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Assessment the ecotoxicity and inhibition of imidazolium ionic liquids by respiration inhibition assays

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ABSTRACT

The ecotoxicity and inhibition of 12 imidazolium ionic liquids (ILs) with alkyl chain from C4 to C10 and chloride (Cl^-), tetrafluoroborate (BF_4^-) and bis(trifluoromethanesulfonyl)imide (NTf_2^-) anions have been studied by means of respiration inhibition assays using activated sludge collected from a wastewater treatment plant. This test represents an alternative easy, economic and quick way to evaluate the true impact of ILs on activated sludge-based wastewater treatment. For comparison purposes, the EC_{50} values were also determined by the Microtox test (*Vibrio fischeri*). It was observed that this widely used microbial test overestimates the effect of the ILs on biological wastewater treatment facilities, especially in the case of ILs with lower ecotoxicity. The results of the biological tests showed that the alkyl chain length plays a crucial role in the ecotoxicity of ILs. A significant increase of the toxicity with the length of the n-alkyl chain was found. Regarding to the impact of the anion, the ecotoxicity measured by respiration inhibition assays follows the order $NTf_2^- > Cl^- > BF_4^-$, being the anion effect higher as decreasing the length of cation alkyl chain. According to the hazard substances ranking for aquatic organisms (Passino and Smith, 1987), imidazolium ILs with C4 alkyl chain can be classified as "practically harmless" compounds whereas those with alkyl chains C8 or C10 correspond to "highly toxic" species.

1. Introduction

In the past 20 years, ionic liquids (ILs) have attracted increasing attention as a new generation of green solvents with potential uses in various industrial fields (Petkovic et al., 2011). ILs are based on combined organic cations and organic or inorganic anions with a melting point below 100 °C. They are characterized by low vapor pressure and high thermal and chemical stabilities. Due to the large number of feasible combinations, it is possible to synthesize ILs with selected properties for numerous applications (Plechkova and Seddon, 2008). The increasing interest for these novel and versatile compounds is mainly focused on the following potential industrial applications: separation processes, catalysis, electrochemistry and materials science. However, the impact that ILs, especially those with high water solubility, can cause on aquatic organisms has been scarcely studied so far (Pham et al., 2010; Docherty et al., 2015).

ILs can be discharged into the aquatic environment due to accidental spills, containers washing operations, leaching from waste disposal sites, as well as waste streams inefficiently treated in current wastewater treatment plants (Tsarpali and Dailianis, 2015). Conventional biological processes are widely used as a cost-effective strategy for wastewater treatment. However, the potential application of such processes to a given effluent must consider some critical issues, as ecotoxicity and biodegradability. Preliminary results have revealed that there is insufficient evidence to confirm the degradation of imidazolium, thiazolium and pyridium ILs, whereas those derived from aliphatic amines and organic acids could be considered as potentially biodegradable (Peric et al., 2013; Neumann et al., 2014; Jordan and Gathergood, 2015; Diaz et al., 2016). In addition, the IL structure plays a key role on the inhibitory effect over the activated sludge performance. As example, alkyl methyl imidazolium ILs have been claimed as non-biodegradable compounds and they do not provoke any toxic effect on activated sludge (Gathergood et al., 2006; Romero et al., 2008; Quijano et al., 2011; Diaz et al., 2016; Rodriguez Castillo et al., 2016). The degradation of easily biodegradable substrates, like sodium n-dodecyl sulfate or glucose in presence of ILs, after long acclimation periods has been reported (Quijano et al., 2011; Rodriguez Castillo et al., 2016). However, the addition of ILs as Aliquat, 1-methyl-3–2-methoxyethyl-imidazolium or 1-methyl-3-butenyl-imidazolium provoked complete inhibition of the microbial community present in activated sludge (Quijano et al., 2011; Rodriguez Castillo et al., 2016).

Different standard toxicity tests have been proposed in the literature and regulations. Several organisms have been used as bioindicators, including invertebrate (*Daphnia magna*, ISO 6341) (Samorì et al., 2010;

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Pham et al., 2010; Stolte et al., 2012), algae (*Selenastrum capricornutum*, ISO 8692) (Cho et al., 2008; Peric et al., 2013; Costa et al., 2015), plant (*Lemna minor*, ISO/CD 20079) (Jastorff et al., 2005; Matzke et al., 2007; Stolte et al., 2007) or mammalian cells (*Rat leukemia cells*, IPC-81) (Ranke et al., 2007; Stolte et al., 2013), as a reproducible IL toxicity screening tool. These micro/organisms are exposed to different concentrations of ILs under controlled conditions and the evolution of a characteristic response of each organism is monitored. Among them, luminescent microbial test with the microorganism *Vibrio fischeri* (ISO 11348) is one of the most used for acute toxicity measurements because it is quick, simple, cost-effective and sensitive to evaluate IL ecotoxicity (Ranke et al., 2004; Johnson, 2005; Romero et al., 2008; Viboud et al., 2012; Ventura et al., 2014; Costa et al., 2015; Hernández-Fernández et al., 2015). However, the main drawback of the aforementioned methods is that the response of the microorganism used does not represent the behavior of the microbial community present in activated sludge from biological wastewater treatment systems. Consequently, the ecotoxicity data from those tests could over/underestimate the effect of ILs on wastewater treatment facilities. Meanwhile, the toxicity of ILs can be studied by a respiration inhibition test (OECD 209, ISO 8192) where the impact of ILs on activated sludge is inferred from specific dissolved oxygen uptake rate measurements, intimately related to the microbial activity. After data collection, the EC_{50} value (defined as the effective concentration of a sample that causes 50% inhibition) can be determined. Markiewicz et al. (2013) reported a pioneer work investigating the influence of ILs on activated sewage sludge communities. A respiration inhibition test was applied using activated sludge from different domestic and industrial sources. Results obtained generally match the IL ecotoxicity trends found for other organism and test systems. It was suggested that EC_{50} values obtained from *Vibrio fischeri* can be reliably used to assess the IL inhibition potential.

The alkyl methyl imidazolium ILs are non-volatile, non-flammable and present high thermal stability, being excellent wide-range solvents (Gordon, 2001; van Rantwijk et al., 2003). Thus, that family has a high potential of entering the water bodies through to industrial discharges. In the current work, the ecotoxicity of 12 imidazolium ILs in aqueous phase has been assessed by obtaining the EC_{50} values upon respiration inhibition assays with an activated sludge from a domestic sewage treatment plant. The effect of the IL structure in the ecotoxicity has been systematically analyzed by studying common cation/anion series, including imidazolium ring with different alkyl chain length (C_4-C_{10}) and three remarkably different anions (Cl^-, BF_4^-, NTf_2^-). For the sake of comparison, EC_{50} values were also obtained by the Microtox standard procedure. Structure-activity relationships were stablished to analyze simultaneously the cation and anion influence. Results obtained from two different tests were compare in order to check if the tests provide analogous information, which could be used to define a treatment strategy to deal with these compounds.

2. Materials and methods

2.1. Ionic liquids

Imidazolium ILs with different chain lengths (4, 6, 8 and 10 carbons) and anions (chloride (Cl^-), tetrafluoroborate (BF_4^-) and bis(trifluoromethilsulfonyl)imide (NTf_2^-)) were selected for this study (Table 1).

ILs are used without previous purification and named according to the number of carbons of the alkyl chain substituent as the butyl group “B”, hexyl group “H”, octyl group “O” and decyl group “D”, and the termination Cl, BF_4 or NTf_2 corresponds to the anion. The suffix “mim” corresponds with the imidazolium group (Table 2).

2.2. Inoculum and culture medium

Activated sludge for the respiration inhibition test (OECD 209, ISO 8192) was collected from a domestic sewage treatment plant (Madrid, Spain), and did not undergo to any acclimation process to the ILs studied. Waste sewage sludge was maintained in a sequencing batch reactor (SBR) at 25 °C and supplied with sodium acetate and glucose as carbon sources (50:50 w/w on chemical oxygen demand (COD) basis) at an organic load rate of 0.4 mg COD/mg VSS·day, referring VSS to volatile suspended solids. The medium was supplemented with ammonium sulfate, phosphoric acid and mineral salts as nitrogen, phosphorous and micronutrients sources ($FeCl_3$, $CaCl_2$, KCl and $MgCl_2$), respectively. A COD:N:P ratio of 100:5:1 (w/w) was fixed and mineral salts were also added as micronutrients supply in a COD: micronutrients (Fe, Ca, K and Mg) ratio of 1:0.05. Biomass concentration in the reactor was maintained at around 3500 mg VSS/L.

2.3. Inhibition assays

Respiration inhibition tests with activated sludge were carried out according to the method proposed by Polo et al. (2011). The procedure consists on short-term respirometric measurements using unacclimated sludge (350 mg VSS/L) where an easily biodegradable substrate (sodium acetate) is fed alone or together with different concentrations of ILs. Assays were carried out in a Liquid-Static-Static (LSS) respirometer (Chica et al., 2007), monitoring the dissolved oxygen concentration decay. Aeration and oxygen probes were controlled by an electronic interface. The respirometer operated with two independent reactors simultaneously to check the reproducibility. The reactors have very small headspace so that oxygen transfer from the gas to the liquid can be neglected. They were placed in a thermostatic bath and continuously stirred by magnetic bars. Nitrification was inhibited by using N-allyltiourea. Fresh activated sludge was used in each test to avoid partial acclimation of the microorganisms to the target chemicals, which could lead to possible underestimation of the toxic effects. The biomass activity was measured in terms of specific exogenous oxygen uptake rate (SOUR). The inhibition [1] is defined as function of the parameter γ [2] and the ratio the specific exogenous oxygen uptake rate for the reference substance (sodium acetate) in presence of a given concentration of the ILs ($SOUR_T$) and SOUR the obtained value for the reference substance ($SOUR_R$). Both measures are corrected by the value for the endogenous SOUR.

$$\text{Inhibition}(\%) = (\gamma)\cdot\frac{SOUR_T}{SOUR_R} \quad (1)$$

$$\gamma = \frac{SOUR_R - SOUR_T}{SOUR_T} \quad (2)$$

Inhibition caused by the ILs was assessed in terms of EC_{50}, defined as the effective concentration causing 50% reduction of SOUR ($\gamma = 1$). It is calculated using i) a logistic model that establish a relationship between the inhibition percentage and the logarithm compound concentration [3] and ii) a linear fit between the logarithm of parameter γ and the logarithm of IL concentration [4]:

$$\text{Inhibition}(\%) = \frac{100}{1+10^{-k(\log C - \log EC_{50})}} \quad (3)$$

$$\log\gamma = a + b\log C; EC_{50} = 10^{-a/b} \quad (4)$$

2.4. Ecotoxicity test

Ecotoxicity measurements were also carried out following the standard Microtox ® test procedure (ISO, 11348-3, 1998). This test is based on the decrease of light emission by the marine bacteria *Vibrio fischeri* (*Photobacterium phosphoreum*). A Microtox M500 Analyzer (Azur Environmental) was used to measure the inhibition of the light emitted by the bacteria after 15 min contact time with the sample. Previously, the pH was adjusted into the range 6–8. The results were expressed as EC_{50} defined as the effective concentration causing 50% reduction of

Table 1
Information on ionic liquids used in this work.

Ionic liquid	Supplier	Purity % (w/w)	K_{OW}
1-Butyl-3-methylimidazolium chloride (BmimCl)	Sigma-Aldrich	> 98%	0.001[a]
1-Hexyl-3-methylimidazolium chloride (HmimCl)	Sigma-Aldrich	> 97%	0.013[a]
1-Methyl-3-octylimidazolium chloride (OmimCl)	Solchemar	> 98%	0.123[a]
1-Decyl-3-methylimidazolium chloride (DmimCl)	Sigma-Aldrich	> 96%	0.520[b]
1-Butyl-3-methylimidazolium tetrafluoroborate ($BmimBF_4$)	Iolitec	> 99%	0.036[c]
1-Hexyl-3-methylimidazolium tetrafluoroborate ($HmimBF_4$)	Iolitec	> 99%	0.195[c]
1-Methyl-3-octylimidazolium tetrafluoroborate ($OmimBF_4$)	Solchemar	> 98%	0.209[d]
1-Decyl-3-methylimidazolium tetrafluoroborate ($DmimBF_4$)	Iolitec	> 98%	–
1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ($BmimNTf_2$)	Iolitec	99%	0.110[e]
1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ($HmimNTf_2$)	Iolitec	99%	1.42[e]
1-Methyl-3-octylimidazolium bis(trifluoromethylsulfonyl) imide ($OmimNTf_2$)	Iolitec	99%	6.30[e]
1-Decyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ($DmimNTf_2$)	Iolitec	> 98%	–

[a] Palomar et al. (2009).
[b] Domanska et al. (2008).
[c] Stepnowski and Storoniak (2005).
[d] Montalbán et al. (2015).
[e] Ropel et al. (2005).

light emission, calculated using the abovementioned linear model [4].

3. Results and discussion

Table 3 shows the EC_{50} values obtained from the Microtox test for the ILs tested and, for the sake of comparison, also includes values from the literature. As can be seen, there is a good agreement between the values of the current work and those previously reported by other authors. Moreover, the low standard deviation values confirm the reproducibility of the test. It can be clearly observed a general trend towards ecotoxicity increase (lower EC_{50}) with the length of the linear alkyl chain of the ILs. That effect becomes more pronounced for ILs with longer chains, so that the ILs with octyl and decyl groups have frankly high ecotoxicities compared to those of same species recognized as highly toxic, like hydroquinone (Zazo et al., 2007), benzoquinone (Zazo et al., 2007) or chlorophenols (Calvo et al., 2004). Even the less ecotoxic IL (the butyl ones) yielded EC_{50} values significantly lower (higher ecotoxicity) than those reported for conventional solvents like methanol (EC_{50}, μM: 10^7), acetone (EC_{50}, μM: $3.1{\cdot}10^5$) and acetonitrile (EC_{50}, μM: $6.3{\cdot}10^5$) (Kaiser and Palabrica, 1991).

In the respirometric tests with activated sludge, the EC_{50} values were obtained by two fitting methods: logistic and linear (Polo et al., 2011). The former requires inhibition extension up to almost 100% to be reached in order to obtain good fitting parameters. This fact limits its applicability since some ILs have water solubility below the concentration required to provoke high inhibition. However, as can be seen in Table 3, in most of the studied cases, the EC_{50} values obtained from both fitting methods were similar. Previous studies about the inhibition of ILs on activated sludge respiration have revealed differences in the EC_{50} values depending on the source of inoculum. This fact seems to be

Table 2
Cations and anions used in this study.

Anion	Alkyl chain length
Cl^-	$Bmim^+$
BF_4^-	$Hmim^+$
NTf_2^-	$Omim^+$
	$Dmim^+$

Table 3
EC_{50} values (mean ± standard deviation; n = 2) for imidazolium ILs from Microtox and inhibition respiration tests (r^2: determination coefficient).

ILs	Microtox Test				Respiration Inhibition Test			
	This study		Other studies		Logistic fit		Linear fit	
	EC_{50} (μM)	r^2	EC_{50} (μM)	References	EC_{50} (μM)	r^2	EC_{50} (μM)	r^2
BmimCl	3162 ± 2	0.994	891–5128	Docherty and Kulpa (2005); Garcia et al. (2005); Couling et al. (2006); Stolte et al. (2007); Romero et al. (2008); Peric et al. (2013); Montalbán et al. (2016)	2089 ± 1259	0.757	2398 ± 174	0.992
HmimCl	182 ± 1	0.998	87.1–813	Ranke et al. (2004); Garcia et al. (2005); Luis et al. (2007); Romero et al. (2008); Montalbán et al. (2016)	339 ± 229	0.963	331 ± 51	0.993
OmimCl	4.79 ± 1.45	0.979	4.26–15.5	Garcia et al. (2005); Luis et al. (2007); Stolte et al. (2007); Romero et al. (2008); Montalbán et al. (2016)	23.4 ± 8.3	0.932	23.4 ± 7.1	0.989
DmimCl	0.69 ± 1.02	0.999	0.59–3.16	Stolte et al. (2007)	3.47 ± 1.10	0.992	3.31 ± 1.10	0.996
$BmimBF_4$	3236 ± 3	0.983	1259–3548	Ranke et al. (2004); Garcia et al. (2005); Samorì et al. (2007); Samorì et al. (2010); Montalbán et al. (2016)	15849 ± 200	0.838	14125 ± 178	0.998
$HmimBF_4$	331 ± 2.2	0.985	1513	Ranke et al. (2004)	1202 ± 71	0.925	1174 ± 68	0.949
$OmimBF_4$	10.0 ± 1.2	0.994	8.13–25.7	Ranke et al. (2004); Matzke et al. (2007); Montalbán et al. (2016)	45.7 ± 6.6	0.907	49.1 ± 5.1	0.939
$DmimBF_4$	1.41 ± 1.01	0.994	0.66	Ranke et al. (2004)	6.31 ± 1.07	0.992	6.31 ± 1.07	0.991
$BmimNTf_2$	871 ± 2	0.987	295–4677	Garcia et al. (2005); Couling et al. (2006); Matzke et al. (2007); Montalbán et al. (2016)	347 ± 44	0.917	346 ± 37	0.996
$HmimNTf_2$	81.3 ± 1.2	0.997	66.1	Montalbán et al. (2016)	91.2 ± 37.2	0.983	91.2 ± 12.9	0.990
$OmimNTf_2$	15.8 ± 1.7	0.986	9.77	Montalbán et al. (2016)	ND	ND	11.7 ± 1.4	0.971
$DmimNTf_2$	2.95 ± 1.12	0.978			1.95 ± 1.05	0.991	1.95 ± 1.05	0.992

ND: not determined.

related to the adaptability of a taxonomically complex community, as activated sludge, to degrade xenobiotic compounds (Gutiérrez et al., 2002). That was the case for imidazolium ILs with four sewage sludge sources (EC_{50} values (μM): HmimCl: 708–15136; OmimCl: 141–1820; DmimCl: 12.6–105) (Markiewicz et al., 2013).

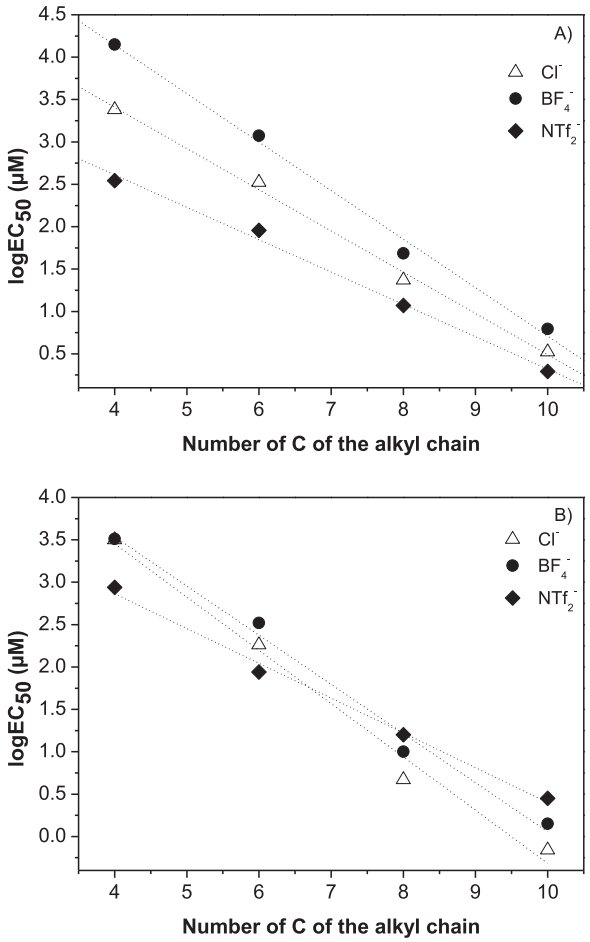

Fig. 1. Relationship between $logEC_{50}$ and the length of alkyl chain for A) inhibition respiration (linear fit) assay and B) Microtox test.

Fig. 1 depicts the EC_{50} values (expressed as log) for both the inhibition bioassay with activated sludge and the Microtox test using *Vibrio fischeri*. It can be observed a similar general trend towards ecotoxicity increase with the length of the linear alkyl chain, depending on the IL anion. However, the EC_{50} values in Table 3 reveal that the respiration test provides a significant wider range of EC50 values depending on IL structure than Microtox test; in addition, it is generally observed higher toxicity effects measured by Microtox Test. In fact, the EC_{50} values from *Vibrio fischeri* can be used to evaluate the sludge inhibition potential of ILs, at least for the less toxic ones.

The wide range of toxicity values for the ILs tested (Table 3) is related to the diversity of their cationic and anionic groups. Fig. 1 also illustrates the cation and anion effects on the $logEC_{50}$ values of the ILs studied in this work. A clear linear relationship between $logEC_{50}$ and the alkyl chain length can be observed. Table 4 summarizes the linear regression equations and the corresponding values of the determination coefficient. Goodness of fit yielded somewhat better for the respiration inhibition assays than for the Microtox test, although it was fairly good in all cases. Both tests show an ecotoxicity increase with the length of the linear chain, which has been related to the loss of polarity or the accentuation of their lipophilic character at increasing chain length (Ranke et al., 2007; Costello et al., 2009; Cvjetko Bubalo et al., 2013; Diaz et al., 2016;). Consequently, EC_{50} ecotoxicity values of Table 3 can be related to the octanol-water partition coefficient (K_{OW} in Table 1) that increased with the length of the imidazolium cation alkyl chain and the hydrophobicity of the anion (Deng et al., 2011; Montalbán et al., 2015, 2018). This has been associated with a higher capacity to accumulate the ILs in the biological membranes that are essentially non-polar interfaces (Garcia et al., 2005). Stolte et al. (2007) observed a

Table 4
Fitting equations of $logEC_{50}$ (μM) vs the length of alkyl chain (number of carbons, C).

Anions	Respiration inhibition assay*		Microtox test	
	Linear fit	r^2	Linear fit	r^2
Cl^-	$logEC_{50}$ = 5.35–0.49·C	0.998	$logEC_{50}$ = 5.97–0.63·C	0.981
BF_4^-	$logEC_{50}$ = 6.43–0.57·C	0.997	$logEC_{50}$ = 5.86–0.58·C	0.983
NTf_2^-	$logEC_{50}$ = 4.14–0.38·C	0.997	$logEC_{50}$ = 4.51–0.41·C	0.991

* EC_{50} values obtained from linear fit in Table 3.

Table 5
Ecotoxicity of the tested ILs according to Passino and Smith classification (Passino and Smith, 1987).

Ionic liquid	Harmless EC_{50} > 1000 mg L^{-1}		Practically harmless EC_{50}: 100–1000 mg L^{-1}		Moderately toxic EC_{50}: 10–100 mg L^{-1}		Highly toxic EC_{50} < 10 mg L^{-1}	
	Activated sludge	*Vibrio fischeri*	Activated sludge	*Vibrio fischeri*	Activated sludge	*Vibrio fischeri*	Activated sludge	*Vibrio fischeri*
$BmimNTf_2$			✓	✓				
$BmimBF_4$	✓			✓				
BmimCl			✓	✓				
$HmimNTf_2$					✓	✓		
$HmimBF_4$			✓			✓		
HmimCl					✓	✓		
$OmimNTf_2$							✓	✓
$OmimBF_4$					✓			✓
OmimCl							✓	✓
$DmimNTf_2$							✓	✓
$DmimBF_4$							✓	✓
DmimCl							✓	✓

strong interaction of hydrophobic IL cations, using a variety of microorganisms, with two different types of common biological lipidic bilayers.

Regarding the anion, it has been commonly considered of minor effect (and nearly constant) on the toxicity in the literature so far (Ranke et al., 2004; Couling et al., 2006; Luis et al., 2007; Romero et al., 2008; Pretti et al., 2009). However, the results of respirometric tests show a clear effect of the nature of the IL anion on the inhibition of activated sludge (Fig. 1A), particularly in the case of ILs with imidazolium cation and short alkyl chain. The values of the intercept of the aforementioned linear relationship (Table 4) reveal some significant differences between the IL families with different anions, increasing the IL ecotoxicity in the order NTf_2^- > Cl^- > BF_4^-. The higher toxicity values obtained for the ILs with NTf_2^- has been also related to its K_{OW} value, more than one order of magnitude higher than ILs containing other anions such as BF_4^- or Cl^- (Ropel et al., 2005; Kamath et al., 2012; Montalbán et al., 2015). The different slope and intercept values of Table 4 imply mixed cation-anion inhibitory effects on the activated sludge performance rather than an additive effect of the intrinsic toxicities of the cations and anions. The anion toxic effects are clearly stronger in the ILs containing shorter cation alkyl chain, whereas increasing the chain length mitigates the toxic effect of the anion (as example, the EC_{50} ratios for $BmimBF_4$ / $BmimNTf_2$ and $DmimBF_4$ / $DmimNTf_2$ are, respectively, 41 and 3, see Table 3). These different anion effects were already observed in previous works but in a lesser extent (Torrecilla et al., 2010).

Summarizing, from respiration inhibition tests, the ILs with short side chain (≤ 6 C) are less likely to interact with membranes and a stronger influence of the anion has been reported (Mester et al., 2015; Montalbán et al., 2016). In contrast, the anion effects observed from the Microtox test seem to be less significant (Fig. 1B) and less quantitatively influenced by the cation (see EC_{50} values in Table 3). The ILs with NTf_2^- anion yielded the highest toxicity in the respiration inhibition tests, consistently with the high hydrophobicity due to its low-polar character (Stolte et al., 2006). On the other hand, looking at the EC_{50} values from the Microtox test, the NTf_2^- anion behaves as stronger toxicophore than Cl^- or BF_4^- in the ILs with $Bmim^+$ cation, while it shows the opposite behavior with long side chain, as $Dmim^+$ cation. Similar changes in IL toxicity trends with the anion nature have been reported before and assigned to the non-additive mixing effects of toxicophores constituting the IL structures (Torrecilla et al., 2010).

Looking at the hazard ranking for aquatic organisms (Passino and Smith, 1987), the ILs tested can be classified from harmless to highly toxic according to their EC_{50} values expressed in mg/L. As can be seen in Table 5, several differences have been observed in the classification of some ILs depending on the test used to evaluate the ecotoxicity. *Vibrio fischeri* shows a higher sensitivity to ILs than activated sludge, but regardless the test used, $Bmim^+$ IL can be classified as "practically harmless" compounds whereas $Omin^+$ and $Dmim^+$ IL are "highly toxic" ones.

4. Conclusions

The Microtox test, using the microorganism *Vibrio fischeri,* and respiration inhibition assays with activated sludge collected from a wastewater treatment plant have been used to analyze the toxicity and the inhibition effect of different ILs. Both tests have showed similar ecotoxicity trends, although EC_{50} values tend to be lower in the case of Microtox Test.

A well-fitting linear correlation between the toxicity ($logEC_{50}$) and the length of the alkyl chain substituent (C4-C10) of imidazolium ILs has been found in most of the cases. The toxicity increases significantly with the chain length, which can be related to the loss of IL polarity or their lipophilic character. The NTf_2^- anion was more toxic than BF_4^- or Cl, but its relative impact on toxicity is reduced for ILs with long alkyl side chain (> 6 C atoms). Attending to the obtained results, imidazolium ILs with C4 alkyl chain can be classified as "practically harmless" compounds whereas those with alkyl chains C8 or C10 correspond to "highly toxic" species.

Taking into account the high water solubility of these ILs, their high ecotoxicity inhibition potential and poor biodegradability of most of them, they would be recalcitrant in conventional activated sludge wastewater treatment. Moreover, some of the ILs can cause severe undesirable effects on the microbial consortium, which can destabilize the biological system. Thus, it is necessary to develop cost-effective solutions to avoid or mitigate their negative impact in aquatic environments.

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References

Calvo, L., Mohedano, A.F., Casas, J.A., Gilarranz, M.A., Rodriguez, J.J., 2004. Treatment of chlorophenols-bearing wastewaters through hydrodechlorination using Pd/activated carbon catalysts. Carbon 42, 1377–1381.
Chica, A.F., Martin, A., Vazquez, F.J., Carmona, F.J., Mohedo, J.J., 2007. Respirometer to analyze measure dissolved oxygen and oxygen demand of microbes in leachate from municipal waste. ES 2283171.
Cho, C.W., Jeon, Y.C., Pham, T.P.T., Vijayaraghavan, K., Yun, Y.S., 2008. The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum*

capricornutum. Ecotoxicol. Environ. Saf. 71, 166–171.
Costa, S.P.F., Pinto, P.C.A.G., Saraiva, M.L.M.F.S., Rocha, F.R.P., Santos, J.R.P., Monteiro, R.T.R., 2015. The aquatic impact of ionic liquids on freshwater organisms. Chemosphere 139, 288–294.
Costello, D.M., Brown, L.M., Lamberti, G.A., 2009. Acute toxic effects of ionic liquids on zebra mussel (*Dreissena polymorpha*) survival and feeding. Green Chem. 11, 548–553.
Couling, D.J., Bernot, R.J., Docherty, K.M., Dixon, J.K., Maginn, E.J., 2006. Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure-property relationship modeling. Green Chem. 8, 82–90.
Cvjetko Bubalo, M., Radošević, K., Redovniković, I., Halambek, J., Gaurina Srček, V., 2013. A brief overview of the potential environmental hazards of ionic liquids. Ecotoxicol. Environ. Saf. 99, 1–12.
Deng, Y., Besse-Hoggan, P., Sancelme, M., Delort, A.-M., Husson, P., Costa Gomes, M.F., 2011. Influence of oxygen functionalities on the environmental impact of imidazolium based ionic liquids. J. Hazard. Mater. 198, 165–174.
Diaz, E., Monsalvo, V., Palomar, J., Mohedano, A.F., 2016. In: Atwood, David A. (Ed.), Ionic Liquids: Bacterial Degradation in Wastewater Treatment Plants, in Encyclopedia of Inorganic and Bioinorganic Chemistry. John Wiley & Sons, Ltd, Chichester, UK. http://dx.doi.org/10.1002/9781119951438.eibc2393.
Docherty, K.M., Kulpa, J.C.F., 2005. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. Green Chem. 7, 185–189.
Docherty, K.M., Aiello, S.W., Buehler, B.K., Jones, S.E., Szymczyna, B.R., Walker, K.A., 2015. Ionic liquid biodegradability depends on specific wastewater microbial consortia. Chemosphere 136, 160–166.
Domanska, U., Rekawek, A., Marciniak, A., 2008. Solubility of 1-Alkyl-3-ethylimidazolium-Based Ionic liquids in water and 1-Octanol. J. Chem. Eng. Data 53, 1126–1132.
Garcia, M.T., Gathergood, N., Scammells, P.J., 2005. Biodegradable ionic liquids Part II. Effect of the anion and toxicology. Green Chem. 7, 9–14.
Gathergood, N., Scammells, P.J., Garcia, M.T., 2006. Biodegradable ionic liquids Part III. The first readily biodegradable ionic liquids. Green Chem. 8, 156–160.
Gordon, C.M., 2001. New developments in catalysis using ionic liquids. Appl. Catal. A: Gen. 222, 101–117.
Gutiérrez, M., Etxebarria, J., De las Fuentes, L., 2002. Evaluation of wastewater toxicity: comparative study between Microtox and activated sludge oxygen uptake inhibition. Water Res. 36, 919–924.
Hernández-Fernández, F.J., Bayo, J., Pérez de los Ríos, A., Vicente, M.A., Bernal, F.J., Quesada-Medina, J., 2015. Discovering less toxic ionic liquids by using the Microtox toxicity test. Ecotoxicol. Environ. Saf. 116, 29–33.
ISO 11348-3, 1998. water quality – determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3: method using freeze-dried bacteria, 1998.
Jastorff, B., Molter, K., Behrend, P., Bottin-Weber, U., Filser, J., Heimers, A., Ondruschka, B., Ranke, J., Schaefer, M., Schroder, H., Stark, A., Stepnowski, P., Stock, F., Stormann, R., Stolte, S., Welz-Biermann, U., Ziegert, S., Thoming, J., 2005. Progress in evaluation of risk potential of ionic liquids—basis for an eco-design of sustainable products. Green Chem. 7, 362–372.
Johnson, B.T., 2005. In: Blaise, C., Férard, J.F. (Eds.), Small-Scale Freshwater Toxicity Investigations-Microtoxs Acute Toxicity Test,1. Springer, Netherlands, pp. 69–105.
Jordan, A., Gathergood, N., 2015. Biodegradation of ionic liquids – a critical review. Chem. Soc. Rev. 44, 8200–8237.
Kaiser, K.L.E., Palabrica, V.S., 1991. *Photobacterium phosphoreum* toxicity data index. Water Pollut. Res. J. Can. 26, 361–431.
Kamath, G., Bhatnagar, N., Baker, G.A., Baker, S.N., Potoff, J.J., 2012. Computational prediction of ionic liquid 1-octanol/water partition coefficients. Phys. Chem. Chem. Phys. 14, 4339–4342.
Luis, P., Ortiz, I., Aldaco, R., Irabien, A., 2007. A novel group contribution method in the development of a QSAR for predicting the toxicity (*Vibrio fischeri* EC_{50}) of ionic liquids. Ecotoxicol. Environ. Saf. 67, 423–429.
Markiewicz, M., Piszora, M., Caicedo, N., Jungnickel, C., Stolte, S., 2013. Toxicity of ionic liquid cations and anions towards activated sewage sludge organisms from different sources – Consequences for biodegradation testing and wastewater treatment plant operation. Water Res. 47, 2921–2928.
Matzke, M., Stolte, S., Thiele, K., Juffernholz, T., Arning, J., Ranke, J., Welz-Biermann, U., Jastorff, B., 2007. The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco) toxicological test battery. Green Chem. 9, 1198–1207.
Mester, P., Wagner, M., Rossmanith, P., 2015. Antimicrobial effects of short chained imidazolium-based ionic liquids—Influence of anion chaotropicity. Ecotoxicol. Environ. Saf. 111, 96–101.
Montalbán, M.G., Collado-González, M., Trigo, R., Díaz Baños, F.G., Víllora, G., 2015. Experimental measurements of octanol-water partition coefficients of ionic liquids. J. Adv. Chem. Eng. 5, 133.
Montalbán, M.G., Hidalgo, J.M., Collado-González, M., Díaz Baños, F.G., Víllora, G., 2016. Assesing chemical toxicity of ionic liquids on *Vibrio fischeri*: correlation with structure and composition. Chemosphere 155, 405–414.
Montalbán, Víllora, G., Licence, P., 2018. Ecotoxicity assessment of dicationic versus monocationic ionic liquids as a more environmentally friendly alternative. Ecotoxicol. Environ. Saf. 150, 129–135.
Neumann, J., Pawlik, M., Bryniok, D., Thöming, J., Stolte, S., 2014. Biodegradation potential of cyano-based ionic liquid anions in a culture of *Cupriavidus spp*. and their in vitro enzymatic hydrolysis by nitrile hydratase. Environ. Sci. Pollut. Res. 21, 9495–9505.
Palomar, J., Lemus, J., Gilarranz, M.A., Rodriguez, J.J., 2009. Adsorption of ionic liquids from aqueous effluents by activated carbon. Carbon 47, 1846–1856.
Passino, D.R.M., Smith, S.B., 1987. Acute bioassays and hazard evaluation of representative contaminants detected in Great lakes fish. Environ. Chem. 6, 901–907.
Peric, B., Sierra, J., Martí, E., Cruanas, R., Garau, M.A., Arning, J., Bottin-Weber, U., Stolte, S., 2013. Ecotoxicity and biodegradability of selected protic and aprotic ionic liquids. J. Hazard. Mater. 261, 99–105.
Petkovic, M., Seddon, K.R., Rebelo, L.P.N., Pereira, C.S., 2011. Ionic liquids: a pathway to environmental acceptability. Chem. Soc. Rev. 40, 1383–1403.
Plechkova, N.V., Seddon, K.R., 2008. Applications of ionic liquids in the chemical industry. Chem. Soc. Rev. 37, 123–150.
Pham, T.P.T., Cho, C.W., Yun, Y.S., 2010. Environmental fate and toxicity of ionic liquids: a review. Water Res. 44, 352–372.
Polo, A.M., Tobajas, M., Sanchis, S., Mohedano, A.F., Rodriguez, J.J., 2011. Comparison of experimental methods for determination of toxicity and biodegradability of xenobiotic compounds. Biodegradation 22, 751–761.
Pretti, C., Chiappe, C., Baldetti, I., Brunini, S., Monni, G., Intorre, L., 2009. Acute toxicity of ionic liquids for three freshwater organisms: *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. Ecotoxicol. Environ. Saf. 72, 1170–1176.
Quijano, G., Couvert, A., Amrane, A., Darracq, G., Couriol, C., Le Cloirec, P., Paquin, L., Carrié, D., 2011. Toxicity and biodegradability of ionic liquids: new perspectives towards whole-cell biotechnological applications. Chem. Eng. J. 174, 27–32.
Ranke, J., Mölter, K., Stock, F., Bottin-Weber, U., Poczobutt, J., Hoffmann, J., Ondruschka, B., Filser, J., Jastorff, B., 2004. Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays. Ecotoxicol. Environ. Saf. 58, 396–404.
Ranke, J., Muller, A., Bottin-Weber, U., Stock, F., Stolte, S., Arning, J., Stormann, R., Jastorff, B., 2007. Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. Ecotoxicol. Environ. Saf. 67, 430–438.
Rodriguez Castillo, A.S., Guihéneuf, S., Le Guéveld, R., Biard, P.-F., Paquin, L., Amrane, A., Couvert, A., 2016. Synthesis and toxicity evaluation of hydrophobic ionic liquids for volatile organic compounds biodegradation in a two-phase partitioning bioreactor. J. Hazard. Mater. 307, 221–230.
Romero, A., Santos, A., Tojo, J., Rodríguez, A., 2008. Toxicity and biodegradability of imidazolium ionic liquids. J. Hazard. Mater. 151, 268–273.
Ropel, L., Belvèze, L.S., Aki, S.N.V.K., Stadtherr, M.A., Brennecke, J.F., 2005. Octanol–water partition coefficients of imidazolium-based ionic liquids. Green Chem. 7, 83–90.
Samorì, C., Pasteris, A., Galletti, P., Tagliavini, E., 2007. Acute toxicity of oxygenated and nonoxygenated imidazolium-based ionic liquids to *Daphnia magna* and *Vibrio fischeri*. Environ. Toxicol. Chem. 26, 2379–2382.
Samorì, 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.
Stepnowski, P., Storoniak, P., 2005. Lipophilicity and metabolic route prediction of imidazolium ionic liquids. Environ. Sci. Pollut. Res. 12, 199–204.
Stolte, S., Arning, J., Bottin-Weber, U., Matze, M., Stock, F., Thiele, K., Uerdingen, M., Welz-Biermann, U., Jastorff, B., Ranke, J., 2006. Anion effects on the cytoxicity of ionic liquids. Green Chem. 8, 621–629.
Stolte, S., Matzke, M., Arning, J., Boschen, A., Pitner, W.R., Welz-Biermann, U., Jastorff, B., Ranke, J., 2007. Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. Green Chem. 9, 1170–1179.
Stolte, S., Steudte, S., Areitioaurtena, O., Pagano, F., Thöming, J., Stepnowski, P., Igartua, A., 2012. Ionic liquids as lubricants or lubrication additives: an ecotoxicity and biodegradability assessment. Chemosphere 89, 1135–1141.
Stolte, S., Schulz, T., Cho, C.W., Arning, J., Strassner, T., 2013. Synthesis, toxicity, and biodegradation of tuneable aryl alkyl ionic liquids (TAAILs). Sustain. Chem. Eng. 1, 410–418 (ACS).
Torrecilla, J.S., Palomar, J., Lemus, J., Rodriguez, F., 2010. A quantum-chemical-based guide to analyze/quantify the cytotoxicity of ionic liquids. Green Chem. 12, 123–134.
Tsarpali, V., Dailianis, S., 2015. Toxicity of two imidazolium ionic liquids, [bmim][BF_4] and [omim][BF_4], to standard aquatic test organisms: role of acetone in the induced toxicity. Ecotoxicol. Environ. Saf. 117, 62–71.
van Rantwijk, F., Madeira Lau, R., Sheldon, R.A., 2003. Biocatalytic transformations in ionic liquids. Trends Biotechnol. 21, 131–138.
Ventura, S.P.M., Silva, F.A., Gonçalves, A.M.M., Pereira, J.L., Gonçalves, F., Coutinho, J.A.P., 2014. Ecotoxicity analysis of cholinium-based ionic liquids to *Vibrio fischeri* marine bacteria. Ecotoxicol. Environ. Saf. 102, 48–54.
Viboud, S., Papaiconomou, N., Cortesi, A., Chatel, G., Draye, M., Fontvieille, D., 2012. Correlating the structure and composition of ionic liquids with their toxicity on *Vibrio fischeri*: a systematic study. J. Hazard. Mater. 215– 216, 40–48.
Zazo, J.A., Casas, J.A., Molina, C.B., Quintanilla, A., Rodriguez, J.J., 2007. Evolution of ecotoxicity upon Fenton's oxidation of phenol in water. Environ. Sci. Technol. 41, 7164–7170.