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## **Cation and anion effect on the biodegradability and toxicity of imidazolium- and choline-based ionic liquids**

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**Cation and anion effect on the biodegradability and toxicity of imidazolium– and choline–based ionic liquids**

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**Abstract**

This work studies the effect of the cation and anion on the biodegradability and inhibition of imidazolium– and choline–based ionic liquids (ILs) using activated sludge. Six commercial ILs, formed by combination of 1–Butyl–3–methylimidazolium (Bmim<sup>+</sup>) and N,N,N–trimethylethanolammonium (Choline<sup>+</sup>) cations and chloride (Cl<sup>–</sup>), acetate (Ac<sup>–</sup>) and bis(trifluoromethanesulfonyl)imide (NTf<sub>2</sub><sup>–</sup>) anions were evaluated, all representative counter–ions with markedly different toxicity and biodegradability. Inherent and fast biodegradability tests were used to evaluate both the microorganism inhibition and the IL biodegradability. In addition, the ecotoxicological response (EC<sub>50</sub>) of the ILs was studied using activated sludge and *Vibrio fischeri* (Microtox<sup>®</sup> test). Bmim<sup>+</sup> and NTf<sub>2</sub><sup>–</sup> can be considered as non–biodegradable, whereas aerobic microorganisms easily degraded Choline<sup>+</sup> and Ac<sup>–</sup>. The biodegradation pattern of each cation/anion is nearly unaffected by counter–ion nature. Moreover, concentrations of CholineNTf<sub>2</sub> higher than 50 mg/L caused a partial inhibition on microbial activity, in good concordance with its low EC<sub>50</sub> (54 mg/L) measured by respiration inhibition test, which alerts on the negative environmental impact of NTf<sub>2</sub>–containing ILs on the performance of sewage treatment plants.

Keywords: Activated sludge; biodegradability; choline; ecotoxicity; imidazolium.

## **1. Introduction**

Ionic liquids are salts with a melting point lower than 100 °C at 1 atm formed through the combination of an organic cation and organic or inorganic anion with interesting properties such as low vapor pressure and physical and chemical stability. They have been designed to be robust at several reaction conditions and, consequently, to become essential chemicals as reaction solvents, battery electrolytes, lubricants, surfactants, catalysts, dielectric fluids as transformers and capacitors (Olivier–Bourbigou et al., 2010; Plechkova and Seddon, 2008). However, several studies have questioned the impact of these compounds on the environment suggesting that not all of them can be considered as green chemicals (Jordan and Gathergood, 2015; Richardson and Ternes, 2014). Recent works have made evident how the release of ILs to the aquatic environment could cause an environmental concern, due to some of them are considered as toxic and non-biodegradable (Amde et al., 2015; Chatel et al., 2017; Diaz et al., 2016; Frade and Afonso, 2010; Thuy Pham et al., 2010). In this context, these aspects must be included in the studies focused on the whole life cycle of ILs in order to establish a real eco–design strategy of the IL.

Some biodegradability assays can be useful to determine ILs biodegradability in the environment and minimize negative impacts due to its persistence and/or accumulation (Polo et al., 2011; Sanchis et al., 2014). Ecotoxicological determinations, which are dependent on the studied model and environment conditions, can be used at a first assessment of the potential effect of ILs on a specific group of aquatic organisms (Costa et al., 2015b; Khan et al., 2016). In both cases, it is essential to select those assays whose results provide realistic effects of the exposure risk to ILs, and let i) know more

about the impact of these compounds on sewage wastewater treatment; and ii) select an efficient degradation process to remove them from water (Diaz et al., 2016; Peric et al., 2013; Richardson and Kimura, 2016).

The choice of an adequate test must consider the end-point discharge of wastewater and, consequently, try to predict the behavior of ILs in sewage treatment plants. Different biodegradation methods have been adopted by OECD and ISO (Diaz et al., 2016; Jordan and Gathergood, 2015; Pagga, 1997) to provide biodegradation data as the OECD 301B (biodegradation test, CO<sub>2</sub> evolution) (Stolte et al., 2012), the OECD 301D (biodegradation closed bottle test) (Stolte et al., 2008), the OECD 301F (biodegradation test, O<sub>2</sub> consumption) (Neumann et al., 2014b) and the OECD 310 (ready biodegradability, CO<sub>2</sub> in sealed vessels) (Atefi et al., 2009; Ford et al., 2010; Pretti et al., 2011). Specifically, Zahn–Wellens test (OECD 302B) evaluates the inherent biodegradability of water pollutants along 28 days, being the main disadvantage the great amount of time required to complete the assay. In this sense, a simple and fast respirometric test, based on the typical operation conditions of an activated sludge process, which provides data from the activity of the microorganisms and pollutant removal, has been proposed to obtain biodegradability data in a relatively short-time (100 h) (Polo et al., 2011).

Previous results focused on the biodegradability of ILs have established some rules of thumb. According to structural design, both the head group and the substituted side chain have influence on the IL degradability. The following sequence of biodegradability could be established regarding head group as long as the same side chain is substituted: pyrrolidinium  $\approx$  pyridinium > piperidinium  $\approx$  morpholinium >> imidazolium (Neumann et al., 2014a). In general, regardless IL family, the biodegradability increases as the length of alkyl chain of cation or by the introduction in

these chains of functional groups such as hydroxyl, carboxylic, alcohol or ether (Egorova and Ananikov, 2014; Jordan and Gathergood, 2015). On the other hand, the use of natural building blocks as choline or nicotinic acid as IL cations has a positive influence on the biodegradability. In relation to the anion, organic anions (carboxylic acids, amino acids, carbohydrates) can be considered as biodegradable. In the case of inorganic anions, their biodegradation cannot take into account, but while halide anions (chloride or bromide) do not interfere on the potential biodegradability of the IL, the degradation of ILs with perfluorinated anions ( $\text{PF}_6^-$ ,  $\text{BF}_4^-$ ) can lead to more toxic compounds (Freire et al., 2010; Gathergood et al., 2006; Jordan and Gathergood, 2015).

With regard to the ecotoxicity, standardized tests can provide different behavior depending on the microorganism employed in the assays (Rizzo, 2011), and again, underestimate or overestimate the behavior of the aquatic system in the presence of a certain pollutant. The standardized toxicity tests traditionally used are: ISO 11348, to determine the inhibitory effect of water samples (*Vibrio fischeri*) (Costa et al., 2015a; Diaz et al., 2018; Docherty and Kulpa, Jr., 2005; Garcia et al., 2005; Montalbán et al., 2018; Oulego et al., 2018; Ventura et al., 2014; Viboud et al., 2012); ISO 8692, to determine the growth inhibition of unicellular green algae by substances and mixtures contained in water (*Selenastrum capricornutum*) (Cho et al., 2008; Stolte et al., 2012); USEPA–ISO 6341, to determine the inhibition of the mobility of *Daphnia magna* *Straus* (acute toxicity test) (Costa et al., 2015b; Samori et al., 2010; Stolte et al., 2012; Vieira et al., 2019); or OECD 201, to determine the effect of a substance on the growth of freshwater microalgae and/or cyanobacteria (*Scenedesmus rubescens*) (Tsarpali and Dailianis, 2015). Despite of Microtox® test is not a standard toxicity test defined in the European Union legislation, it is widely accepted to establish a first–approach of a toxicity of a xenobiotic compound due to be fast, simple and cost–efficient (Sintra et al.,

2017). However, *Vibrio fischeri* is not representative of the microorganisms that constitute a sewage sludge. In this sense and following the idea to obtain useful ecotoxicity data to establish a reliable assessment of potential toxicity of a xenobiotic in a sewage treatment plant, a respiration inhibition test using activated sludge (OECD 209 – Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)) is recommendable (Polo et al., 2011; Rizzo, 2011). Although it is well known that toxicity of ILs depends on the nature of a biological system, there are some factors that seem to modulate the ILs toxicity: i) length of an alkyl chain in the cation; ii) degree and nature of functionalization in the side chain of the cation; iii) nature of the anion; iv) nature of the cation; and v) mutual influence of anion and cation (Egorova and Ananikov, 2014). In general, an increase of the alkyl chain length causes a drastic increase of the toxicity, which seems to be related to the high lipophilic character, and the introduction of oxygenated groups provokes a decrease on IL toxicity (Costello et al., 2009; Cvjetko Bubalo et al., 2014; Diaz et al., 2016; Ranke et al., 2007; Stolte et al., 2007). Despite of the fact that the anion nature contributes less than the cation to the overall IL toxicity, the toxicity of anions as  $\text{NTf}_2^-$  and  $\text{C}_8\text{SO}_4^-$  must not be underestimated, being this contribution more evident in the case of IL with short cation alkyl chain (Diaz et al., 2018; Petkovic et al., 2011).

Moreover, in order to study the called PBT assessment (persistence, bioaccumulation, toxicity), some authors have focused their research in the bioconcentration potential of the ILs (Bingham and Ballone, 2012; Dołzonek et al., 2017; Jing et al., 2016; Yoo et al., 2014). In general, imidazolium ILs containing cations with long alkyl side chain ( $> 8$  C atoms) can intercalate into the lipid membrane bilayer (Dołzonek et al., 2017; Jing et al., 2016). In terms of anions, ILs constituted by  $\text{NTf}_2^-$  present higher bilayer disruption than those with contain chloride anion (Jing et al., 2016).

ILs show in general high thermal and chemical stability together with low biodegradability, which do not favor their removal from aqueous effluents. Adsorption of ILs on different materials, such as active carbon, alumina or silica, provides a way to recover them (Lemus et al., 2012; Neves et al., 2014). Moreover, ILs have been effectively degraded by means of AOPS such as Fenton oxidation (Dominguez et al., 2014; Munoz et al., 2015; Gomez-Herrero et al., 2018), catalytic wet peroxide oxidation (Mena et al., 2019b; Munoz et al., 2016), photodegradation (Bedia et al., 2019; Leonardo da Silva et al., 2019; Stepnowski et al., 2005), electrooxidation (Mena et al., 2019a; Pieczyńska et al., 2015) or electro-Fenton processes (Bocos et al., 2017; Poza-Nogueiras et al., 2018).

In this work, we study the toxicity and biodegradability of several imidazolium- and choline-based ILs in order to analyze their potential behavior in a sewage treatment plant, taking into account the effect of anion and cation. Thus, inherent (Zahn-Wellens) and fast biodegradability tests have been performed. Respiration inhibition test, using activated sludge, has been employed to evaluate ILs toxicity. With respect to ILs, imidazolium family presents a huge potential for industrial applications (Chatel et al., 2017). They have been used as surfactants (Bhadani et al., 2016; Nandwani et al., 2017), in CO<sub>2</sub> absorption (Karadas et al., 2013) and as solvents (Cull et al., 2000) and homogeneous catalysts in organic reactions (Pârvulescu and Hardacre, 2007). Bmim<sup>+</sup>, used as representative species of the imidazolium family, has been studied in electro synthesis of organic compounds (Kathiresan and Velayutham, 2015), CO<sub>2</sub> absorption (Moya et al., 2016) and as catalysts in organic reactions, such as hydrogenation or alkylation (Zhao et al., 2002). On the other hand, choline-based ILs refers to a quaternary ammonium salts with a counter anion, with application in organic synthesis and polymerization reactions (Liu et al., 2015; Plechkova and Seddon, 2008; Zhang et



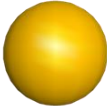
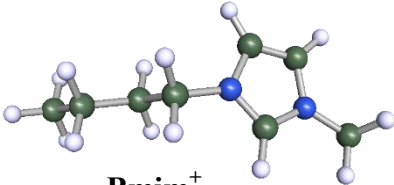
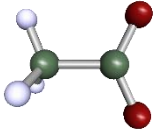
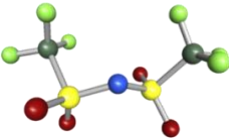
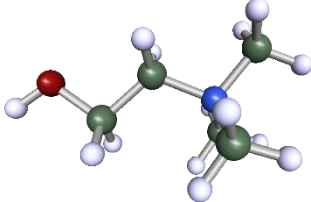
al., 2012),  $\text{NH}_3$  and  $\text{CO}_2$  absorption (Palomar et al., 2011; Pandey et al., 2013),  
fractionation of lignocellulosic biomass for biofuel production (An et al., 2015),  
cellulose decrystallization (Zhang et al., 2012) and dyes degradation (Sekar et al.,  
2012). In the last years, the latter have shown a huge potential to replace traditional  
organic solvents, which have apparently shown a lower adverse environmental impact  
than imidazolium-based ILs (Gadilohar and Shankarling, 2017; Petkovic et al., 2011).  
The selected anions were chloride ( $\text{Cl}^-$ ) and acetate ( $\text{Ac}^-$ ), representative hydrophilic  
inorganic and organic anions, respectively, and also bis(trifluoromethanesulfonyl)imide  
( $\text{NTf}_2^-$ ) as hydrophobic anion.

## 2. Materials and methods

### 2.1. Ionic liquids

Table 1 collects the ionic liquids from imidazolium (1-Butyl-3-methylimidazolium, Bmim<sup>+</sup>) and choline (N,N,N-trimethylethanolammonium, Choline<sup>+</sup>) families with chloride (Cl<sup>-</sup>), acetate (Ac<sup>-</sup>) and bis(trifluoromethanesulfonyl)imide (NTf<sub>2</sub><sup>-</sup>) as anions, used in this study, together with their nomenclature. The ILs were supplied by Sigma-Aldrich and Iolitec with a purity higher than 95 %.

**Table 1.** Chemical structure and nomenclature of imidazolium- and choline-based ILs.

Anion	Cation
 <p>Cl<sup>-</sup></p>	 <p>Bmim<sup>+</sup></p>
 <p>Ac<sup>-</sup></p>	
 <p>NTf<sub>2</sub><sup>-</sup></p>	 <p>Choline<sup>+</sup></p>

### 2.2. Inoculum and culture medium

Unacclimated activated sludge, obtained from a domestic sewage treatment plant, has been used as inoculum in the biodegradability assays (inherent biodegradability and fast biodegradability tests) and in the respiration inhibition tests (OECD 209, ISO 8192). The sludge was maintained in a sequencing batch reactor (SBR) operated at room

temperature using an organic loading rate (sodium acetate and glucose) of 0.4 mg COD/mg VSS·day. Ammonium sulphate and phosphoric acid were used as nitrogen and phosphorous sources, respectively. A COD:N:P of 100:5:1 (w/w) was fixed and mineral salts ( $\text{FeCl}_3$ ,  $\text{CaCl}_2$ ,  $\text{KCl}$  and  $\text{MgCl}_2$ ) were also added as micronutrients supply in a COD:micronutrients (Fe, Ca, K and Mg) ratio of 100:0.05 (w/w).

### 2.3. Inherent biodegradability test

The Zahn–Wellens test (OECD 302 B 1992) was carried by triplicate in amber glass reactors (2.5 L) with 10 mg/L of IL in aqueous solution. The IL carbon content to inoculum (dry–weight) ratio was 1:4 and mineral medium (phosphate buffer with  $\text{CaCl}_2$  (27.5 mg/L),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (22.5 mg/L) and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.25 mg/L)) was also added. The mixture was agitated and aerated at room temperature for 28 days. A control test using ethylene glycol was carried out in order to ensure the activity of the sludge. Samples were taken periodically to quantify the TOC and IL concentration. The results reported are the average values from duplicate runs, the standard errors being always lower than 10%.

### 2.4. Fast biodegradability test

Fast biodegradability tests were carried out in close reactors with a mixture of unacclimated activated sludge (350 mg/L), IL (10 and 50 mg/L) and mineral medium as previously described (Polo et al., 2011). The evolution of IL concentration, TOC and specific oxygen uptake rate (SOUR) profile was registered. Experiments were carried out in duplicate. The standard errors of the measurements were always lower than 10%.

### 2.5. Respirometric inhibition assay

Respiration inhibition tests for activated sludge were carried out according to the method proposed by Polo et al. (2011). The procedure consisted in short–term respirometric measurements carried out in a Liquid–Static–Static (LSS) respirometer

(Chica et al., 2007), using unacclimated sludge (350 mgVSS/L) in the presence of an easily biodegradable substrate (sodium acetate) alone or together with different concentrations of the ILs. The biomass activity was measured in terms of SOUR. Inhibition was also estimated in terms of  $EC_{50}$  defined as the effective concentration of a sample that causes 50% reduction of SOUR. Experiments were performed in triplicate, being the standard deviation below 10 %.

## 2.6. Ecotoxicity test

Ecotoxicity measurements were performed according to standard Microtox test procedure (ISO 11348, 2007), based on the decreasing of light emission by the marine bacteria *Vibrio fischeri* (*Photobacterium phosphoreum*) after 15 min in presence of IL at pH within 6–8, using a Microtox M500 Analyzer (Azur Environmental). The results were expressed in terms of  $EC_{50}$ , defined as the effective concentration of IL that causes a 50 % inhibitory effect. The results reported were the average values from triplicate measurements. The standard errors of the measurements were always lower than 10%.

## 2.7. Analytical methods

Bmim<sup>+</sup> was analyzed by means of HPLC (Varian Pro–Start 240) with a visible UV detector (Prostar 325 UV–Vis) at 218 nm. A Synergy 4 mm Polar–RP 80 A (Phenomenex) column (15 cm length, 4.6 mm diameter) was used as stationary phase and a mixture of acetonitrile and water (5:95, v/v) at 0.75 mL/min as mobile phase.

Choline<sup>+</sup> identification was carried out by means of ionic chromatography (Metrohm 790 Persona IC) fitted with a Metrosep C4–250/4.0 column as stationary phase. The mobile phase (0.9 mL/min) was a mixture of 0.7 mM of 2,6–pyridinedicarboxylic acid and 1.7 mM of HNO<sub>3</sub>. Ion chromatography with chemical suppression (DIONEX ICS–900) was employed to detect the anionic species (chloride and acetate) using a Dionex IonPac AS22 4 x 250 mm column at 1 mL/min of 1.4 mM NaHCO<sub>3</sub>/4.5 mM Na<sub>2</sub>CO<sub>3</sub> as

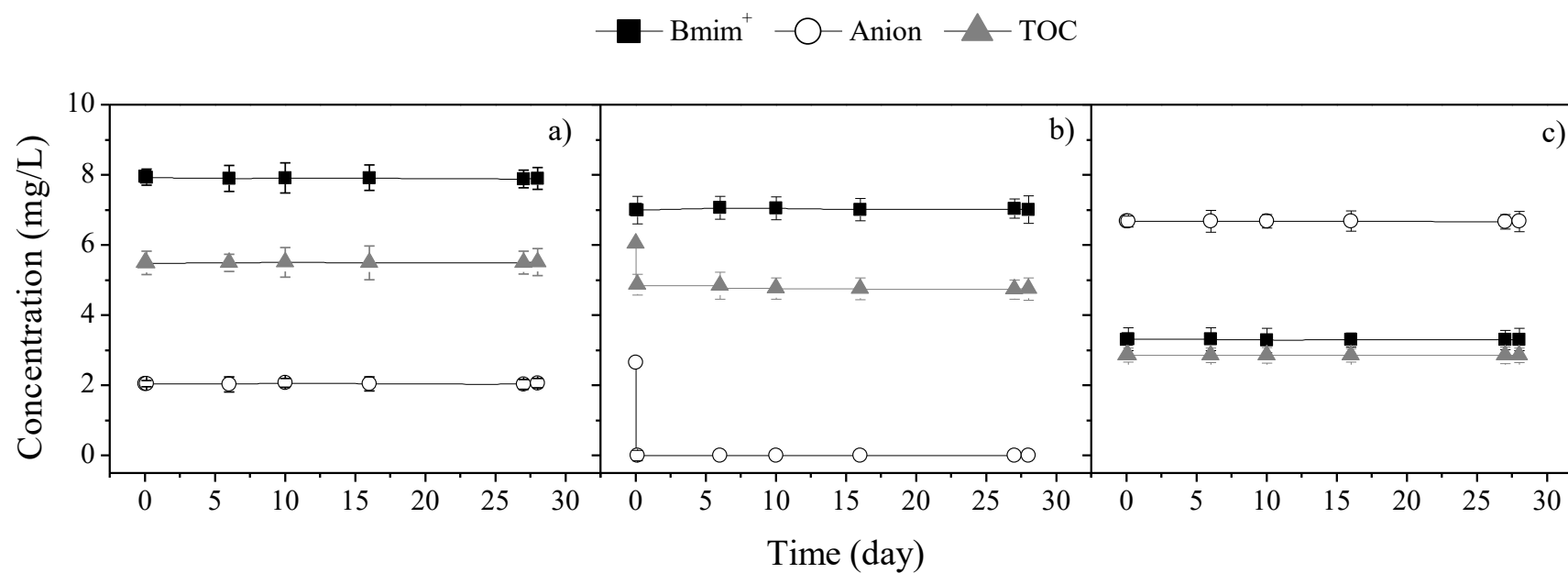
mobile phase. The  $\text{NTf}_2^-$  anion was identified by liquid chromatography coupled with mass spectrometry (LC/MS SQ Agilent) fitted with an ACE Excel 3 C18–Amide, 250 x 2.1 mm column at 40 °C as stationary phase. A formic acid (0.1 % v/v) and acetonitrile (10:90 % v/v) at 0.2 mL/min was used as mobile phase. Total organic carbon (TOC) was measured using a TOC apparatus from Shimadzu (Shimadzu TOC–VCSH). Before analysis, samples were filtered through 0.45  $\mu\text{m}$  syringe filters (OlimPeak PTFE, 25 mm, Teknokroma).

### 3. Results and discussion

Figure 1 show the biodegradability of Bmim–based ILs by Zahn–Wellens test. In preliminary tests, neither ILs volatilization nor adsorption occurred during the testing period. In the case of Bmim–based ILs, no biodegradation was observed for BmimCl and BmimNTf<sub>2</sub>. This fact has also observed by previous studies by means of the Closed Bottle Test (OECD 301D) (Garcia et al., 2005) and the biological oxygen demand test (BOD<sub>5</sub>) (Quijano et al., 2011; Romero et al., 2008). Regarding the Bmim<sup>+</sup> removal, Quijano et al. (2011) did not find any evidence of degradation of Bmim<sup>+</sup> (BmimNTf<sub>2</sub>, 10 mg/L) in a long–term biodegradability test for 30 days using activated sludge. However, a partial Bmim<sup>+</sup> biodegradation (BmimCl, 130 mg/L), without transformation of the imidazolium core, can be reached in less than 40 days using an enriched activated sludge (Alisawi et al., 2017; Docherty et al., 2015). In this study, only a partial biodegradation happened for BmimAc IL (Figure 1b), which was attributed to the complete mineralization of acetate anion in the first 3h. Along the experimental time, biomass did not adapt to degrade Bmim<sup>+</sup> or NTf<sub>2</sub><sup>−</sup>. On the other hand, organic acid anions, as acetate, appear to be the best selection for promoting biodegradability of ILs (Jordan and Gathergood, 2015; Quijano et al., 2011).

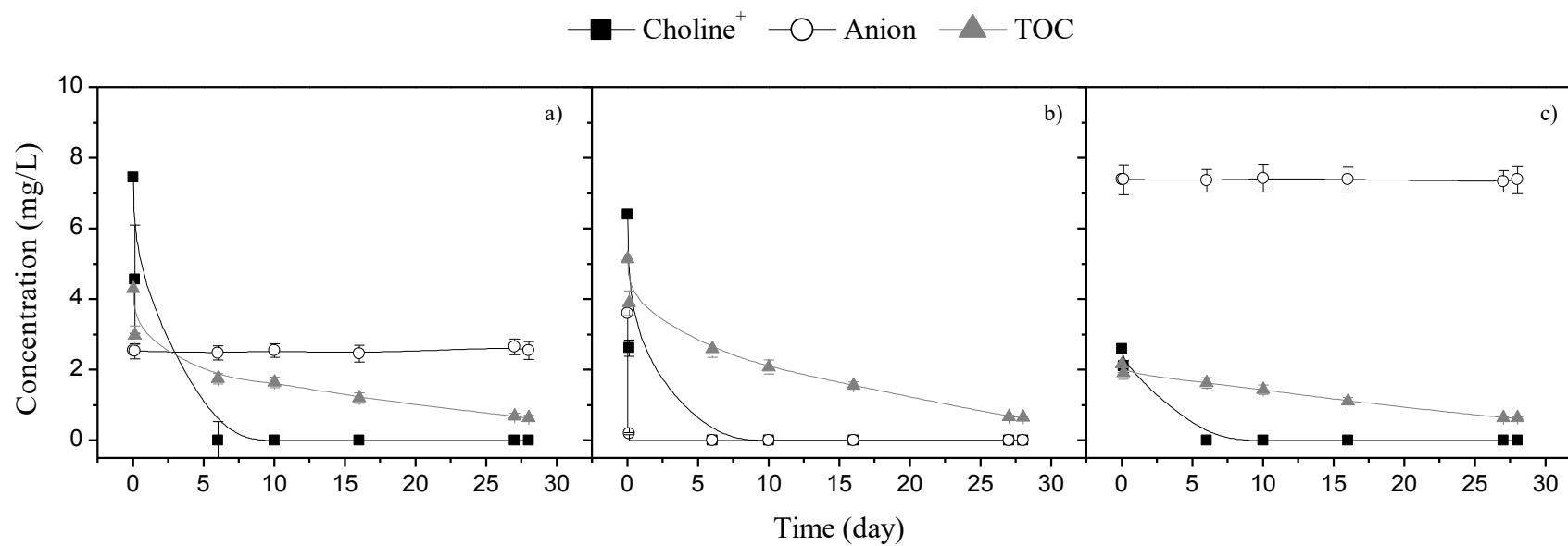
In the case of choline-based ILs (Figure 2), choline cation can be considered biodegradable, showing complete removal in less than 6 days. In a recent work we identified some species, like ethanol, and methyl-, dimethyl- and trimethylamine in the degradation pathway of Choline<sup>+</sup> in a sequencing batch reactor using conventional activated sludge (Mena et al., 2019c). However, only acetate anion (CholineAc) was totally degraded in few hours. As can be seen in Figures 1 and 2, the separately observed biodegradability of each cation or anion does not seem be strongly affected by the nature of the counter-ion. It suggests that IL biodegradation occurs through dissociated ionic species in water, rather than ion-pair or associated species. TOC significantly decreased during the first hours, and after that time, a lower TOC removal rate was observed. TOC conversion achieved 85 and 87 % for CholineCl and CholineAc, respectively, and consequently, both compounds can be considered biodegradables. The biodegradability of CholineCl has been previously observed according to the ready biodegradability test 301D OECD, reaching more than 90 % removal within 14 days (Radošević et al., 2015), and by means of the biological oxygen demand test (BOD<sub>5</sub>) (Gadilohar et al. 2014). For CholineNTf<sub>2</sub>, Radošević et al. (2015) evidenced Choline<sup>+</sup> degradation together with a TOC removal of ~70 % corresponding to the cation mineralization.

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270 **Figure 1.** Time course of (a) BmimCl, (b) BmimAc, (c) BmimNTf<sub>2</sub> and TOC removal along Zahn–Wellens test.

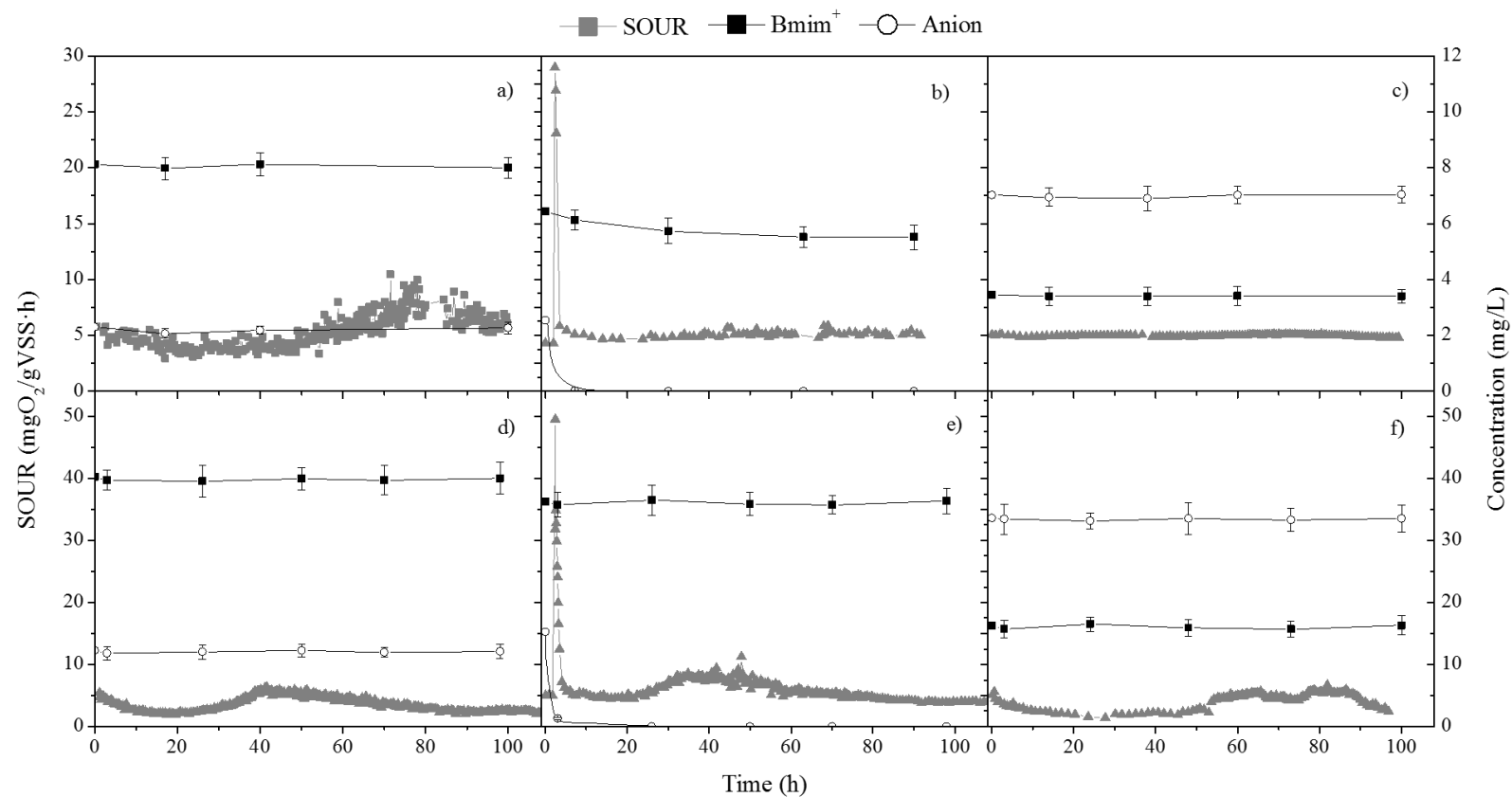


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272 **Figure 2.** Time course of CholineCl (a), CholineAc (b), and CholineNTf<sub>2</sub> (c) and TOC removal along Zahn–Wellens test.



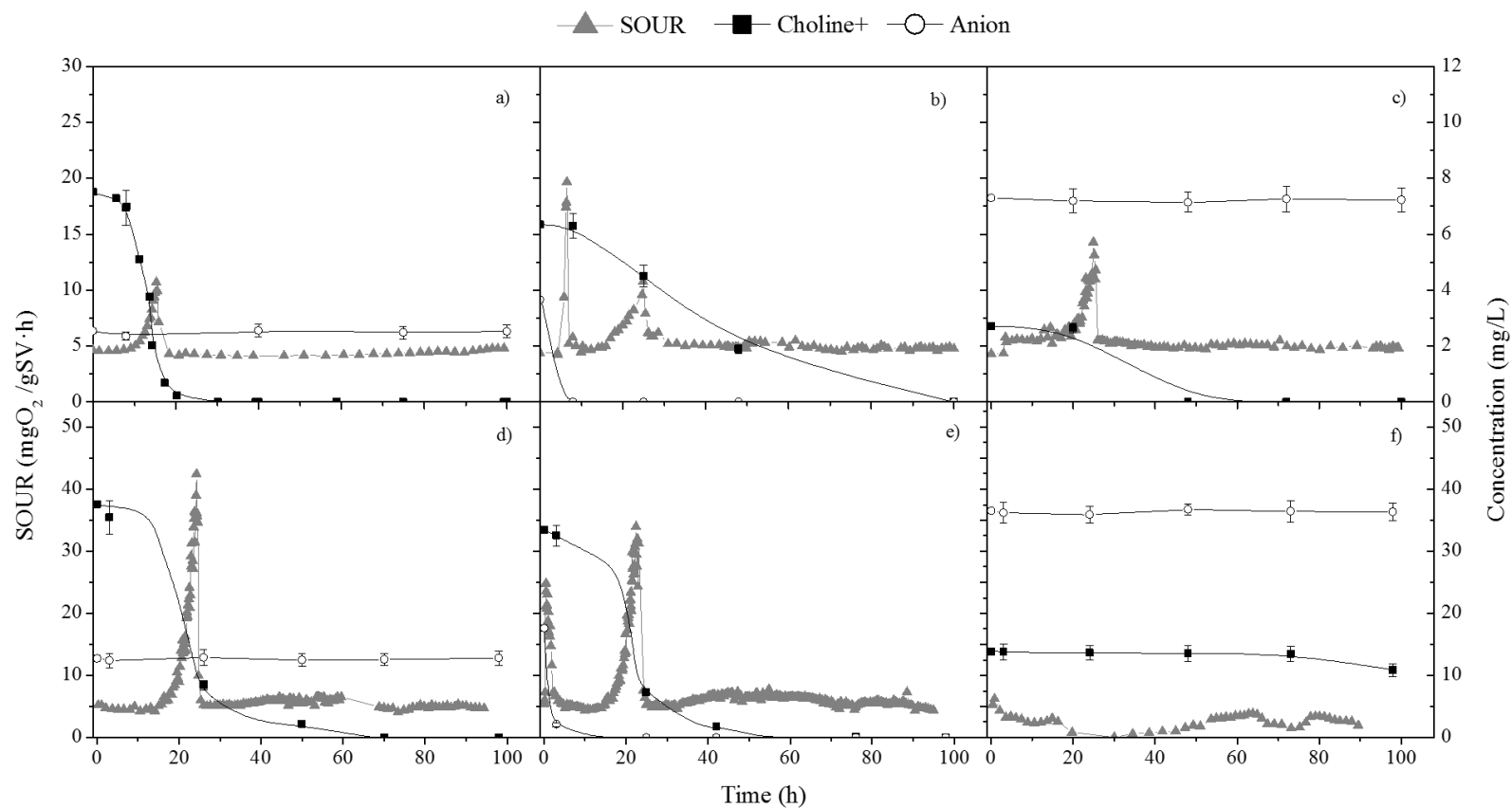
ILs biodegradability was further measured by the fast biodegradability respirometric test (Polo et al., 2011). Figure 3 shows the time course of SOUR and the concentration of each cation and anion for Bmim-based ILs using an initial concentration of 10 and 50 mg/L. Time-evolution ILs concentration is maintained practically unalterable for BmimCl and BmimNTf<sub>2</sub>, regardless of the IL initial concentration. The respirometric profiles did not suffer any significant changes for the lowest IL concentration (10 mg/L), whereas an initial decay of endogenous respiration was observed for the highest one (50 mg/L), which could be attributed to the partial inhibition of the microbial activity, followed by a slight increase of the sludge respiration after an adaptation time. The unacclimated sludge was able to degrade acetate concentrations of 3 and 15 mg/L in the first 8 h for BmimAc. SOUR values were proportional to the initial acetate concentration, and after the complete decay of this substrate, the microbial activity remained unalterable. The results of the fast biodegradability test are comparable to the obtained by the Zahn–Wellens test, although the former also indicates that a concentration of BmimNTf<sub>2</sub> superior to 50 mg/L can cause detrimental effects on the biomass, which seems to be related to the highest toxicity attributed to NTf<sub>2</sub><sup>−</sup> (Diaz et al., 2018; Santos et al., 2014).



290

291 **Figure 3.** Time course of IL concentration and SOUR profiles along the respirometric assays for an initial concentration of 10 mg/L (BmimCl  
 292 (a), BmimAc (b), and BmimNTf<sub>2</sub> (c)) and 50 mg/L (BmimCl (d), BmimAc (e), and BmimNTf<sub>2</sub> (f)).

The respirometric profiles obtained using 10 mg/L choline ILs (Figure 4) showed an increase of sludge respiration, after an acclimation time, which is associated with Choline<sup>+</sup> degradation. In the case of CholineCl, the presence of Cl<sup>-</sup> did not interfere to Choline<sup>+</sup> removal, whereas in presence of organic anions as Ac<sup>-</sup> and NTf<sub>2</sub><sup>-</sup>, the cation transformation happened at a lower rate. It could be related, on the one hand, to the fast assimilation of acetate as carbon source for the microorganisms, which took place in the first 8 h of the experiment, and on the other hand, to the presence of a non-biodegradable compound in the reaction medium as NTf<sub>2</sub><sup>-</sup>. An increase of the choline-based ILs concentration (50 mg/L) did not cause any important effect on the microbial activity in the case of CholineCl and CholineAc. In both cases, the respirometric profiles were similar to that obtained with a lower concentration, except that the maximum value of the SOUR was higher for the highest IL concentration. It may be related to the hydrophilic nature of Cl<sup>-</sup> and Ac<sup>-</sup> anions, which promotes their solvation by water molecular and the dominant presence of dissociated ionic species in the solution (Li et al., 2007; Palomar et al., 2007; Stassen et al., 2015). In the case of CholineNTf<sub>2</sub>, a fall of the endogenous respiration took place along the first hours of the assay and neither degradation of Choline<sup>+</sup> nor NTf<sub>2</sub><sup>-</sup> were observed. This suggests that CholineNTf<sub>2</sub>, at the highest concentration used (50 mg/L), caused a negative effect on the microbial diversity of sewage sludge, probably due to the hydrophobic nature of this anion, which promotes the presence of aggregated cation-anion species as increasing IL concentration (Li et al., 2007; Stassen et al., 2015). Ion-pair structures are less polar and more lipophilic than dissociated ions (González et al., 2018), increasing the toxic effects of the IL compound and preventing its biodegradation (Torrecilla et al., 2010).

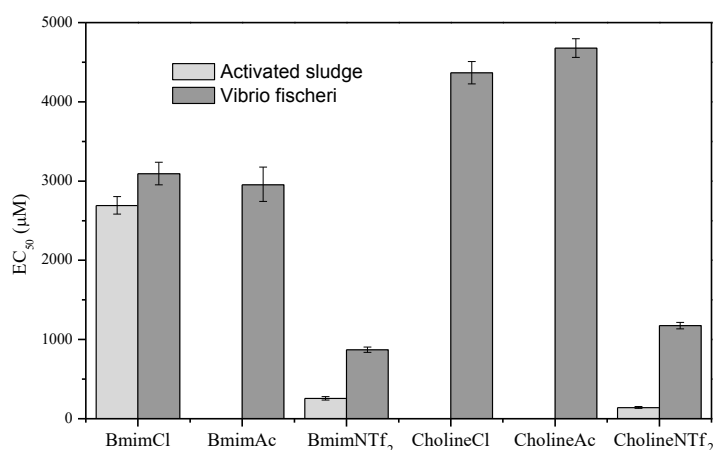


317

318 **Figure 4.** Time course of IL concentration and SOUR profiles along the respirometric assays for an initial concentration of 10 mg/L (CholineCl  
 319 (a), CholineAc (b), and CholineNTf<sub>2</sub> (c)) and 50 mg/L (CholineCl (d), CholineAc (e), and CholineNTf<sub>2</sub> (f)).

In order to gain information on the toxicity of Bmim- and choline-based ILs, a series of respirometric tests were carried out for each IL over a wide range of concentrations (10 – 500 mg/L), with the aim of establishing the inhibition of the activated sludge in terms of EC<sub>50</sub> values. In spite of the fact that the required time to carry out the experiments is less than 30 min, it was not possible to obtain values of EC<sub>50</sub> for compounds constituted by an easily biodegradable substrate as acetate, since in these cases, an increase in the BmimAc or CholineAc concentration did not cause a proportional decrease on the respiration rate of the microorganisms. This was also observed in the case of the experiments performed adding different concentrations of CholineCl, because of the high biodegradability of Choline<sup>+</sup>, and Cl<sup>-</sup> did not show an intrinsic toxicity effect. However, as can be seen in the Figure 5, the high toxicity associated with the anion NTf<sub>2</sub><sup>-</sup> allowed establishing an EC<sub>50</sub> value of 140 µM using activated sludge for CholineNTf<sub>2</sub>, which is equivalent to 54 mg/L. Additionally, the NTf<sub>2</sub><sup>-</sup> can be considered as a potential toxic species due to the possible formation of hydrofluoric acid from the hydrolysis of the fluorine component (Costa et al., 2015). Furthermore, the high toxicity associated to NTf<sub>2</sub><sup>-</sup> has been related to its high hydrophobicity (Costa et al., 2015b; Ranke et al., 2007) and the ability of the anion to permeate on the cell membrane, essentially non-polar (Dołzonek et al., 2017). This negative effect of NTf<sub>2</sub><sup>-</sup> respect Cl<sup>-</sup> and Ac<sup>-</sup> is accentuated because the cations studied are less likely to interact with membranes (Diaz et al., 2018), making evident the anion role on the ILs ecotoxicity. This value could explain the behavior exhibited by the microbial population of the activated sludge in the biodegradability assay (Figure 4f), which was exposed to an inhibitory concentration of CholineNTf<sub>2</sub>. Moreover, EC<sub>50</sub> values of 2691 µM for BmimCl and 257 µM for BmimNTf<sub>2</sub>, which are equivalent to 470 and 108 mg/L, respectively, could be also determined, due to both ILs did not present biodegradability.

For comparison purposes, the EC<sub>50</sub> values determined by Microtox® test are also showed in Figure 5. Among the ILs studied, those constituted by Cl<sup>-</sup> and Ac<sup>-</sup> presented the highest EC<sub>50</sub> values, evidencing their environmentally friendly behavior (Couling et al., 2006), being slightly lower the EC<sub>50</sub> values associated to Bmim ILs than to choline ILs. This fact agrees with the ILs with aromatic rings present more toxicity than those with non-aromatic groups (Costa et al., 2015b). On the other hand, the presence of the anion NTf<sub>2</sub><sup>-</sup> in the IL structure caused a considerable ecotoxicity increase. In general, the influence of the anion on the toxicity of IL has been considered of minor importance, but the results of this test makes evident that some anions, specifically those that contain fluorine atoms in their structure, can affect drastically to the IL toxicity (Costa et al., 2015b; Diaz et al., 2018; Matzke et al., 2007; Montalbán et al., 2016). The comparison of the results obtained for both tests lets conclude that the Microtox test can be used to establish a toxicity trend about the behavior of ILs on the activated sludge of a wastewater treatment plant. However, it is important to take into account that this test may not anticipate the toxicity of an IL on the activated sludge of wastewater plants, being more evident in the cases of the ILs constituted by NTf<sub>2</sub><sup>-</sup>.



**Figure 5.** EC<sub>50</sub> values (µM) for imidazolium and choline based ILs towards activated sludge and *Vibrio fischeri*.

#### 4. Conclusions

The detailed analysis of the evolution of the ILs concentration along biodegradability studies revealed that BmimNTf<sub>2</sub> could not be degraded by an activated sludge, even at low concentrations (10 mg/L), whereas CholineAc concentrations up to 50 mg/L can be completely removed. Moreover, the microorganisms can assimilate Choline<sup>+</sup> and acetate as constituents of other ILs, depending on the structure and the concentration of the counter-ion. A partial inhibition of the microbial activity was observed in the fast biodegradability test performed with 50 mg/L of CholineNTf<sub>2</sub>.

EC<sub>50</sub> values from Microtox Test for BmimCl and CholineCl lead the conclusion that toxic effects of these ILs are directly related to both ILs constituents. Choline<sup>+</sup> was slightly less toxic than Bmim<sup>+</sup> and that the presence of NTf<sub>2</sub><sup>-</sup> increased considerably the toxicity of ILs. According to the results from inhibition tests, ILs formed by a natural building block as Choline<sup>+</sup> can lead to nontoxic ILs for activated sludge microbiota, as well as occurred with Ac<sup>-</sup> in BmimAc and CholineAc. However, the presence of an anion with intrinsic toxicity as NTf<sub>2</sub><sup>-</sup> must not be underestimated, being this negative contribution more evident in Choline<sup>+</sup> ILs than in Bmim<sup>+</sup> ones. Consequently, the negative environmental impact of NTf<sub>2</sub>-containing ILs should take into account for their use in chemical processes, which can generate aqueous wastes.

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## References

- Alisawi, W.A., Rahbarirad, S., Walker, K.A., Venter, A.R., Docherty, K.M., Szymczyna, B.R., 2017. Identification of metabolites produced during the complete biodegradation of 1-butyl-3-methylimidazolium chloride by an enriched activated sludge microbial community. *Chemosphere* 167, 53–61.
- Amde, M., Liu, J., Pang, L., 2015. Environmental application, fate, effects and concerns of ionic liquids: A review. *Environ. Sci. Technol.* 49, 12611–12627. doi:10.1021/acs.est.5b03123.
- An, Y.X., Zong, M.H., Wu, H., Li, N., 2015. Pretreatment of lignocellulosic biomass with renewable cholinium ionic liquids: Biomass fractionation, enzymatic digestion and ionic liquid reuse. *Bioresour. Technol.* 192, 165–171. doi:10.1016/j.biortech.2015.05.064.
- Atefi, F., Garcia, M.T., Singer, R.D., Scammells, P.J., 2009. Phosphonium ionic liquids: design, synthesis and evaluation of biodegradability. *Green Chem.* 11, 1595–1604. doi:10.1039/b913057h.
- Bedia, J., Rodriguez, J.J., Moreno, D., Palomar, J., Belver, C., 2019. Photostability and photocatalytic degradation of ionic liquids in water under solar light. *RSC Adv.* 9, 2026–2033. doi:10.1039/c8ra07867j.
- Bhadani, A., Misono, T., Singh, S., Sakai, K., Sakai, H., Abe, M., 2016. Structural diversity, physicochemical properties and application of imidazolium surfactants: Recent advances. *Adv. Colloid Interface Sci.* 231, 36–58. doi:10.1016/j.cis.2016.03.005.
- Bingham, R.J., Ballone, P., 2012. Computational study of room-temperature ionic liquids interacting with a POPC phospholipid bilayer. *J. Phys. Chem. B* 116, 11205–11216. doi:10.1021/jp306126q.
- Bocos, E., González-Romero, E., Pazos, M., Sanromán, M.A., 2017. Application of electro-Fenton treatment for the elimination of 1-Butyl-3-methylimidazolium triflate from polluted water. *Chem. Eng. J.* 318, 19–28. doi:10.1016/j.cej.2016.04.058.



417 Chatel, G., Naffrechoux, E., Draye, M., 2017. Avoid the PCB mistakes: A more  
 418 sustainable future for ionic liquids. *J. Hazard. Mater.* 324, 773–780.  
 419 doi:10.1016/j.jhazmat.2016.11.060.

420 Chica, A.F., Martin, A., Vazquez, F.J., Carmona, F.J., Mohedo, J. Respirometer to  
 421 analyze measure dissolved oxygen and oxygen demand of microbes in leachate  
 422 from municipal waste. ES 2283171, 2007.

423 Cho, C.-W., Jeon, Y.-C., Pham, T.P.T., Vijayaraghavan, K., Yun, Y.-S., 2008. The  
 424 ecotoxicity of ionic liquids and traditional organic solvents on microalga  
 425 *Selenastrum capricornutum*. *Ecotoxicol. Environ. Saf.* 71, 166–171.  
 426 doi:10.1016/j.ecoenv.2007.07.001.

427 Costa, S.P.F., Pinto, P.C.A.G., Lapa, R.A.S., Saraiva, M.L.M.F.S., 2015a. Toxicity  
 428 assessment of ionic liquids with *Vibrio fischeri*: An alternative fully automated  
 429 methodology. *J. Hazard. Mater.* 284, 136–142.  
 430 doi:10.1016/j.jhazmat.2014.10.049.

431 Costa, S.P.F., Pinto, P.C.A.G., Saraiva, M.L.M.F.S., Rocha, F.R.P., Santos, J.R.P.,  
 432 Monteiro, R.T.R., 2015b. The aquatic impact of ionic liquids on freshwater  
 433 organisms. *Chemosphere* 139, 288–294. doi:10.1016/j.chemosphere.2015.05.100.

434 Costello, D.M., Brown, L.M., Lamberti, G.A., 2009. Acute toxic effects of ionic liquids  
 435 on zebra mussel (*Dreissena polymorpha*) survival and feeding. *Green Chem.* 11,  
 436 548–553. doi:10.1039/b822347e.

437 Couling, D.J., Bernot, R.J., Docherty, K.M., Dixon, J.K., Maginn, E.J., 2006. Assessing  
 438 the factors responsible for ionic liquid toxicity to aquatic organisms via  
 439 quantitative structure–property relationship modeling. *Green Chem.* 8, 82–90.  
 440 doi:10.1039/B511333D.

441 Cull, S.G., Holbrey, J.D., Vargas–Mora, V., Seddon, K.R., Lye, G.J., 2000. Room-  
 442 temperature ionic liquids as replacements for organic solvents in multiphase  
 443 bioprocess operations. *Biotechnol. Bioeng.* 69, 227–233. doi:10.1002/(SICI)1097-  
 444 0290(20000720)69:2%3C227::AID-BIT12%3E3.0.CO;2-0.

445 Cvjetko Bubalo, M., Radošević, K., Radojčić Redovniković, I., Halambek, J., Gaurina  
 446 Srček, V., 2014. A brief overview of the potential environmental hazards of ionic

liquids. *Ecotoxicol. Environ. Saf.* 99, 1–12. doi:10.1016/j.ecoenv.2013.10.019.

Diaz, E., Monsalvo, V., Palomar, J., Mohedano, A.F., 2016. Ionic liquids: Bacterial degradation in wastewater treatment plants, *Encycl. Inorg. Bioinorg. Chem.*, John Wiley & Sons, Ltd. doi:10.1002/9781119951438.eibc2393.

Diaz, E., Monsalvo, V.M., Lopez, J., Mena, I.F., Palomar, J., Rodriguez, J.J., Mohedano, A.F., 2018. Assessment the ecotoxicity and inhibition of imidazolium ionic liquids by respiration inhibition assays. *Ecotoxicol. Environ. Saf.* 162, 29–34. doi:10.1016/j.ecoenv.2018.06.057.

Docherty, K.M., Kulpa Jr., C.F., 2005. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chem.* 7, 185–189. doi:10.1039/b419172b.

Docherty, K.M., Aiello, S.W., Buehler, B.K., Jones, S.E., Szymczyna, B.R., Walker, K.A.C.F., 2015. Ionic liquid biodegradability depends on specific wastewater microbial consortia. *Chemosphere* 136, 160–166. doi:10.1016/j.chemosphere.2015.05.016.

Dołzonek, J., Cho, C.-W., Stepnowski, P., Markiewicz, M., Thöming, J., Stolte, S., 2017. Membrane partitioning of ionic liquids cations, anions, and ion pairs – Estimating the bioconcentration potential of organic ions. *Environ. Pollut.* 228, 378–389. doi:10.1016/j.envpol.2017.04.079.

Dominguez, C.M., Munoz, M., Quintanilla, A., de Pedro, Z.M., Ventura, S.P.M., Coutinho, J.A.P., Casas, J.A., Rodriguez, J.J., 2014. Degradation of imidazolium-based ionic liquids in aqueous solution by Fenton oxidation. *J. Chem. Technol. Biotechnol.* 89, 1197–1202. doi:10.1002/jctb.4366.

Egorova, K.S., Ananikov, V.P., 2014. Toxicity of ionic liquids: Eco(cyto)activity as complicated, but unavoidable parameter for task-specific optimization. *ChemSusChem.* 7, 336–360. doi:10.1002/cssc.201300459.

Fabiańska, A., Ossowski, T., Stepnowski, P., Stolte, S., Thöming, J., Siedlecka, E.M., 2012. Electrochemical oxidation of imidazolium-based ionic liquids: The influence of anions. *Chem. Eng. J.* 198–199, 338–345. doi:10.1016/j.cej.2012.05.108.

477 Ford, L., Harjani, J.R., Atefi, F., Garcia, M.T., Singer, R.D., Scammells, P.J., 2010.  
 478 Further studies on the biodegradation of ionic liquids. *Green Chem.* 12, 1783–  
 479 1789. doi:10.1039/c0gc00082e.

480 Frade, R.F.M., Afonso, C.A.M., 2010. Impact of ionic liquids in environment and  
 481 humans: An overview. *Hum. Exp. Toxicol.* 29, 1038–1054.  
 482 doi:10.1177/0960327110371259.

483 Freire, M.G., Neves, C.M.S.S., Marrucho, I.M., Coutinho, J.A.P., Fernandes, A.M.,  
 484 2010. Hydrolysis of tetrafluoroborate and hexafluorophosphate counter ions in  
 485 imidazolium-based ionic liquids. *J. Phys. Chem. A.* 114, 3744–3749.  
 486 doi:10.1021/jp903292n.

487 Gadilohar, B.L., Kumbhar, H.S., Shankarling, G.S., 2014. Choline Peroxydisulfate:  
 488 Environmentally friendly biodegradable oxidizing TSIL for selective and rapid  
 489 oxidation of alcohols. *Ind. Eng. Chem. Res.* 53, 19010–19018.  
 490 doi:10.1021/ie5032919.

491 Gadilohar, B.L., Shankarling, G.S., 2018. Choline based ionic liquids and their  
 492 applications in organic transformation. *J. Mol. Liq.* 227, 234–261.  
 493 doi:10.1016/j.molliq.2016.11.136.

494 Garcia, M.T., Gathergood, N., Scammells, P.J., 2005. Biodegradable ionic liquids - Part  
 495 II. Effect of the anion and toxicology. *Green Chem.* 7, 9–14.  
 496 doi:10.1039/B411922c.

497 Gathergood, N., Scammells, P.J., Garcia, M.T., 2006. Biodegradable ionic liquids - Part  
 498 III. The first readily biodegradable ionic liquids. *Green Chem.* 8, 156–160.  
 499 doi:10.1039/B516206H.

500 Gomez-Herrero, E., Tobajas, M., Polo, A., Rodriguez, J.J., Mohedano, A.F., 2018.  
 501 Removal of imidazolium- and pyridinium-based ionic liquids by Fenton  
 502 oxidation. *Environ. Sci. Pollut. Res.* 25, 34930–34937. doi:10.1007/s11356-017-  
 503 0867-4.

504 González, E.J., Palomar, J., Navarro, P., Larriba, M., García, J., Rodríguez, F., 2018. On  
 505 the volatility of aromatic hydrocarbons in ionic liquids: Vapor-liquid equilibrium  
 506 measurements and theoretical analysis. *J. Mol. Liq.* 250, 9–18.

doi:10.1016/j.molliq.2017.11.134.

Jing, B., Lan, N., Qiu, J., Zhu, Y., 2016. Interaction of ionic liquids with a lipid bilayer: a biophysical study of ionic liquid cytotoxicity. *J. Phys. Chem. B* 120, 2781–2789. doi:10.1021/acs.jpcc.6b00362.

Jordan, A., Gathergood, N., 2015. Biodegradation of ionic liquids - a critical review. *Chem. Soc. Rev.* 44, 8200–8237. doi:10.1039/c5cs00444f.

Karadas, F., Köz, B., Jacquemin, J., Deniz, E., Rooney, D., Thompson, J., Yavuz, C.T., Khraisheh, M., Aparicio, S., Atihan, M., 2013. High pressure CO<sub>2</sub> absorption studies on imidazolium-based ionic liquids: Experimental and simulation approaches. *Fluid Phase Equilib.* 351, 74–86. doi:10.1016/j.fluid.2012.10.022.

Kathiresan, M., Velayutham, D., 2015. Ionic liquids as an electrolyte for the electro synthesis of organic compounds. *Chem. Commun.* 51, 17499–17516. doi:10.1039/C5CC06961K.

Khan, M.I., Zaini, D., Shariff, A.M., 2016. Framework for ecotoxicological risk assessment of ionic liquids. *Procedia Eng.* 148, 1141–1148. doi:10.1016/j.proeng.2016.06.567.

Lemus, J., Palomar, J., Heras, F., Gilarranz, M.A., Rodriguez, J.J., 2012. Developing criteria for the recovery of ionic liquids from aqueous phase by adsorption with activated carbon. *Sep. Purif. Technol.* 97, 11–19. doi:10.1016/j.seppur.2012.02.027.

Leonardo da Silva, W., Caroline Leal, B., Luiza Ziulkoski, A., van Leeuwen, P. W. N. M., Henrique Zimnoch dos Santos, J., Stephan Schrekker, H., 2019. Petrochemical residue-derived silica-supported titania-magnesium catalysts for the photocatalytic degradation of imidazolium ionic liquids in water. *Sep. Purif. Technol.* 218, 191–199. doi:10.1016/j.seppur.2019.01.066.

Li, W., Zhang, Z., Han, B., Hu, S., Xie, Y., Yang, G., 2007. Effect of water and organic solvents on-the ionic dissociation of ionic liquids. *J. Phys. Chem. B.* 111, 6452–6456. doi:10.1021/jp071051m.

Liu, H., Zhang, X., Chen, C., Du, S., Dong, Y., 2015. Effects of imidazolium chloride

536 ionic liquids and their toxicity to *Scenedesmus obliquus*. *Ecotoxicol. Environ. Saf.*  
537 122, 83–90. doi:10.1016/j.ecoenv.2015.07.010.

538 Matzke, M., Stolte, S., Thiele, K., Juffernholz, T., Arning, J., Ranke, J., Welz-Birmann,  
539 U., Jastorff, B., 2007. The influence of anion species on the toxicity of 1-alkyl-3-  
540 methylimidazolium ionic liquids observed in an (eco)toxicological test battery.  
541 *Green Chem.* 9, 1198–1207. doi:10.1039/B705795D.

542 Mena, I.F., Cotillas, S., Díaz, E., Sáez, C., Mohedano, A.F., Rodrigo, M.A., 2019a.  
543 Sono- and photoelectrocatalytic processes for the removal of ionic liquids based  
544 on the 1-butyl-3-methylimidazolium cation. *J. Hazard. Mat.* 372, 77–84.  
545 doi:10.1016/j.jhazmat.2017.12.015.

546 Mena, I.F., Díaz, E., Pérez-Farías, C., Stolte, S., Moreno-Andrade, I., Rodriguez, J.J.,  
547 Mohedano, A.F., 2019b. Catalytic wet peroxide oxidation of imidazolium-based  
548 ionic liquids: Catalyst stability and biodegradability enhancement. *Chem. Eng. J.*  
549 (In press). doi:10.1016/j.cej.2018.11.129.

550 Mena, I.F., Díaz, E., Rodriguez, J.J., Mohedano, A.F., 2019c. Biological oxidation of  
551 choline-based ionic liquids in sequencing batch reactors. *J. Chem. Technol.*  
552 *Biotechnol.* (In press). doi:10.1002/jctb.5954.

553 Montalbán, M.G., Hidalgo, J.M., Collado-González, M., Díaz Baños, F.G., Villora, G.,  
554 2016. Assessing chemical toxicity of ionic liquids on *Vibrio fischeri*: Correlation  
555 with structure and composition. *Chemosphere.* 155, 405–414.  
556 doi:10.1016/j.chemosphere.2016.04.042.

557 Montalbán, M.G., Villora, G., Licence, P., 2018. Ecotoxicity assessment of dicationic  
558 versus monocationic ionic liquids as a more environmentally friendly alternative.  
559 *Ecotoxicol. Environ. Saf.* 150, 129–135. doi:10.1016/j.ecoenv.2017.11.073.

560 Moya, C., Alonso-Morales, N., Gilarranz, M.A., Rodriguez, J.J., Palomar, J., 2016.  
561 Encapsulated Ionic Liquids for CO<sub>2</sub> Capture: Using 1-Butyl-methylimidazolium  
562 Acetate for Quick and Reversible CO<sub>2</sub> Chemical Absorption. *ChemPhysChem* 17,  
563 3891–3899. doi:10.1002/cphc.201600977.

564 Munoz, M., Domínguez, C.M., De Pedro, Z.M., Quintanilla, A., Casas, J.A., Ventura,  
565 S.P.M., Coutinho, J.A.P., 2015. Role of the chemical structure of ionic liquids in

566 their ecotoxicity and reactivity towards Fenton oxidation, Sep. Purif. Technol.  
 567 150, 252–256. doi:10.1016/j.seppur.2015.07.014.

568 Munoz, M., Domínguez, C.M., De Pedro, Z.M., Quintanilla, A., Casas, J.A., Rodriguez,  
 569 J.J., 2016. Degradation of imidazolium-based ionic liquids by catalytic wet  
 570 peroxide oxidation with carbon and magnetic iron catalysts. J. Chem. Technol.  
 571 Biotechnol. 91, 2882–2887. doi:10.1002/jctb.4904.

572 Nandwani, S.K., Malek, N.I., Lad, V.N., Chakraborty, M., Gupta, S., 2017. Study on  
 573 interfacial properties of Imidazolium ionic liquids as surfactant and their  
 574 application in enhanced oil recovery. Colloids Surfaces A Physicochem. Eng.  
 575 Asp. 516, 383–393. doi:10.1016/j.colsurfa.2016.12.037.

576 Neumann, J., Pawlik, M., Bryniok, D., Thöming, J., Stolte, S., 2014a. Biodegradation  
 577 potential of cyano-based ionic liquid anions in a culture of *Cupriavidus spp.* and  
 578 their in vitro enzymatic hydrolysis by nitrile hydratase. Environ. Sci. Pollut. Res.  
 579 21, 9495–9505. doi:10.1007/s11356-013-2341-2.

580 Neumann, J., Steudte, S., Cho, C.-W., Thöming, J., Stolte, S., 2014b. Biodegradability  
 581 of 27 pyrrolidinium, morpholinium, piperidinium, imidazolium and pyridinium  
 582 ionic liquid cations under aerobic conditions. Green Chem. 16, 2174–2184.  
 583 doi:10.1039/C3GC41997E.

584 Neves, C.M.S.S., Lemus, J., Freire, M.G., Palomar, J., Coutinho, J.A.P., 2014.  
 585 Enhancing the adsorption of ionic liquids onto activated carbon by the addition of  
 586 inorganic salts. Chem. Eng. J. 252, 305–310. doi:10.1016/j.cej.2014.05.009.

587 Olivier-Bourbigou, H., Magna, L., Morvan, D., 2010. Ionic liquids and catalysis: Recent  
 588 progress from knowledge to applications. Appl. Catal. A Gen. 373, 1–56.  
 589 doi:10.1016/j.apcata.2009.10.008.

590 Oulego, P., Blanco, D., Ramos, D., Viesca, J.L., Díaz, M., Hernández Battez, A., 2018.  
 591 Environmental properties of phosphonium, imidazolium and ammonium cation-  
 592 based ionic liquids as potential lubricant additives, J. Mol. Liq. 272, 937–947.  
 593 doi:10.1016/j.molliq.2018.10.106.

594 Pagga, U., 1997. Testing biodegradability with standardized methods, Chemosphere 35,  
 595 2953–2972.

596 Palomar, J., Ferro, V.R., Gilarranz, M.A., Rodriguez, J.J., 2007. Computational  
597 approach to nuclear magnetic resonance in 1-alkyl-3-methylimidazolium ionic  
598 liquids, *J. Phys. Chem. B.* 111, 168–180. doi:10.1021/jp063527s.

599 Palomar, J., Gonzalez-Miquel, M., Bedia, J., Rodriguez, F., Rodriguez, J.J., 2011. Task-  
600 specific ionic liquids for efficient ammonia absorption, *Sep. Purif. Technol.* 82,  
601 43–52. doi:10.1016/j.seppur.2011.08.014.

602 Pandey, S., Baker, G.A., Sze, L., Pandey, S., Kamath, G., Zhao, H., Baker, S.N., 2013.  
603 Ionic liquids containing fluorinated  $\beta$ -diketonate anions: synthesis,  
604 characterization and potential applications, *New J. Chem.* 37, 909–919.  
605 doi:10.1039/c3nj40855h.

606 Pârvulescu, V.I., Hardacre, C., 2007. Catalysis in ionic liquids. *Chem. Rev.* 107, 2615–  
607 2665. doi:10.1021/cr050948h.

608 Peric, B., Sierra, J., Martí, E., Cruaños, R., Garau, M.A., Arning, J., Bottin-Weber, U.,  
609 Stolte, S., 2013. (Eco)toxicity and biodegradability of selected protic and aprotic  
610 ionic liquids, *J. Hazard. Mater.* 261, 99–105. doi:10.1016/j.jhazmat.2013.06.070.

611 Petkovic, M., Seddon, K.R., Rebelo, L.P.N., Pereira, C.S., 2011. Ionic liquids: a  
612 pathway to environmental acceptability, *Chem. Soc. Rev.* 40, 1383–1403.  
613 doi:10.1039/c004968a.

614 Pieczyńska, A., Ofiarska, A., Borzyszkowska, A.F., Białk-Bielińska, A., Stepnowski,  
615 P., Stolte, S., Siedlecka, E.M., 2015. A comparative study of electrochemical  
616 degradation of imidazolium and pyridinium ionic liquids: A reaction pathway and  
617 ecotoxicity evaluation. *Sep. Purif. Technol.* 156, 522–534.  
618 doi:10.1016/j.seppur.2015.10.045.

619 Plechkova, N.V., Seddon, K.R., 2008. Applications of ionic liquids in the chemical  
620 industry, *Chem. Soc. Rev.* 37, 123–150. doi:10.1039/b006677j.

621 Polo, A.M., Tobajas, M., Sanchis, S., Mohedano, A.F., Rodriguez, J.J., 2011.  
622 Comparison of experimental methods for determination of toxicity and  
623 biodegradability of xenobiotic compounds, *Biodegradation.* 22, 751–761.  
624 doi:10.1007/s10532-010-9448-7.

625 Poza–Nogueiras, V., Arellano, M., Rosales, E., Pazos, M., González–Romero, E.,  
 626 Sanromán, M.A., 2018. Heterogeneous electro-Fenton as plausible technology for  
 627 the degradation of imidazolinium–based ionic liquids. *Chemosphere* 199, 68–75.  
 628 doi:10.1016/j.chemosphere.2018.01.174.

629 Pretti, C., Renzi, M., Focardi, S.E., Giovani, A., Monni, G., Melai, B., Rajamani, S.,  
 630 Chiappe, C., 2011. Acute toxicity and biodegradability of N-alkyl-N-  
 631 methylmorpholinium and N-alkyl-DABCO based ionic liquids, *Ecotoxicol.*  
 632 *Environ. Saf.* 74, 748–753. doi:10.1016/j.ecoenv.2010.10.032.

633 Quijano, G., Couvert, A., Amrane, A., Darracq, G., Couriol, C., Le Cloirec, P., Paquin,  
 634 L., Carrié, D., 2011. Toxicity and biodegradability of ionic liquids: New  
 635 perspectives towards whole-cell biotechnological applications, *Chem. Eng. J.* 174,  
 636 27–32. doi:10.1016/j.cej.2011.07.055.

637 Radošević, K., Cvjetko Bubalo, M., Gaurina Srček, V., Grgas, D., Landeka Dragičević,  
 638 T., Redovniković, R.I., 2015. Evaluation of toxicity and biodegradability of  
 639 choline chloride based deep eutectic solvents, *Ecotoxicol. Environ. Saf.* 112, 46–  
 640 53. doi:10.1016/j.ecoenv.2014.09.034.

641 Ranke, J., Müller, A., Bottin-Weber, U., Stock, F., Stolte, S., Arning, J., Störmann, R.,  
 642 Jastorff, B., 2007. Lipophilicity parameters for ionic liquid cations and their  
 643 correlation to in vitro cytotoxicity, *Ecotoxicol. Environ. Saf.* 67, 430–438.  
 644 doi:10.1016/j.ecoenv.2006.08.008.

645 Richardson, S.D., Kimura, S.Y., 2016. Water analysis: Emerging contaminants and  
 646 current issues, *Anal. Chem.* 88, 546–582.  
 647 <http://pubs.acs.org/doi/10.1021/acs.analchem.5b04493>.

648 Richardson, S.D., Ternes, T.A., 2014. Water analysis: Emerging contaminants and  
 649 current issues, *Anal. Chem.* 86, 2813–2848. doi:10.1021/ac500508t.

650 Rizzo, L., 2011. Bioassays as a tool for evaluating advanced oxidation processes in  
 651 water and wastewater treatment, *Water Res.* 45, 4311–4340.  
 652 doi:10.1016/j.watres.2011.05.035.

653 Romero, A., Santos, A., Tojo, J., Rodriguez, A., 2008. Toxicity and biodegradability of  
 654 imidazolium ionic liquids, *J. Hazard. Mater.* 151, 268–273.



doi:10.1016/j.jhazmat.2007.10.079.

Samori, C., Malferrari, D., Valbonesi, P., Montecavalli, A., Moretti, F., Galletti, P., Sartor, G., Tagliavini, E., Fabbri, E., Pasteris, A., 2010. Introduction of oxygenated side chain into imidazolium ionic liquids: Evaluation of the effects at different biological organization levels, *Ecotoxicol. Environ. Saf.* 73, 1456–1464. doi:10.1016/j.ecoenv.2010.07.020.

Sanchis, S., Polo, A.M., Tobajas, M., Rodriguez, J.J., Mohedano, A.F., 2014. Strategies to evaluate biodegradability: Application to chlorinated herbicides, *Environ. Sci. Pollut. Res.* 21, 9445–9452. doi:10.1007/s11356-013-2130-y.

Santos, A.G., Ribeiro, B.D., Alviano, D.S., Coelho, M.A.Z., 2014. Toxicity of ionic liquids toward microorganisms interesting to the food industry, *RSC Adv.* 4, 37157–37163. doi:10.1039/C4RA05295A.

Sekar, S., Surianarayanan, M., Ranganathan, V., Macfarlane, D.R., Mandal, A.B., 2012. Choline-Based Ionic Liquids-Enhanced Biodegradation of Azo Dyes, *Environ. Sci. Technol.* 46, 4902–4908.

Sintra, T.E., Nasirpour, M., Siopa, F., Rosatella, A.A., Gonçalves, F., Coutinho, J.A.P., Afonso, C.A.M., Ventura, S.P.M., 2017. Ecotoxicological evaluation of magnetic ionic liquids, *Ecotoxicol. Environ. Saf.* 143, 315–321. doi:10.1016/j.ecoenv.2017.05.034.

Stassen, H.K., Ludwig, R., Wulf, A., Dupont, J., 2015. Imidazolium salt ion Pairs in solution, *Chem. - A Eur. J.* 21, 8324–8335. doi:10.1002/chem.201500239.

Stepnowski, P., Zaleska, A., 2005. Comparison of different advanced oxidation processes for the degradation of room temperature ionic liquids. *J. Photochem. Photobiol. A Chem.* 170, 45–50. doi:10.1016/j.jphotochem.2004.07.019.

Stolte, S., Abdulkarim, S., Arning, J., Blomeyer-Nienstedt, A.K., Bottin-Weber, U., Matzke, M., Ranke J., Jastorff, B., Thöming, J., 2008. Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds, *Green Chem.* 10, 214–224. doi:10.1039/b713095c.

684 Stolte, S., Arning, J., Bottin-Weber, U., Müller, A., Pitner, W.-R., Welz-Biermann, U.,  
685 Jastorff, B., Ranke, J., 2007. Effects of different head groups and functionalised  
686 side chains on the cytotoxicity of ionic liquids, *Green Chem.* 9, 1170–1179.  
687 doi:10.1039/B615326G.

688 Stolte, S., Steudte, S., Areitioaurtena, O., Pagano, F., Thöming, J., Stepnowski, P.,  
689 Igartua A., 2012. Ionic liquids as lubricants or lubrication additives: An  
690 ecotoxicity and biodegradability assessment, *Chemosphere.* 89, 1135–1141.  
691 doi:10.1016/j.chemosphere.2012.05.102.

692 Thuy Pham, T.P., Cho, C.W., Yun, Y.S., 2010. Environmental fate and toxicity of ionic  
693 liquids: A review, *Water Res.* 44, 352–372. doi:10.1016/j.watres.2009.09.030.

694 Torrecilla, J.S., Palomar, J., Lemus, J., Rodríguez, F. A., 2010. Quantum-chemical-  
695 based guide to analyze/quantify the cytotoxicity of ionic liquids, *Green Chem.* 12,  
696 123–134. doi:10.1039/b919806g.

697 Tsarpali, V., Dailianis, S., 2015. Toxicity of two imidazolium ionic liquids,  
698 [bmim][BF<sub>4</sub>] and [omim][BF<sub>4</sub>], to standard aquatic test organisms: Role of  
699 acetone in the induced toxicity, *Ecotoxicol. Environ. Saf.* 117, 62–71.  
700 doi:10.1016/j.ecoenv.2015.03.026.

701 Ventura, S.P.M., e Silva, F.A., Gonçalves, A.M.M., Pereira, J.L., Gonçalves, F.,  
702 Coutinho, J.A.P., 2014. Ecotoxicity analysis of cholinium-based ionic liquids to  
703 *Vibrio fischeri* marine bacteria, *Ecotoxicol. Environ. Saf.* 102, 48–54.  
704 doi:10.1016/j.ecoenv.2014.01.003.

705 Viboud, S., Papaiconomou, N., Cortesi, A., Chatel, G., Draye, M., Fontvieille, D., 2012.  
706 Correlating the structure and composition of ionic liquids with their toxicity on  
707 *Vibrio fischeri*: A systematic study, *J. Hazard. Mater.* 215-216, 40–48.  
708 doi:10.1016/j.jhazmat.2012.02.019.

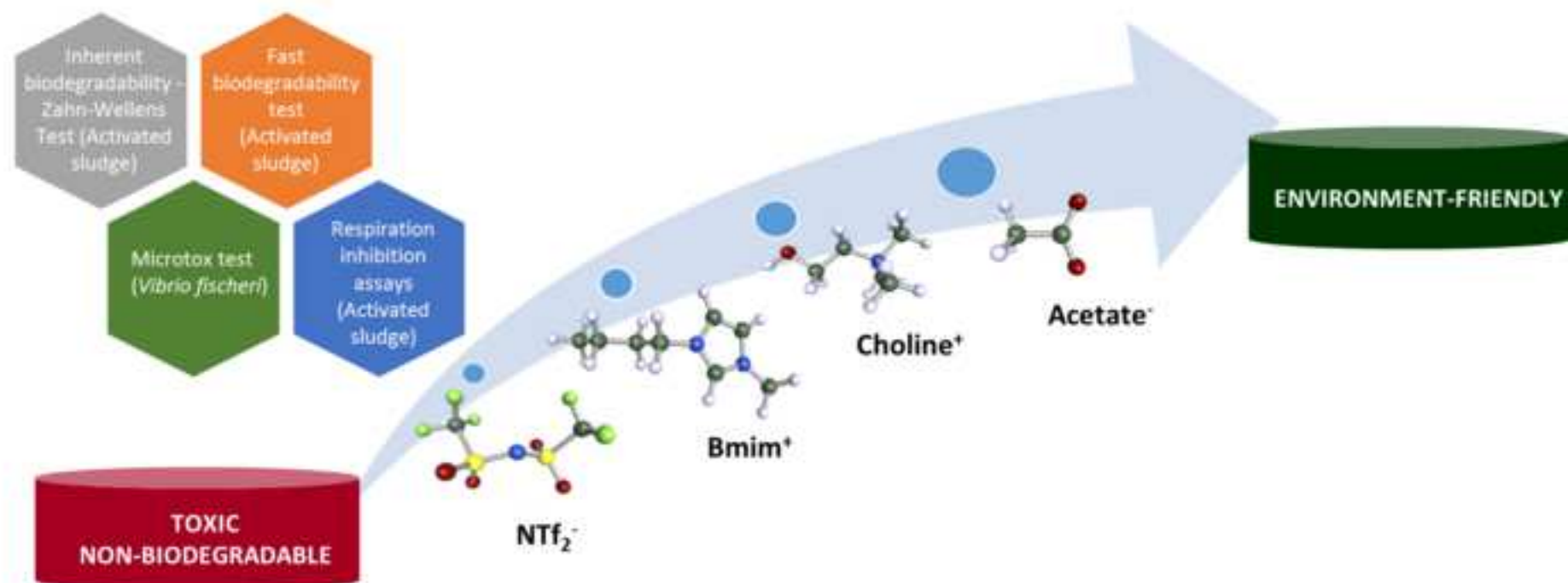
709 Vieira, N.S.M., Stolte, S., Araújo, J.M.M., Rebelo, L.P.N., Pereiro, A.B., Markiewicz,  
710 M., 2019. Acute Aquatic Toxicity and Biodegradability of Fluorinated Ionic  
711 Liquids, *ACS Sustain. Chem. Eng.* 7, 3733–3741.  
712 doi:10.1021/acssuschemeng.8b03653.

713 Yoo, B., Shah, J.K., Zhu, Y., Maginn, E.J., 2014. Amphiphilic interactions of ionic

714 liquids with lipid biomembranes: a molecular simulation study. *Soft Matter* 10,  
715 8641–8651. doi:10.1039/C4SM01528B

716 Zhang, Q., De Oliveira Vigier, K., Royer, S., Jérôme, F., 2012. Deep eutectic solvents:  
717 syntheses, properties and applications, *Chem. Soc. Rev.* 41, 7108.  
718 doi:10.1039/c2cs35178a.

719 Zhao, D., Wu, M., Kou, Y., Min, E., 2002. Ionic liquids: applications in catalysis, *Catal.*  
720 *Today* 74, 157–189. doi:10.1016/S0920-5861(01)00541-7.



**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: