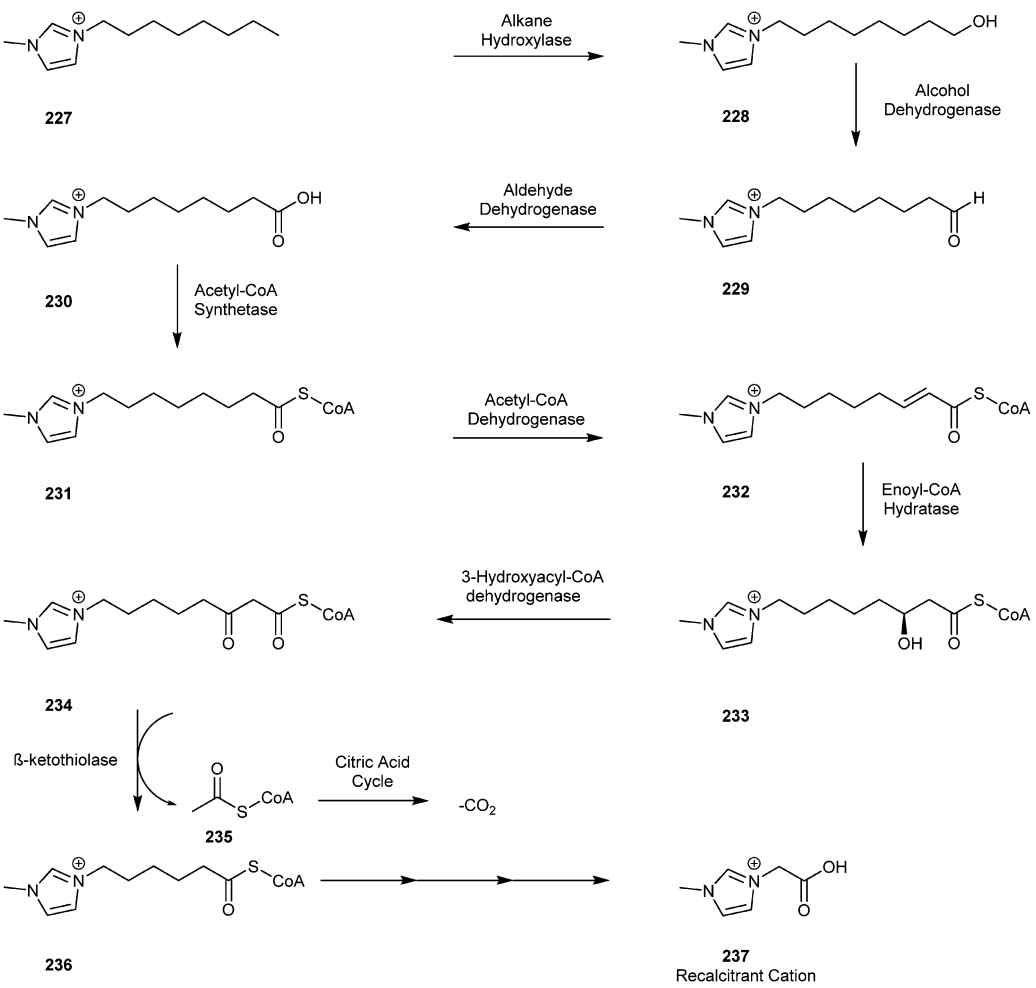
- Amino acid cations are generally readily biodegradable
- Organic acid anions are generally readily biodegradable
- Cholinium cations are generally readily biodegradable but modification leads to a rapid drop off in biodegradation
- Tetrabutylammonium cations are not readily biodegradable yet the DMDBA cations are when paired with an organic anion
- Hexyl and cyclohexyl substituted phosphonium ILs are too sterically hindered to undergo biodegradation
- Non-aromatic cycles can be classed as readily biodegradable but only if an *N*-substituted alcohol of appropriate length is used or an alkyl chain of >8 carbons (however longer chains can increase toxicity)
- Dicationic ILs examined did not undergo biodegradation of $>5\%$

5. Metabolite studies

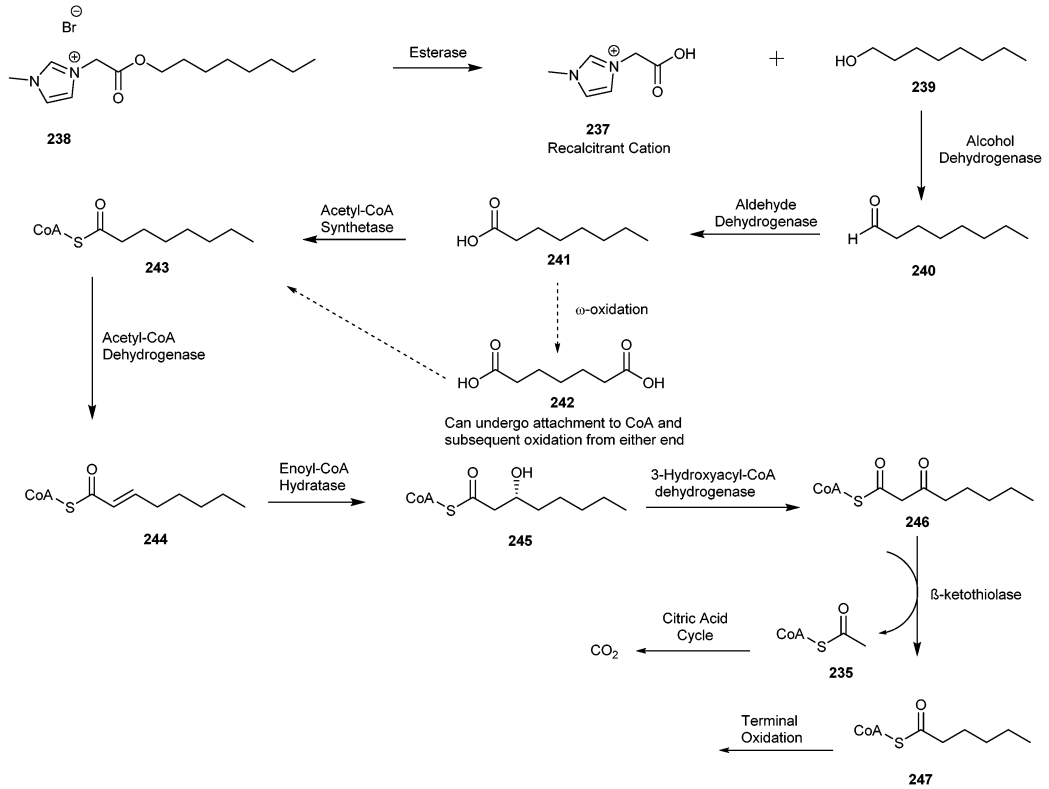
An active area in IL biodegradation research has been metabolite studies. It has been acknowledged that a molecule can pass the readily biodegradable threshold but a portion may remain indefinitely in the environment. If a non-biodegradable metabolite (or even a metabolite that degrades at a slower pace) is toxic then its release into the environment through biodegradation pathways is a detrimental effect in what would otherwise seem to be a green process. The 4th Principle of Green Chemistry tells us that chemicals should be designed to be both functional with minimal toxicity; therefore examining the toxicity of metabolites is a parameter of consequence.[7]

Multiple methods of metabolite detection have been successfully employed including LC/MS techniques,[96] MALDI,[97] GC/MS[36] *etc.* In the previous review published by Coleman and Gathergood in 2008, the metabolite studies of 1-butyl-3-methylpyridinium bromide[98] and 1-octyl-3-methylimidazolium chloride[61] were discussed in detail. Since then a number of metabolite studies have been performed analysing the breakdown paths of ILs in OECD approved biodegradation tests and in more complex systems. Forays into predicting breakdown pathways have also been made using computer modelling.

Two of the most common pathways examined in biodegradation and metabolism prediction are β-oxidation and/or ω-oxidation of fatty acid or long alkyl chain residues, Scheme 1 (**227** to **237**), or the hydrolysis of susceptible bonds such as esters and amides followed by the β-oxidation and/or ω-oxidation of any fatty



Scheme 1 Potential β-oxidation pathway of an alkyl imidazolium IL.



Scheme 2 Potential β-oxidation pathway of an ester derived imidazolium IL.

acid residues, Scheme 2 (**238–247**). ω-Oxidation occurs with oxidation occurring on both ends of the alkane residue to from a dicarboxylic acid (**242**).[99]

Using a combination of IC and LC-MS, pyrrolidinium, morpholinium, piperidinium and imidazolium ILs were examined for headgroup biodegradation, by Stolte *et al.* in 2014.[83] The biodegradation and metabolites are shown in Fig. 55 for the pyrrolidinium headgroup.

Stolte also showed that a number of cations (pyrrolidinium (**1**, **154**); morpholinium (**131**); piperidinium (**142**, **143**) and pyridinium (**111**, **112**)) could be classified as inherently biodegradable after extended periods of time, >28 days, Fig. 56. For piperidinium (**142**, **143**) up to 80% biodegradation after 60 days was observed, again classifying these compounds as inherently biodegradable. Supporting other aforementioned data on the imidazolium headgroup, no biodegradation was observed for these compounds.

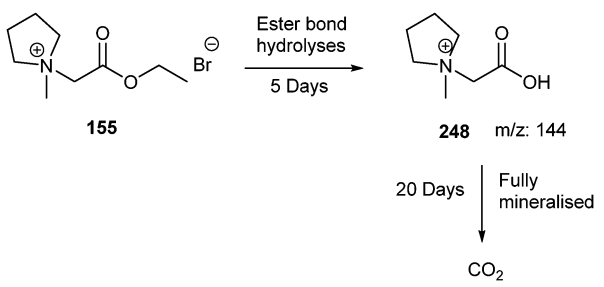


Fig. 55 Biodegradation of pyrrolidinium headgroup.

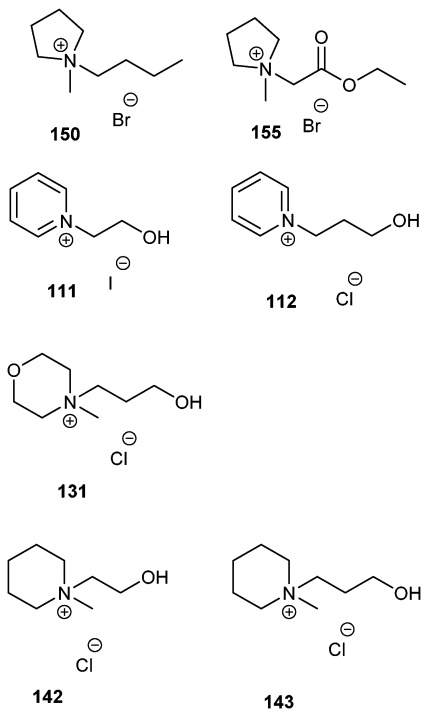


Fig. 56 Inherently biodegradable ILs; effect of cations by Stolte.[83]

For more robust ILs the aerobic degradation process is much longer, as was observed by Gendaszewska *et al.* 2014.[43] After 28 days, limited aerobic oxidation of the 1-methylimidazolium

hexyl side chain (**100**), Fig. 29, to an aldehyde had been detected and a greater proportion of the hexyl substituted methylimidazolium IL was detected by MS-MS showing that the biodegradation had progressed along the β-pathway very little (total biodegradation of ~9%).

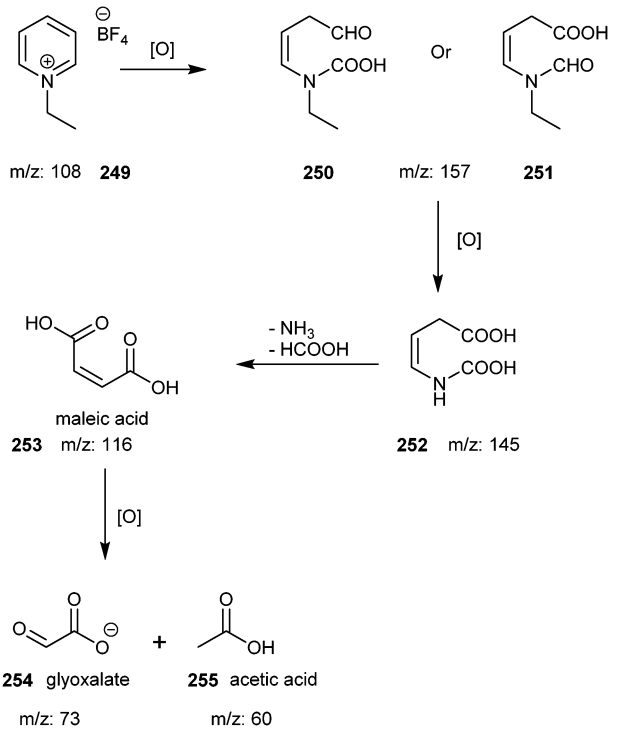
5.1 Aerobic degradation metabolite studies

Docherty *et al.* 2010 reported the breakdown of three pyridinium ILs, [bmpyr][Br], [hmpyr][Br], [ompyr][Br] into their respective metabolites by GC-MS analysis. The degradation pathways were investigated and in this seminal work, metabolites were screened for their toxicity against *daphnia magnia*. The conclusion of this study on IL metabolites showed that the metabolites of these particular pyridinium ILs were less toxic than the parent ILs.[96] The results were discussed in the previous review published by Gathergood *et al.*[21]

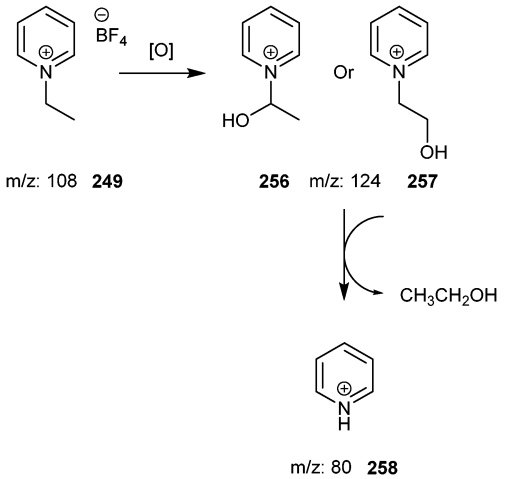
5.2 Pyridinium

The total metabolism of two pyridinium ILs [EtPy][BF_4] and [EtPy][CF_3COO] was successfully demonstrated by Zhang *et al.* 2010 when an axenic culture of bacteria (*Corynebacterium* sp.) demonstrated ring opening of the pyridinium cation (**250** or **251**) before the ethyl chain underwent oxidation to (**252**), Scheme 3. The study also demonstrated that the same bacteria could not successfully breakdown a methylimidazolium IL [bmim][PF_6] and would suggest that different cultures of bacteria are involved in degrading different classes of ILs.[63]

The metabolic breakdown of [EtPy][BF_4] by an axenic culture of *P. fluorescens* was successfully analysed by Zhang *et al.* 2011,



Scheme 3 Breakdown pathway of [EtPy][BF_4] by an axenic culture of *Corynebacterium*.



Scheme 4 Metabolism of [EtPy][BF_4] by an axenic culture of *P. fluorescens*.[100]

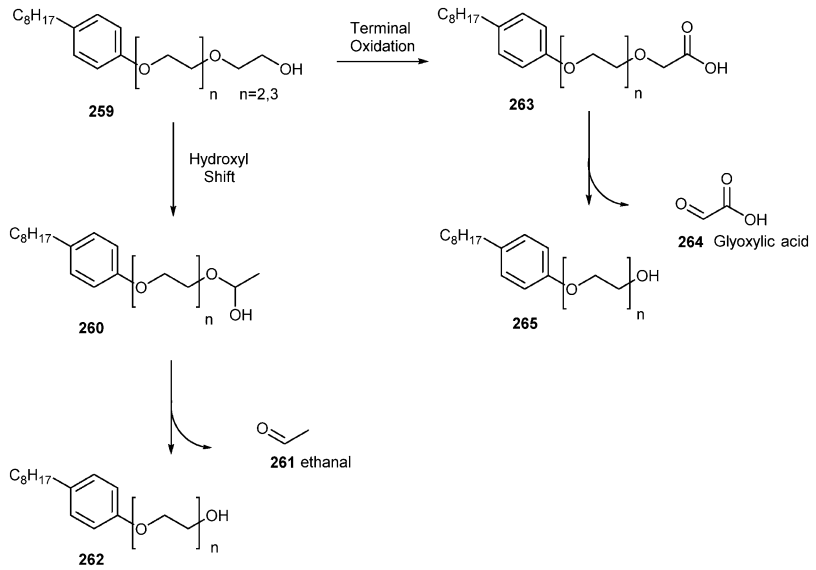
Scheme 4, when they published the following breakdown pathway. The study suggests that *N*-ethyl pyridinium (**249**) degrades by oxidation of the ethyl side chain (**256** or **257**) followed by the elimination of a molecule of ethanol and the formation of a pyridinium salt (**258**).[100] The choice of bacteria for this biodegradation test appears to have altered the metabolic breakdown pathway. In the first test described in Scheme 3, the pyridinium ring is completely mineralised by the *Corynebacterium* strain, yet *P. fluorescens* cannot completely mineralise the ring within the 48 hours of the test described in Scheme 4.

5.3 Sidechain studies

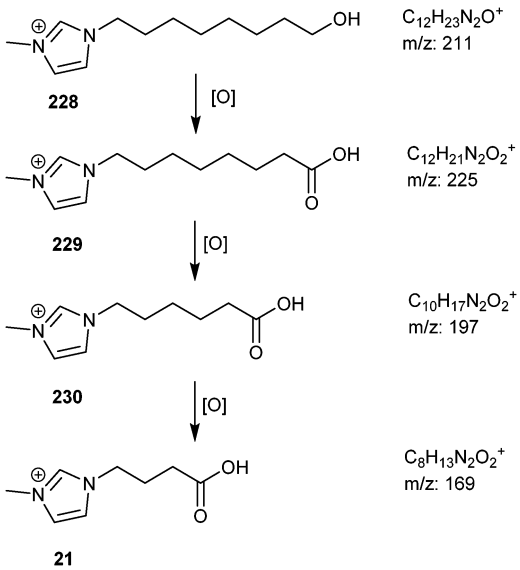
Other metabolic pathways for ethoxylated molecules include terminal oxidation and hydroxyl shift metabolism, Scheme 5. Sato *et al.* 2001 demonstrated with the surfactant octylphenol polyethoxylate that the chain could be broken down by two possible pathways. Though these molecules are not ILs, a lot can be learned from potential biodegradation pathways of ethoxylated chains which have been previously employed in IL synthesis.[67] The metabolites were analysed by MALDI-MS.[97]

5.4 Anaerobic degradation metabolite studies

The 2010 investigation carried out by Neumann *et al.* on the anaerobic biodegradation of ILs included a GC-MS analysis of the imidazolium IL (**228**), see anaerobic breakdown pathway Scheme 6. It is important to note that under the anaerobic denitrifying conditions, biodegradation does not initiate without the presence of the free alcohol site on the alkyl chain. The anaerobic degradation pathways differ to the aerobic pathways in that oxygenase enzymes cannot insert oxygen into the terminus of alkyl chains under anaerobic conditions. The addition of a terminal alcohol could therefore allow some methylimidazolium ILs an alternate biodegradation pathway, however 318 days were required for the last metabolite identified (**21**) to be detected.[52] Anaerobic degradation can also take place by a fumarate addition mechanism.[101]



Scheme 5 Breakdown pathway of a poly ethoxylated sidechain.



Scheme 6 Anaerobic biodegradation of an imidazolium IL.[52]

6. Computer modelling, predictability and databases available containing IL biodegradation data

- ADMET – absorption, distribution, metabolism, excretion, toxicity
- TSAR – thinking in structure–activity relationships
- SBR – structure biodegradation relationships

Aside from the physical aspects of measuring biodegradability of ILs there has emerged a drive to predict biodegradation using *in silico* techniques. Modelling software has been successfully used during drug development to screen drug activity and ADMET parameters and it is this ideology and technology that has been adapted for use in IL and surfactant biodegradation prediction. Current software that is available includes TOPKAT,[102] META,[103] BIOWIN,[104] VEGA,[105] START,[106] (also part of ToxTree) and CATABOL.[107] The software suites can have mixed results in predicting biodegradation pathways, in part due to the lack of available biodegradation data sets. In 2013, Benfenati *et al.* conducted an in depth study of the a number of the most popular biodegradation prediction suites (VEGA, TOPKAT, BIOWIN 5 and 6 and START) for the potential use in regulation under the EU REACH legislation or the "Registration Evaluation Authorization and Restriction of Chemicals". A dataset of 722 compounds was chosen and the software packages were examined under the following headings: accuracy, sensitivity, specificity and Matthew's correlation coefficient (MCC). BIOWIN, TOPKAT and VEGA performed the best out of the examined software. VEGA and TOPKAT gave 88 and 87% accuracy in biodegradation prediction with BIOWIN 6 and 5 performing slightly lower at 83 and 82% and START following up with 70%. Overall it was concluded that BIOWIN, TOPKAT and VEGA could be employed for legislative purposes.[108] However in a critical review by Rucker *et al.* 2012 it was demonstrated that there were issues present with using prediction software. The major downfalls being the difficulty in reproducing biodegradation data due to the inherent issues associated with bacteria and the variability they introduce into a system. Other issues include the secrecy behind some of the datasets used due to their sensitive nature in industry. Other biodegradation data never sees the light of day and remains under industry redaction permanently.[109]

Currently a centralised database of some IL biodegradation data has been established by MERCK in association with UFT Bremen. There currently resides an ever growing library of data on IL – and other associated materials such as starting materials – biodegradation. With the ever increasing datasets available in "UFT/Merck ILs Biological Effects Database" more information about potential eco-persistence posed by ILs can potentially be determined before their synthesis even begins.[110]

The SBR approach in conjunction with Boethling's rules of thumb for designing biodegradable compounds[74] has led to successfully producing some of the first biodegradable ILs. Inspired by design strategies successfully implemented by the surfactant industry, the incorporation of ester linkages was demonstrated to promote biodegradability in a range of previously recalcitrant imidazolium ILs.[80] Similar design strategies include adding susceptible oxygenated groups such as aldehydes, carboxylic acids and alcohols have all shown to promote biodegradation. Hydrolysable amides, although more stable to chemical hydrolysis than esters, can also potentially promote biodegradation. By considering how these molecules and their functional groups will interact in the environment and especially in WWTP's will allow for a more streamlined synthesis that promotes increased biodegradability. Will a particular bond interact with an enzyme to enhance breakdown? Or can this particular anion be used as a carbon source for aerobic digestion? Will a particular cation adsorb to a sewage floc or into soil and therefore be removed from aerobic degradation pathways altogether? These are the types of design questions that need to be considered when discussing an ILs structure and its potential interactions in the environment.

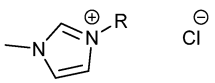
Combining the SBR approach with computer modelling will be the ultimate goal for rational design of sustainable and biodegradable green ILs. A well informed and designed synthesis, under the 10th principle of green chemistry ''Design for Degradation'', in conjunction with computer modelling of biodegradation can and will aid the design of the next generation of ILs.

The greatest challenge with using *in silico* methods to predict biodegradation of ILs is that in many cases charged compounds, especially QAC's, are not suitable candidates for these models, (*e.g.* BIOWIN). In addition, as the majority of ILs screened up to 2010 did not pass readily biodegradable tests, despite the diversity of structures present, they collectively formed a biased training set for the model. However, as this review illustrates, the number of readily biodegradable ILs is increasing, and we believe a more balanced training set is now available.

7. Abiotic degradation – chemically induced degradation in advanced systems

Currently there is interest in the area of abiotic degradation of ILs due to the relatively low numbers of ILs passing readily biodegradable tests.[21] Thus, solving the problem of poor biodegradability by investigating alternative methods to biological degradation has been examined including: advanced oxidation systems,[36] peroxide,[39] UV degradation,[111] electrochemical[37,38] and ultrasonic degradation.[39]

With the increasing use of ILs in technological applications, the chance of release into the environment has grown. Environmental persistence of these compounds has already been postulated and current advanced oxidation process (AOP) technology



8, R = C_2H_5
9, R = C_4H_9
12, R = C_6H_{13}
13, R = C_8H_{17}

Fig. 57 Commercially available ILs tested under UV/H_2O_2 oxidation conditions.[36]

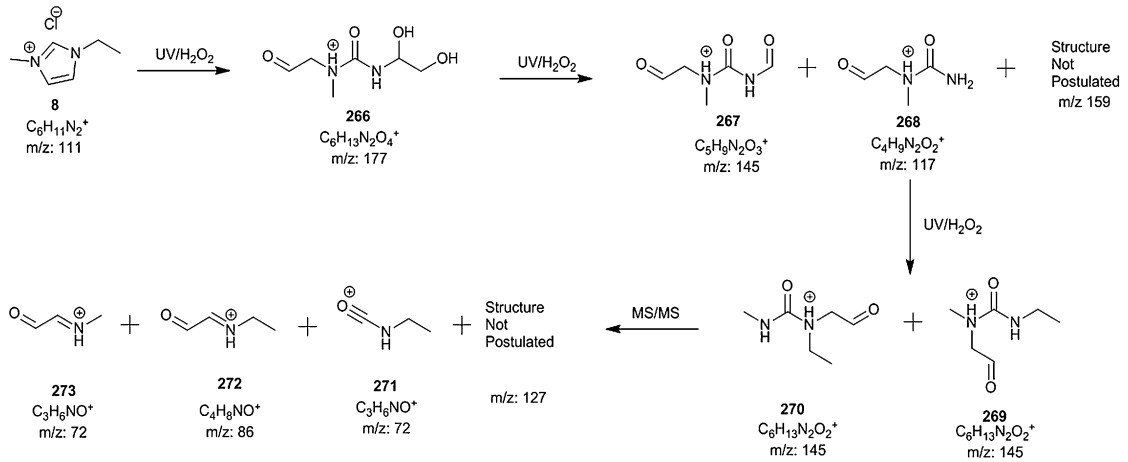
has demonstrated that where ILs have failed biodegradation tests, AOP's can offer an alternative method of degradation. One of the most common AOP's that can potentially be used in WWTP's is the photolysis of hydrogen peroxide. This process involves irradiating H_2O_2 with 400 nm UV light causing rapid generation of hydroxyl radicals.

Stepnowski *et al.*[36] showed, by examining the degradation products formed under an advanced oxidation system (hydrogen peroxide 0.2% treatment time 2 h UV Lamp 254/366 nm), that the imidazolium core of ILs (**8, 9, 12, 13**), Fig. 57, is the first part of the molecule to be affected and not the alkyl chain. It was also shown that the imidazolium ring was cleaved and lost its aromatic properties under the test conditions. Other observations include various hydroxyl groups inserted on the alkyl chains and carbonyl insertion into the cleaved imidazolium ring, some of the observed degradation products (**266–273**) for [emim][Cl] are illustrated in Scheme 7.[36]

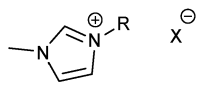
A real, applicable, benefit of an oxidative pre-treatment is that the imidazolium core, which has been shown not to break down under standard aerobic biodegradation conditions, has ring opened (**266**), Scheme 7. The insertion of carbonyl groups and hydroxyl functionalities also potentially allows for more rapid biodegradation as free hydroxyl groups have previously shown to enhance biodegradation of alkyl chains.[21]

Apart from UV/H_2O_2 oxidation, a number of other methods have been successful in the area of abiotic degradation. Ultrasonic breakdown of ILs in solution was demonstrated by Tsang *et al.* when they showed that a number of [Rmim] ILs (R = C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}), Fig. 58, could be broken down in a H_2O_2/acetic acid/ultrasonic system at a temperature of 50 °C. This system performed to a much higher degree than systems based on the individual components used in the final degradation experiment, see Fig. 59. Under the optimised test conditions >74% degradation was achieved for all compounds examined after 3 hours, and after 12 hours of test time >93% degradation of all ILs examined had been reached. >98% degradation of all 8 of the commercially available ILs was achieved within 72 h, Table 12.[39] The degradation pathways were also examined using GC-MS, Scheme 8. The proposed degradation products (**276–283**) were similar to a number of those reported by Stepnowski *et al.*, Scheme 7.[36] Two of the end products were determined to be biurea and acetoxyacetic acid, both ''low-toxicity'' molecules.[39]

Further methods of IL degradation in AOP's include ozonolysis[112] or employing the Fenton reaction.[113] Ozonolysis results published by Pernak *et al.* 2004 demonstrated the effective



Scheme 7 Possible breakdown products detected by HPLC/MS for [emim][Cl] IL. Further fragmentation was analysed by MS/MS.



3, R = C_4H_8, X = BF_4
4, R = C_4H_8, X = PF_6
8, R = C_2H_5, X = Cl
27, R = C_4H_8, X = Br
31, R = C_6H_{13}, X = BF_4
274, R = C_3H_7, X = Cl
275, R = C_5H_{11}, X = Cl

Fig. 58 Structures of the commercially available ILs screened.[39]

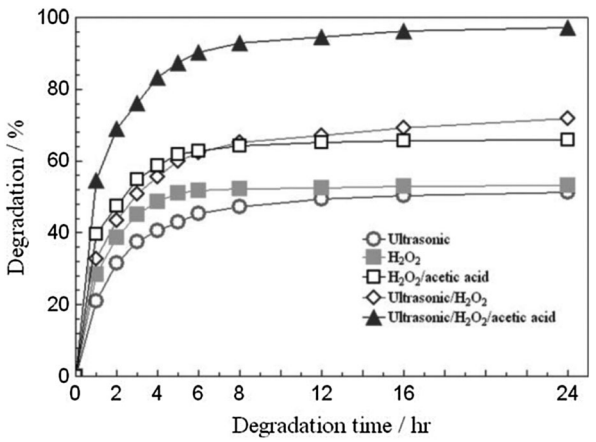


Fig. 59 Radical oxidation of [bmim][BF_4] under various oxidising environments.[39] Reproduced from ref. 39 with permission from the Royal Society of Chemistry.

degradation of 98 pyridinium salts by ozonation within 30 minutes, examples of which include IL (**284**) Fig. 60 and 61. The destruction of the IL is rapid and complete (100% degradation).

In the Fenton reaction, H_2O_2 in combination with an iron catalyst, are used to produce hydroxyl radicals. However, a strong anion effect was observed on reaction time with the ability of [Cl] ions from the IL to form Fe^{3+} chloride complexes and reduce the rate of radical production, Fig. 62. [bmim][CF_3SO_4] was completely

Table 12 Oxidative degradation of alkyl imidazolium ILs assisted by acetic acid and ultrasonic irradiation[a].[39] Reproduced from ref. 39 with permission from the Royal Society of Chemistry

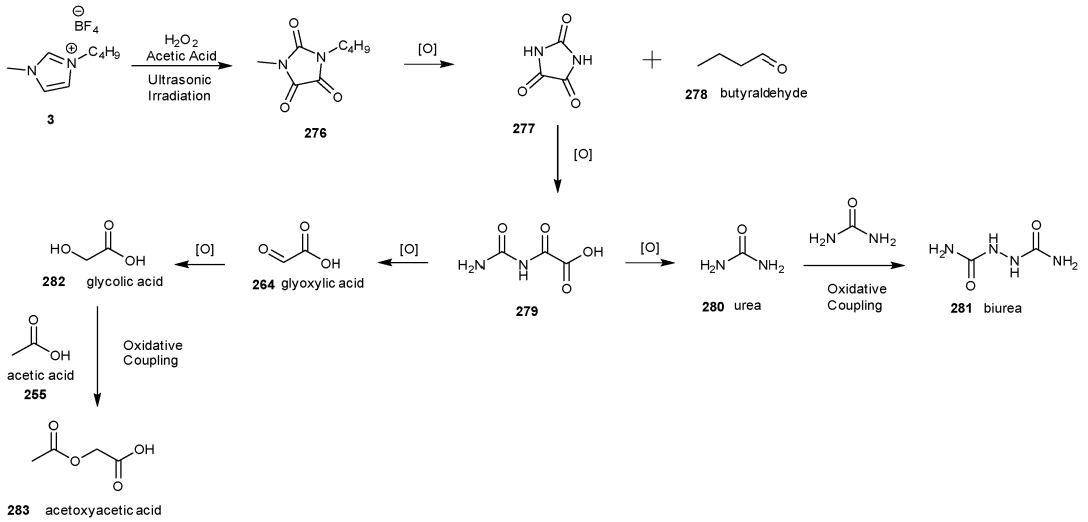
	Degradation (%)					
ILs	1 h	3 h	5 h	12 h	24 h	72 h
C_2mimCl	53.49	76.55	86.62	95.09	96.27	98.35
C_3mimCl	52.65	74.26	85.71	93.08	96.22	98.13
C_4mimCl	54.60	76.07	87.45	94.58	97.25	99.16
C_4mimBr	55.35	76.91	87.33	93.66	96.47	98.75
C_4mimBF_4	54.78	75.25	86.29	94.18	96.87	98.89
C_4mimPF_6	53.26	77.47	87.63	95.38	97.76	98.47
C_5mimCl	55.11	75.63	86.71	93.54	97.18	99.31
C_6mimBF_4	54.38	76.25	87.32	94.61	96.55	98.68

[a] 25 mL solution, ILs: 2.5 mM, H_2O_2: 21 mM, CH_3COOH: 10 mM, at 50 °C.

degraded after 45 minutes and its tricyanomethide analogue within one hour.[113]

ILs have also been examined for their use in electrochemical systems as electrolytes due to their attractive properties as non-volatile tuneable solvents.[27] The harsh conditions potentially present in some electrochemical applications has caused reported breakdowns of some of the ILs used.[37] Witkamp *et al.*[37] demonstrated in 2006 that the use of semi-empirical PM3 quantum calculations allowed for the successful prediction of IL breakdown in their electrochemical systems, including the range of decomposition products. The electrochemical conditions involved applying a voltage difference of 8 V across the electrodes for 3 hours at room temperature. The decomposition products were subsequently detected by GC-MS. ILs examined were [bmim][BF_4] (**3**) and [bmpyrrol][NTf_2] (**47**), Fig. 63.

Pyrrole derivative (**47**) was observed to break down into a range of products including methylpyrrolidine, octanes, octenes, 2-butanol, dibutylmethylamine and butylpyrrolidine, Scheme 9, whereas with the 1-methylimidazole derivative (**3**), radicals were formed that reacted with each other in a radical–radical coupling reaction and in a disproportionation reaction.[37] The successful application of quantum calculations in decomposition products shows the potential for predicting IL



Scheme 8 Proposed degradation scheme by Li *et al.*[39]

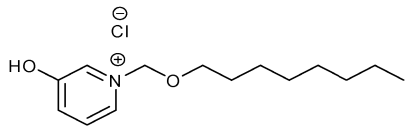


Fig. 60 Example of a pyridinium salt synthesised by Pernak *et al.*[112] and examined for degradation in an AOP.

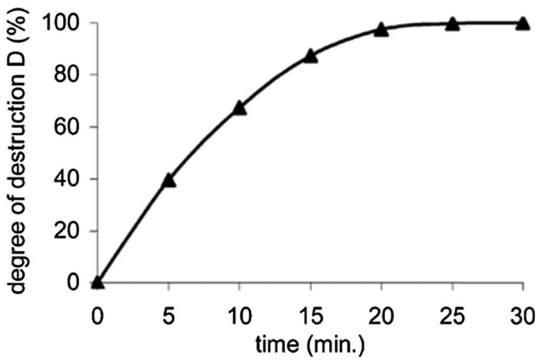


Fig. 61 Degree of destruction (%) *vs.* time (min) for a pyridinium salt (**284**), examined by Pernak *et al.*[112] Reprinted with permission from J. Pernak and M. Branicka, *Ind. Eng. Chem. Res.*, 2004, **43**, 1966–1974. Copyright 2004 American Chemical Society.

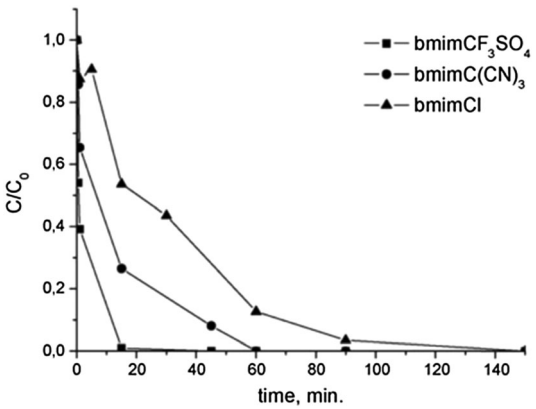


Fig. 62 Observed rate of degradation for [bmim][Cl] is slower than [bmim][CF_3SO_4] or [bmim][$C(CN)_3$].[113] Reprinted from E. M. Siedlecka, M. Golebiowski, Z. Kaczynski, J. Czupryniak, T. Ossowski and P. Stepnowski, *Appl. Catal., B*, 2009, **91**, 573–579. With permission from Elsevier. Copyright (2009).

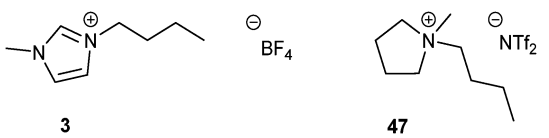
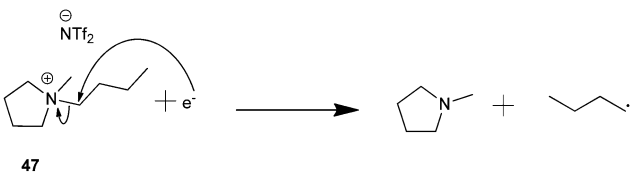


Fig. 63 [bmim][BF_4] (**3**) and [bmpyrrol][NTf_2] (**47**).

suitability for task and how the decompositions will affect their usefulness.

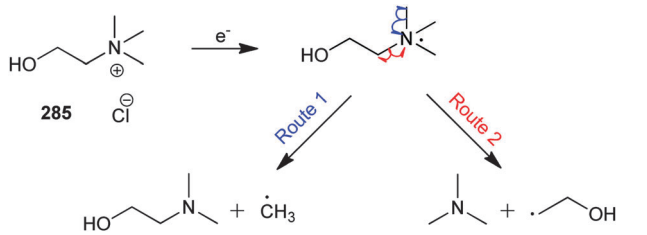
Similarly Van der Bruggen *et al.* demonstrated the breakdown of choline based ILs in a range of electrochemical applications, Scheme 10.[38]



Scheme 9 Example of electrochemical decomposition of a pyrrolidinium IL.

8. Biodegradable surfactants: an IL by another name

Current designs of biodegradable ILs are based on mid-20th century successes in the field of biodegradable surfactants.[114] The drive to replace persistent and low biodegradability surfactants and fabric softeners has seen some great advances in the past 50 years. For instance, the replacement of "DHTDMAC"



Scheme 10 Electrochemical decomposition of a choline chloride (**285**).[38]

(di-hydrogenated tallow dimethylammonium chloride) with derivatives incorporating hydrolysable amide or ester linkages, example DEEDMAC,[115] demonstrated increased biodegradability and reduced foaming in sewage treatment plants. There are four major classes of surfactant: non-ionic, anionic, cationic and zwitterionic. ILs hold much in common with the ionic derivatives as the cationic surfactants are usually derived from quaternised nitrogen salts, and the anionic derivatives derive their charge often from sulphonate salts. The drive to produce biodegradable surfactants is now cemented by the European Detergents Regulation which came into law on October 8 2005 and states that "*all surfactants used for domestic detergents have to be ultimately biodegradable*". An exception is also made for some surfactants for use in the industrial and institutional sector "*which are primarily but not ultimately biodegradable only for very special purposes and after having obtained a derogation based on risk assessment and benefits evaluations*".[116] With this regulation in place, designing surfactants for use in the European market requires that they are biodegradable by law.

Since the implementation of the law a number of biocompatible surfactant designs have been published. In 2010 Perez *et al.* published results on arginine based surfactants synthesised using enzymatic catalysis.[117] Three candidates were screened for their ready biodegradability and all three passed the CBT with 79–90% biodegradation values being recorded. All three surfactants contain amide and ester bonds and none showed recalcitrance to biodegradation. It is postulated that the use of natural building blocks in surfactants and ILs, such as amino acids, allow for easier recognition by enzymes and accessibility to a wider range of amino acid specific degradation pathways.

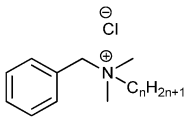
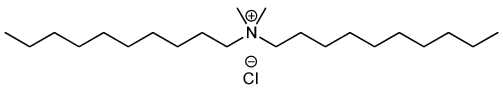
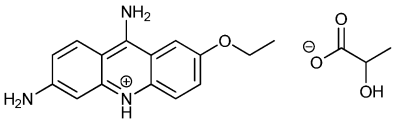
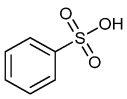
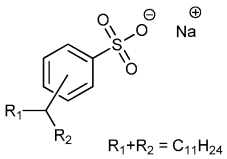
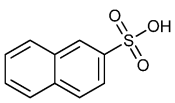
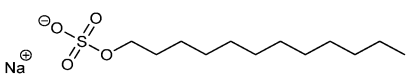
Further work carried out on bis-arginine gemini surfactants by Lozano *et al.* 2011 further demonstrated the effectiveness of using natural building block in synthesising biocompatible surfactants. It was suggested that the high levels of biodegradation may be attributed to the formation of biodegradable intermediates during the biodegradation process such as arginine and glycerol.[118]

A number of key points regarding surfactants (also relevant to ILs), were illustrated in the review published by Ranke *et al.* 2007[119] and are summarised as follows:

Cations:

- Medium alkyl chain length pyridinium cations are potential candidates for producing readily biodegradable surfactants
- Alkylpyridinium surfactants are poorly biodegradable
- Dialkyldimethyl-ammonium and alkyldimethylbenzyl-ammonium quaternary compounds are less biodegradable than

Table 13 Biodegradation of surfactants and mixtures of surfactants[120]

Substance	% biodegradability CBT[a]
 n = 8, 10, 12, 14, 16, 18	−8
BAC – benzalkonium chloride 	4
DDMAC – didecyldimethylammonium chloride 	13
EL – ethacridine lactate 	25
BSA – benzene sulphonic acid 	50
LAS – linear alkylbenzene sulphonate 	12
NSA – naphthalene sulphonic acid 	69
SDS – sodium dodecyl sulphate	
BAC : BSA 1 : 1	45
BAC : LAS 1 : 1	13
BAC : NSA 1 : 1	45
BAC : SDS 1 : 1	39
DDMAC : LAS 1 : 1	11
DDMAC : NSA 1 : 1	−2
DDMAC : SDS 1 : 1	15
EL : BSA 1 : 1	1
EL : LAS 1 : 1	23
EL : NSA 1 : 1	16
EL : SDS 1 : 1	36

[a] Biodegradation values assessed by the CBT OECD (TG 301D).

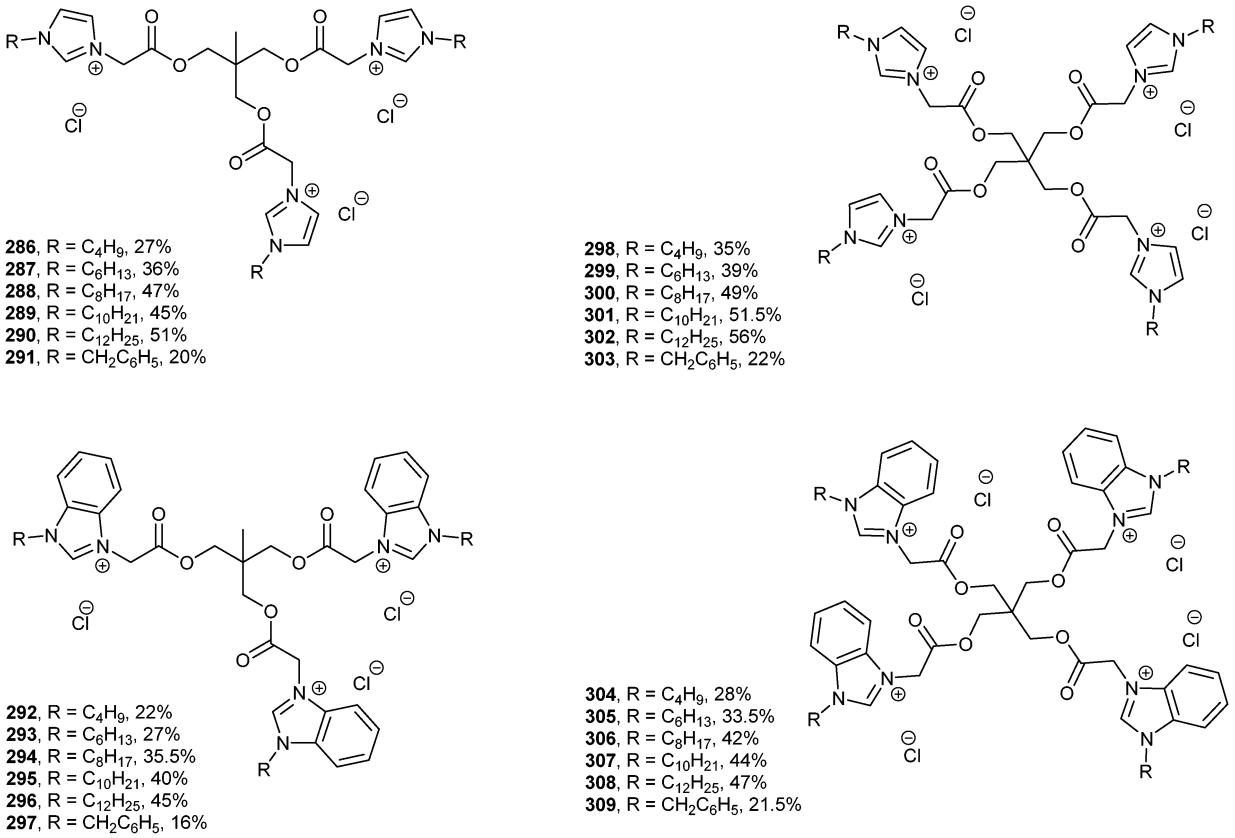


Fig. 64 Tris- and tetrakis-imidazolium and benzimidazolium IL surfactants examine for their biodegradability.

monoalkyltrimethyl-ammonium quaternaries but more biodegradable than alkyl pyridinium surfactants

Biodegradability: monoalkyltrimethyl-ammonium > dialkyldimethyl-ammonium and alkyldimethylbenzyl-ammonium > alkylpyridinium

Anions:

- Linear alkylsulphates exhibit excellent biodegradability
- Linear alkyl sulphonates and alkylbenzene sulphonates show good biodegradability

Surfactants are screened for biodegradation in the same tests that can be employed for ILs, *vide supra*. When released to the environment or indeed to a WWTP, surfactants are not treated as single compounds but as complex mixtures. In 2008 Kümmerer *et al.*[120] screened mixtures of surfactants for their biodegradability and compared the mixtures to the single compound results, Table 13. It was found that ion pair formation between organic anions and quaternary ammonium cations, *e.g.* between LAS (linear alkylbenzene sulphonate) and BAC (benzalkonium chloride) caused a decrease in biodegradation. It was suggested that compounds above 500 Da render the molecule impossible to biodegrade and indeed mixtures of BAC:SDS or DDMAC (didecyldimethylammonium chloride):LAS are all above this molecular weight.

The biodegradability of tris (**286–297**) and tetrakis (**298–309**) imidazolium and benzimidazolium IL surfactants, Fig. 64, using the CBT (OECD 301D) was reported in 2015 by Al-Mohammed *et al.*[15,16] As can be seen from Fig. 64, a number of tris and tetrakis imidazolium and benzimidazolium ILs with various alkyl chain lengths, from C_4 to C_{12} and benzyl, were synthesised and evaluated for their surfactant properties and their biodegradability. The same trend of increased biodegradation with increased chain length can be observed for these series of IL surfactants with C_{12} (**290, 296, 302, 308**) degrading the most (45–56%) and C_4 (**286, 292, 298, 304**) the least (22–35%) and the non-linear *N*-benzyl derivatives (**291, 297, 303, 309**) degrading even less than the C_4 derivatives (16–22%). The tetrakis family (**298–309**) in general appeared to have slightly elevated levels of biodegradability, ~5–10%, when compared to the tris family (**286–297**). The benzimidazolium tris (**292–297**) and tetrakis (**304–309**) families in turn were observed to have lower levels of biodegradability, throughout the homologous series of alkyl chains, than their respective imidazolium families (**286–291, 298–303**). A plateau in the levels of biodegradability was reached after 16 days and the tests were not run for the full 28 day period.

The scope of this review does not include an in depth discussion on biodegradable surfactants, however the authors wish to acknowledge the overlap between IL biodegradation and the lessons that can be learned from biodegradable surfactant research.

9. Outlook and recommendations

To date a number of readily biodegradable ILs have been synthesised, ILs (**54, 55, 60–66, 101, 102, 109, 110, 120, 122, 151,**

156–158, **161–165**, **175**, **176**, **178**, **180**, **310–322**). Nearly 50%, of the readily biodegradable ILs have been comprised of a cholinium cation and organic acid anion, (**61–66**, **157**, **310–322**). The cholinium cation was shown to be highly sensitive to modification, with most modifications reducing biodegradability. The cholinium family of ILs present the most effective route to readily biodegradable compounds examined to date.

Protic analogues of the cholinium core also proved to be good candidates for synthesising biodegradable ILs with seven considered readily biodegradable (**54**, **55**, **161–165**).

Efforts to prepare a readily biodegradable imidazolium IL have demonstrated that the imidazolium core does not degrade under standardised ISO or OECD test conditions. Chemical oxidation is the most effective method to degrade these compounds. Substitution at the imidazolium rings C2 and C4/5 positions with electron withdrawing groups and readily hydrolysable groups such as esters and amides did not significantly improve biodegradation. It is the recommendation of this review that further studies into imidazolium ILs biodegradation are required.

Biodegradable pyridinium ILs have been synthesised (**109**, **110**, **120**, **122**). Three examples (**109**, **110**, **122**) critically include a primary alcohol terminated chain on the *N*-position. Pyridinium ILs are very sensitive to modification on the ring with many derivatives which fail the CBT and CO_2 headspace test known including 3-position and *N*-substituted ethers.

Most tetraalkylammonium cations do not undergo high levels of biodegradation, with the exceptions of TMBA IL (**158**) and 5 DMDBA examples (**60**, **175**, **176**, **178**, **180**). The DMDBA ILs all required a carboxylate counterion for biodegradation to be high enough to pass readily biodegradation tests. The chloride DMDBA derivative (**174**) was found to undergo just 5% biodegradation.

Hexyl and cyclohexyl phosphonium cations appear to sterically hinder enzymatic degradation and further studies in this area are required. It is proposed that shorter, less bulky, alkyl chain substituted phosphonium cations, or an asymmetrically substituted cation paired with an organic acid anion, may undergo a higher level of biodegradation similar to the DMDBA series.

Non-aromatic 6-membered cyclic compounds examined, such as the morpholinium, DABCO and piperidinium ILs, are in general not readily biodegradable. One exception is the derivative (**143**), which degraded 79%. The primary structural feature of (**143**) enabling biodegradation was the presence of an *N*-substituted propyl alcohol terminated chain. The chain length was also of importance, with an ethyl derivative (**142**) undergoing a considerably lower 29% biodegradation.

The 5-membered non-aromatic pyrrolidinium heterocycles underwent similar levels of biodegradation to the 6-membered cycles with the exception of derivatives (**151**) and (**156**), both achieving greater than 60% biodegradation. The key structural feature of (**156**) was an *N*-substituted octyl chain while the critical feature of (**151**) was the presence of an *N*-substituted propyl alcohol. Once more it was observed that the shorter ethyl alcohol substituted IL (**153**) did not undergo biodegradation $>6\%$.

Biodegradation screening of symmetrical ILs has been undertaken since Coleman and Gathergood's review in 2010 with a number of imidazolium, pyridinium, pyrrolidinium ILs studied. The dicationic ILs screened all failed to achieve $>5\%$ biodegradation under their respective test conditions and it has been surmised that the compounds are resistant to biodegrading due to inability of enzymes to access hydrolysable sites, whether this is due to steric effects or electronic considerations is unknown.

Metabolite identification as part of biodegradation studies provides useful data to allow researchers to design ILs which can not only pass ready biodegradability tests but reduce the build-up of persistent breakdown products.

10. Conclusion

To conclude this IL biodegradation review it is necessary to examine the guidelines with which potentially biodegradable ILs are designed. Often, Boethling's rules of thumb are quoted for designing biodegradable ILs but just how compatible are these rules with the ILs that have been synthesised since the previous review in 2010?

When the trends in IL biodegradation are taken as a whole it can be observed that a number of the rules of thumb hold true. Longer unbranched alkyl chains promote biodegradability and the presence of hydrolysable groups (*i.e.* esters) and groups easily oxidised (*i.e.* alcohols) and carboxylic acids are noted as being amongst the prominent features of the readily biodegradables ILs in this review. The use of 'natural' building blocks, for instance choline and nicotinic acid for the cation, can lead to biodegradable derivatives. However, even modest changes to the structure can in some cases greatly inhibit access to biodegradation pathways. Detrimental factors that have been observed, that are in agreement with the rules of thumb, include the presence of aliphatic ethers. It is stated in the rules of thumb that heterocycles are best avoided, making the task of producing heterocyclic ILs a challenge from the very beginning, although every compound is unique and requires careful consideration of all structural features.

The goal of making task specific yet biodegradable low toxicity ILs is a daunting one. Can ILs which are robust under a wide range of applications (*e.g.* chemical reactions, thermal, biocatalysis *etc.*), yet are suitable substrates for breakdown pathways in the environment, leading to ultimate biodegradation be designed? While this task is challenging, from the data collated in this review the authors believe that great progress is being made towards this goal.

To undergo a full eco(toxicity) life cycle assessment is the ultimate goal for any IL that has potential industrial application. The hazard of using the IL *versus* the benefits must be carefully reviewed as ILs move from academic curiosities to large scale industrial applications. Further studies on metabolic pathways and breakdown products for ILs will be required to stimulate advances that are required in producing readily biodegradable ILs. Ultimately (eco)toxicity analysis of these metabolites will also be required, for after all, what is the benefit of nowadays producing a readily biodegradable IL if its persistent breakdown

products are more toxic than the parent compound? We argue that it is the intense debate, of this very issue, at green chemistry, IL and environmental toxicology meetings and conferences and in research papers over the last ten years that has raised awareness to the limitations of standardised biodegradation tests. We propose that the outcome from this process is that current biodegradation studies of ILs are now at the forefront of new initiatives in biodegradation assays and reinterpretation of results. Chemists working together with experts in environmental science and (eco)toxicology can work towards the common interest of the design of safer chemicals. Indeed, although the number of possible cation and anion combinations which lead to salts classed as ILs seems mindboggling large (frequently $> 10^{18}$), this is a small subset of possible simple organic molecules. The journey which scientists that study biodegradation of ILs have travelled is a roadmap to enable other researchers to reduce the potential problems of emergence of persistent chemicals in the environment.

Acknowledgements

The authors would like to thank the EPA in Ireland for financial support. This project has also received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 621364 (TUTIC-Green). We also thank COST Actions CM1206 and TD1203 for their contribution to this study.

References

1 L. P. N. Rebelo, J. N. C. Lopes, J. Esperanca and E. Filipe, *J. Phys. Chem. B*, 2005, **109**, 6040–6043.
2 M. J. Earle, J. Esperanca, M. A. Gilea, J. N. C. Lopes, L. P. N. Rebelo, J. W. Magee, K. R. Seddon and J. A. Widegren, *Nature*, 2006, **439**, 831–834.
3 M. Smiglak, W. M. Reichert, J. D. Holbrey, J. S. Wilkes, L. Y. Sun, J. S. Thrasher, K. Kirichenko, S. Singh, A. R. Katritzky and R. D. Rogers, *Chem. Commun.*, 2006, 2554–2556, DOI: 10.1039/b602086k.
4 P. Walden, *Bull. Acad. Imp. Sci. St.-Petersbourg*, 1914, **8**, 405–422.
5 S. Gabriel and J. Weiner, *Ber. Dtsch. Chem. Ges.*, 1888, **21**, 2669–2679.
6 H. Goldschmidt, *Ber. Dtsch. Chem. Ges.*, 1881, **14**, 1844–1848.
7 P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, New York, 1998, ch. 1, p. 3.
8 B. M. Trost, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 259–281.
9 R. A. Sheldon, *Chem. Ind.*, 1992, 903–906.
10 J. Andraos, *Org. Process Res. Dev.*, 2005, **9**, 149–163.
11 K. M. Docherty, S. Z. Hebbeler and C. F. Kulpa, *Green Chem.*, 2006, **8**, 560–567.
12 N. Sun, H. Rodriguez, M. Rahman and R. D. Rogers, *Chem. Commun.*, 2011, **47**, 1405–1421.
13 D. Parmentier, S. J. Metz and M. C. Kroon, *Green Chem.*, 2013, **15**, 205–209.
14 M. Mahrova, F. Pagano, V. Pejakovic, A. Valea, M. Kalin, A. Igartua and E. Tojo, *Tribol. Int.*, 2015, **82A**, 245–254.
15 N. N. Al-Mohammed, R. S. Duali Hussen, T. H. Ali, Y. Alias and Z. Abdullah, *RSC Adv.*, 2015, **5**, 21865–21876.
16 N. N. Al-Mohammed, R. S. Duali Hussen, Y. Alias and Z. Abdullah, *RSC Adv.*, 2015, **5**, 2869–2881.
17 R. G. Gore, L. Myles, M. Spulak, I. Beadham, T. M. Garcia, S. J. Connon and N. Gathergood, *Green Chem.*, 2013, **15**, 2747–2760.
18 L. Myles, R. Gore, M. Spulak, N. Gathergood and S. J. Connon, *Green Chem.*, 2010, **12**, 1157–1162.
19 L. Myles, R. G. Gore, N. Gathergood and S. J. Connon, *Green Chem.*, 2013, **15**, 2740–2746.
20 R. G. Gore, T.-K.-T. Truong, M. Pour, L. Myles, S. J. Connon and N. Gathergood, *Green Chem.*, 2013, **15**, 2727–2739.
21 D. Coleman and N. Gathergood, *Chem. Soc. Rev.*, 2010, **39**, 600–637.
22 S. Harrad, *Persistent Organic Pollutants*, John Wiley & Sons, Ltd, 2009, ch. 1, pp. 1–4, DOI: 10.1002/9780470684122.
23 S. Milla, S. Depiereux and P. Kestemont, *Ecotoxicology*, 2011, **20**, 305–319.
24 R. P. Herwig, J. P. Gray and D. P. Weston, *Aquaculture*, 1997, **149**, 263–283.
25 P. H. Howard, R. S. Boethling, W. Stiteler, W. Meylan and J. Beauman, *Sci. Total Environ.*, 1991, **109**, 635–641.
26 R. S. Boethling, P. H. Howard, W. Meylan, W. Stiteler, J. Beauman and N. Tirado, *Environ. Sci. Technol.*, 1994, **28**, 459–465.
27 M. Galinski, A. Lewandowski and I. Stepniak, *Electrochim. Acta*, 2006, **51**, 5567–5580.
28 J. G. Huddleston, A. E. Visser, W. M. Reichert, H. D. Willauer, G. A. Broker and R. D. Rogers, *Green Chem.*, 2001, **3**, 156–164.
29 D. M. Fox, W. H. Awad, J. W. Gilman, P. H. Maupin, H. C. De Long and P. C. Trulove, *Green Chem.*, 2003, **5**, 724–727.
30 M. Armand, F. Endres, D. R. MacFarlane, H. Ohno and B. Scrosati, *Nat. Mater.*, 2009, **8**, 621–629.
31 K. M. Docherty, J. K. Dixon and C. F. Kulpa, *Biodegradation*, 2007, **18**, 481–493.
32 G. Quijano, A. Couvert, A. Amrane, G. Darracq, C. Couriol, P. Le Cloirec, L. Paquin and D. Carrié, *Chem. Eng. J.*, 2011, **174**, 27–32.
33 Y. Luo, Q. Wang, Q. Lu, Q. Mu and D. Mao, *Environ. Sci. Technol. Lett.*, 2014, **1**, 266–270.
34 OECD, *Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3*, OECD Publishing, 2006.
35 OECD Guideline for Testing of Chemicals, http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm, accessed 07/07/15.
36 M. Czerwicka, S. Stolte, A. Muller, E. M. Siedlecka, M. Golebiowski, J. Kumirska and P. Stepnowski, *J. Hazard. Mater.*, 2009, **171**, 478–483.
37 M. C. Kroon, W. Buijs, C. J. Peters and G. J. Witkamp, *Green Chem.*, 2006, **8**, 241–245.

38 K. Haerens, E. Matthijs, K. Binnemans and B. Van der Bruggen, *Green Chem.*, 2009, **11**, 1357–1365.
39 X. H. Li, J. G. Zhao, Q. H. Li, L. F. Wang and S. C. Tsang, *Dalton Trans.*, 2007, 1875–1880, DOI: 10.1039/b618384k.
40 M. Markiewicz, M. Piszora, N. Caicedo, C. Jungnickel and S. Stolte, *Water Res.*, 2013, **47**, 2921–2928.
41 M. Markiewicz, C. Jungnickel, A. Markowska, U. Szczepaniak, M. Paszkiewicz and J. Hupka, *Molecules*, 2009, **14**, 4396–4405.
42 V. Gomez, L. Ferreres, E. Pocurull and F. Borrull, *Talanta*, 2011, **84**, 859–866.
43 E. Liwarska-Bizukojc, C. Maton, C. V. Stevens and D. Gendaszewska, *J. Chem. Technol. Biotechnol.*, 2014, **89**, 763–768.
44 A. Modelli, A. Sali, P. Galletti and C. Samori, *Chemosphere*, 2008, **73**, 1322–1327.
45 W. Mrozik, A. Kotłowska, W. Kamysz and P. Stepnowski, *Chemosphere*, 2012, **88**, 1202–1207.
46 C. Jungnickel, W. Mrozik, M. Markiewicz and J. Luczak, *Curr. Org. Chem.*, 2011, **15**, 1928–1945.
47 W. Mrozik, C. Jungnickel, T. Ciborowski, W. R. Pitner, J. Kumirska, Z. Kaczynski and P. Stepnowski, *J. Soils Sediments*, 2009, **9**, 237–245.
48 M. Matzke, S. Stolte, J. Arning, U. Uebers and J. Filser, *Ecotoxicology*, 2009, **18**, 197–203.
49 P. T. P. Thi, C. W. Cho and Y. S. Yun, *Water Res.*, 2010, **44**, 352–372.
50 S. Studzinska and B. Buszewski, *Anal. Bioanal. Chem.*, 2009, **393**, 983–990.
51 F. J. Deive, A. Rodriguez, A. Varela, C. Rodrigues, M. C. Leitao, J. Houbraken, A. B. Pereiro, M. A. Longo, M. A. Sanroman, R. A. Samson, L. P. N. Rebelo and C. S. Pereira, *Green Chem.*, 2011, **13**, 687–696.
52 J. Neumann, O. Grundmann, J. Thoming, M. Schulte and S. Stolte, *Green Chem.*, 2010, **12**, 620–627.
53 M. M. Häggblom and I. D. Bossert, *Microbial Processes and Environmental Applications*, Kluwer Academic Publishers, Dordrecht, 2003.
54 Ridding the world of pops: a guide to the Stockholm Convention on Persistent Organic Pollutants, http://www.pops.int/documents/guidance/beg_guide.pdf, accessed 25/11/2013, 2013.
55 E. Meszaros, R. Sipos, R. Pal, C. Romsics and K. Marialigeti, *Int. Biodeterior. Biodegrad.*, 2013, **84**, 126–133.
56 I. Kranzioch, C. Stoll, A. Holbach, H. Chen, L. J. Wang, B. H. Zheng, S. Norra, Y. H. Bi, K. W. Schramm and A. Tiehm, *Environ. Sci. Pollut. Res.*, 2013, **20**, 7046–7056.
57 B. Kuipers, W. R. Cullen and W. W. Mohn, *Environ. Sci. Technol.*, 1999, **33**, 3579–3585.
58 M. G. Freire, C. M. S. S. Neves, I. M. Marrucho, J. o. A. P. Coutinho and A. M. Fernandes, *J. Phys. Chem. A*, 2009, **114**, 3744–3749.
59 J. Neumann, C.-W. Cho, S. Steudte, J. Koser, M. Uerdingen, J. Thoming and S. Stolte, *Green Chem.*, 2012, **14**, 410–418.
60 C. Abrusci, J. Palomar, J. L. Pablos, F. Rodriguez and F. Catalina, *Green Chem.*, 2011, **13**, 709–717.
61 S. Stolte, S. Abdulkarim, J. Arning, A. K. Blomeyer-Nienstedt, U. Bottin-Weber, M. Matzke, J. Ranke, B. Jastorff and J. Thoming, *Green Chem.*, 2008, **10**, 214–224.
62 M. T. Garcia, N. Gathergood and P. J. Scammells, *Green Chem.*, 2005, **7**, 9–14.
63 C. Zhang, H. Wang, S. V. Malhotra, C. J. Dodge and A. J. Francis, *Green Chem.*, 2010, **12**, 851–858.
64 M. Markiewicz, J. Henke, A. Brillowska-Dabrowska, S. Stolte, J. Luczak and C. Jungnickel, *Int. J. Environ. Sci. Technol.*, 2014, **11**, 1919–1926.
65 E. Liwarska-Bizukojc and D. Gendaszewska, *J. Biosci. Bioeng.*, 2013, **115**, 71–75.
66 L. Carson, P. K. W. Chau, M. J. Earle, M. A. Gilea, B. F. Gilmore, S. P. Gorman, M. T. McCann and K. R. Seddon, *Green Chem.*, 2009, **11**, 492–497.
67 S. Morrissey, B. Pegot, D. Coleman, M. T. Garcia, D. Ferguson, B. Quilty and N. Gathergood, *Green Chem.*, 2009, **11**, 475–483.
68 L. Ford, J. R. Harjani, F. Atefi, M. T. Garcia, R. D. Singer and P. J. Scammells, *Green Chem.*, 2010, **12**, 1783–1789.
69 B. Peric, J. Sierra, E. Martí, R. Cruañas, M. A. Garau, J. Arning, U. Bottin-Weber and S. Stolte, *J. Hazard. Mater.*, 2013, **261**, 99–105.
70 N. Ferlin, M. Courty, A. N. Van Nhien, S. Gatard, M. Pour, B. Quilty, M. Ghavre, A. Haiß, K. Kummerer, N. Gathergood and S. Bouquillon, *RSC Adv.*, 2013, **3**, 26241–26251.
71 N. Ferlin, M. Courty, S. Gatard, M. Spulak, B. Quilty, I. Beadham, M. Ghavre, A. Haiß, K. Kümmerer, N. Gathergood and S. Bouquillon, *Tetrahedron*, 2013, **69**, 6150–6161.
72 F. Boissou, A. Muhlbauer, K. De Oliveira Vigier, L. Leclercq, W. Kunz, S. Marinkovic, B. Estrine, V. Nardello-Rataj and F. Jérôme, *Green Chem.*, 2014, **16**, 2463–2471.
73 X.-D. Hou, Q.-P. Liu, T. J. Smith, N. Li and M.-H. Zong, *PLoS One*, 2013, **8**, e59145, DOI: 10.1371/journal.pone.0059145.
74 R. S. Boethling, E. Sommer and D. DiFiore, *Chem. Rev.*, 2007, **107**, 2207–2227.
75 M. G. Freire, C. M. S. S. Neves, I. M. Marrucho, J. o. A. P. Coutinho and A. M. Fernandes, *J. Phys. Chem. A*, 2009, **114**, 3744–3749.
76 N. V. Plechkova and K. R. Seddon, *Chem. Soc. Rev.*, 2008, **37**, 123–150.
77 A. Romero, A. Santos, J. Tojo and A. Rodríguez, *J. Hazard. Mater.*, 2008, **151**, 268–273.
78 S. Stolte, S. Steudte, A. Igartua and P. Stepnowski, *Curr. Org. Chem.*, 2011, **15**, 1946–1973.
79 S. Stolte, T. Schulz, C.-W. Cho, J. Arning and T. Strassner, *ACS Sustainable Chem. Eng.*, 2013, **1**, 410–418.
80 N. Gathergood, P. J. Scammells and M. T. Garcia, *Green Chem.*, 2006, **8**, 156–160.
81 D. Coleman, M. Spulak, M. T. Garcia and N. Gathergood, *Green Chem.*, 2012, **14**, 1350–1356.
82 The UFT/Merck Ionic Liquids Biological Effects Database, http://www.il-eco.uft.uni-bremen.de/index.php?page=substance&chent_id=IM0&view=degradation&lang=en#l4013, accessed 24/10/14.

83 J. Neumann, S. Steudte, C.-W. Cho, J. Thoming and S. Stolte, *Green Chem.*, 2014, **16**, 2174–2184.
84 J. R. Harjani, R. D. Singer, M. T. Garciac and P. J. Scammells, *Green Chem.*, 2009, **11**, 83–90.
85 C. Pretti, M. Renzi, S. E. Focardi, A. Giovani, G. Monni, B. Melai, S. Rajamani and C. Chiappe, *Ecotoxicol. Environ. Saf.*, 2011, **74**, 748–753.
86 J. Pernak, N. Borucka, F. Walkiewicz, B. Markiewicz, P. Fochtman, S. Stolte, S. Steudte and P. Stepnowski, *Green Chem.*, 2011, **13**, 2901–2910.
87 S. Stolte, S. Steudte, O. Areitioaurtena, F. Pagano, J. Thöming, P. Stepnowski and A. Igartua, *Chemosphere*, 2012, **89**, 1135–1141.
88 OECD, *SIDS Initial Assessment Report For SIAM 19*, OECD Publishing, Berlin, Germany, 2004.
89 K. Radosevic, M. C. Bubalo, V. G. Srcek, D. Grgas, T. L. Dragicevic and I. R. Redovnikovic, *Ecotoxicol. Environ. Saf.*, 2015, **112**, 46–53.
90 S. Sekar, M. Surianarayanan, V. Ranganathan, D. R. MacFarlane and A. B. Mandal, *Environ. Sci. Technol.*, 2012, **46**, 4902–4908.
91 R. Zana, J. Schmidt and Y. Talmon, *Langmuir*, 2005, **21**, 11628–11636.
92 M. L. Ingalsbe, J. D. S. Denis, M. E. McGahan, W. W. Steiner and R. Priefer, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 4984–4987.
93 V. I. Pârvulescu and C. Hardacre, *Chem. Rev.*, 2007, **107**, 2615–2665.
94 F. Atefi, M. T. Garcia, R. D. Singer and P. J. Scammells, *Green Chem.*, 2009, **11**, 1595–1604.
95 S. Steudte, S. Bemowsky, M. Mahrova, U. Bottin-Weber, E. Tojo-Suarez, P. Stepnowski and S. Stolte, *RSC Adv.*, 2014, **4**, 5198–5205.
96 K. M. Docherty, M. V. Joyce, K. J. Kulacki and C. F. Kulpa, *Green Chem.*, 2010, **12**, 701–712.
97 H. Sato, A. Shibata, Y. Wang, H. Yoshikawa and H. Tamura, *Polym. Degrad. Stab.*, 2001, **74**, 69–75.
98 T. P. T. Pham, C. W. Cho, C. O. Jeon, Y. J. Chung, M. W. Lee and Y. S. Yun, *Environ. Sci. Technol.*, 2009, **43**, 516–521.
99 F. Rojo, *Environ. Microbiol.*, 2009, **11**, 2477–2490.
100 C. Zhang, S. V. Malhotra and A. J. Francis, *Chemosphere*, 2011, **82**, 1690–1695.
101 B. Kumari, S. N. Singh and S. Mishra, in *Microbial Degradation of Alkanes*, ed. S. N. Singh, Springer, 2012, ch. 17, pp. 439–449.
102 TOPKAT, http://accelrys.com/products/discovery-studio/predictive-toxicology.html, accessed 09/08/13.
103 META, http://www.multicase.com/products/prod05.htm, accessed 09/08/13.
104 BIOWIN, http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, accessed 09/08/13.
105 VEGA, http://www.vega-qsar.eu/download.html, accessed 14/01/2014.
106 START, http://toxtree.sourceforge.net/start.html, accessed 14/01/2014.
107 CATABOL, http://oasis-lmc.org/?section=software&swid=1, accessed 09/08/13.
108 F. Pizzo, A. Lombardo, A. Manganaro and E. Benfenati, *Sci. Total Environ.*, 2013, **463–464**, 161–168.
109 C. Rucker and K. Kummerer, *Green Chem.*, 2012, **14**, 875–887.
110 UFT, http://www.il-eco.uft.uni-bremen.de/, accessed 14/01/14, 2014.
111 P. Stepnowski and A. Zaleska, *J. Photochem. Photobiol., A*, 2005, **170**, 45–50.
112 J. Pernak and M. Branicka, *Ind. Eng. Chem. Res.*, 2004, **43**, 1966–1974.
113 E. M. Siedlecka, M. Golebiowski, Z. Kaczynski, J. Czupryniak, T. Ossowski and P. Stepnowski, *Appl. Catal., B*, 2009, **91**, 573–579.
114 M. J. Scott and M. N. Jones, *Biochim. Biophys. Acta, Biomembr.*, 2000, **1508**, 235–251.
115 S. T. Giolando, R. A. Rapaport, R. J. Larson, T. W. Federle, M. Stalmans and P. Masscheleyn, *Chemosphere*, 1995, **30**, 1067–1083.
116 AISE, http://www.aise.eu/downloads/05_Fact%20sheet%20biodegradability-updated%2027082012.pdf, accessed 05/01/2014.
117 M. R. Infante, L. Perez, M. C. Moran, R. Pons, M. Mitjans, M. P. Vinardell, M. T. Garcia and A. Pinazo, *Eur. J. Lipid Sci. Technol.*, 2010, **112**, 110–121.
118 A. Pinazo, N. Lozano, L. Perez, M. C. Moran, M. R. Infante and R. Pons, *C. R. Chim.*, 2011, **14**, 726–735.
119 J. Ranke, S. Stolte, R. Stormann, J. Arning and B. Jastorff, *Chem. Rev.*, 2007, **107**, 2183–2206.
120 H. Sütterlin, R. Alexy, A. Coker and K. Kümmerer, *Chemosphere*, 2008, **72**, 479–484.