Research Article

Ecotoxicity evaluation towards *Vibrio fischeri* of imidazolium- and pyridinium-based ionic liquids for their use in separation processes

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Abstract

The non-volatility character of the ionic liquids makes them environmentally attractive to new separation processes development, but their water solubility emphasizes the importance of the study of their impact on the aquatic environment. The present work reports the toxicity of 24 imidazolium- and pyridinium-based ionic liquids through *Vibrio fischeri* inhibition basic test. The toxicity-structure relationship of the ionic liquids has been studied through the anion and cation core, the cation alkyl chain length, and the presence of functional groups on the cation alkyl chain. Ionic liquids whose chemical structure includes pyridinium cations, long cation alkyl chains, and bis(trifluoromethylsulfonyl) imide ($[Tf_2N]$) anions present the highest toxicity. Finally, considering ionic liquids as potential solvents for the aromatic/aliphatic separation process, their toxicity has been compared to that of sulfolane, which is the current organic solvent used in this kind of process. Most of the studied ionic liquids can be classified as harmless or practically harmless; meanwhile, only four of them present a slightly or moderately toxicity, the same than that of sulfolane. The present study suggests that not only the atmospheric pollution would be decreased, but the aquatic impact as well by using ionic liquids as alternative solvents.

Graphic abstract Ionic liquids (ILs) stand as more environmentally friendly solvents in the petrochemical industry. Their toxicity towards *Vibrio fischeri* bacteria is lower than most of the "green" alternative deep eutectic solvents (DESs) and much lower than the current solvent, sulfolane.
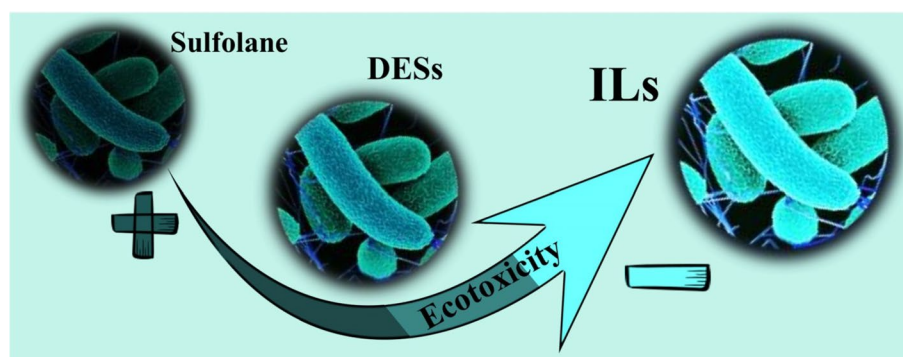
1 Introduction

The attraction and importance of the ionic liquids as a new generation of solvents have been increasing in the past two decades. These non-conventional salts with melting points below 100 °C [1] have been widely studied for their use in several petrochemical applications as they present important advantages, such as negligible vapor pressure, high chemical, thermal, and electrochemical stability, and, mainly, the possibility of tailoring their physicochemical properties for specific applications by merely selecting the cation and the anion [2]. Their non-volatility does not lead to air pollution, but the solubility of water in most of the ionic liquids [3], their combination with organic solvents [4] and their possible presence into the environment in case of an effluent disposal or an accidental discharge highlight the importance of the study of their impact on soils and on aquatic ecosystems [5].

Recently it has been reported that the structure of the ionic liquids plays an essential role in the toxic effects upon aquatic organisms and their microbial activity [6]. Some toxicity studies have demonstrated that the effect of the cation on the ecotoxicity is more significant than that of the anion [7]. It has also been demonstrated that the ecotoxicity of ionic liquids increases with longer cation alkyl chains [8]. In addition, among the most common cations of ionic liquids, pyridinium and imidazolium cations have been proved to be resistant to photodegradation [6] and to present low biodegradability [9, 10]. But contrary to these ecotoxicity data, somewhat unexpected results have suggested that longer alkyl chains (hexyl and octyl) bound to pyridinium cations are easier to mineralize than shorter alkyl chains (butyl) bound to imidazolium cations (considered as non-biodegradable ionic liquids) [9].

Different tests have been used to determine the toxicity of the ionic liquids, such as those based on algae (*Selenastrum capricornutum* or *Pseudokirchneriella subcapitata*, ISO 8692) [11, 12], plants (*Lemna minor*, ISO/CD 20079) [13, 14], invertebrates (*Daphnia magna*, ISO 6341) [11, 15, 16], mammalian cells (*Rat leukemia cells*, IPC-81) [17, 18], and bacteria (mainly *Vibrio fischeri*, ISO 11348) [19–22]. Toxicity testing with the bacterium *Vibrio fischeri* is the standard ecotoxicological method in Europe because it is a rapid, cost effective, sensitive and reproducible assay [21].

The aim of the present work is to determine the aquatic toxicity of a set of 24 imidazolium- and pyridinium-based ionic liquids using the *Vibrio fischeri* inhibition test. The effect of the cation and anion of the ionic liquids and their combinations on the ecotoxicity is

also discussed. To that end, various anions and cations with different cation alkyl chain lengths and functional groups have been tested, along with sulfolane as a benchmark and acetone as one of the most common solvents in the industry. The selection of the studied ionic liquids was based on their good performance as potential solvents in the extraction process of aromatic hydrocarbons. Thus, tricyanomethanide- ([TCM]) and bis(trifluoromethylsulfonyl) imide- ([Tf$_2$N]) based ionic liquids were tested due to their high aromatic distribution ratio and thiocyanate- ([SCN]) and dicyanamide- ([DCA]) based ionic liquids due to their high aromatic/aliphatic selectivity. Toxicity values are given through the EC50 parameter (mg·L^{-1}) and they have been correlated with the structure of the ionic liquids. Beside this, the trend of the toxicity has been compared with the lipophilic character of the ionic liquids through the octanol-water partition coefficients, K_{ow}.

The ecotoxicity of the studied ionic liquids is also compared with those of the deep eutectic solvents (DESs), a new generation of environmentally sustainable solvents. The importance of the DESs has been increasing lately because of their easiness of synthesizing and handling, the non-toxic and biodegradable starting materials and their similar physicochemical properties to those of the ionic liquids. It has been reported that EC50 values for cholinium-based DESs vary from 21 to 661 mg·L^{-1} (cholinium dihydrogenocitrate and benzyldimethyl(2-hydroxyethyl) ammonium chloride, respectively). This moderate toxicity is mainly dominated by the toxicity of the hydrogen bond donor [23, 24]. The studied ionic liquids present a similar or even much lower toxicity to these "green" solvents. Besides this, some previous results have demonstrated that the extractive properties of ionic liquids in separation processes overcome those of the DESs [25]. Therefore, both toxicity and separation properties make ionic liquids more attractive than DESs as alternative solvents.

2 Experimental section

2.1 Chemicals

The used ionic liquids, namely 1-ethyl-3-methylimidazolium dicyanamide ([C$_2$C$_1$im][DCA], > 98 wt%), 1-allyl-3-methylimidazolium dicyanamide ([AC$_1$im][DCA], > 98 wt%), 1-butyl-3-methylimidazolium dicyanamide ([C$_4$C$_1$im][DCA], > 98 wt%), 1-benzyl-3-methylimidazolium dicyanamide ([BzC$_1$im][DCA], > 98 wt%), 1-ethyl-3-methylimidazolium thiocyanate ([C$_2$C$_1$im][SCN], > 98 wt%), 1-butyl-3-methylimidazolium thiocyanate ([C$_4$C$_1$im][SCN], > 98 wt%), 1,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide ([C$_1$C$_1$im][Tf$_2$N], > 99

wt%), 1-ethylimidazolium bis(trifluoromethylsulfonyl) imide ([C_2im][Tf_2N], > 98 wt%), 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_2C_1im][Tf_2N], > 99 wt%), 1-methyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide ([C_3C_1im][Tf_2N], > 99 wt%), 1-allyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([AC_1im][Tf_2N], > 99 wt%), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ([C_4C_1im][Tf_2N], > 99 wt%), 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_6C_1im][Tf_2N], > 99 wt%), 1-ethyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide ([C_2-2,3C_1mim][Tf_2N], > 99 wt%), 1-propyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl) imide ([C_3-2,3C_1im][Tf_2N], > 99 wt%), 1-butyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide ([C_4-2,3C_1im][Tf_2N], > 99 wt%), 1-ethyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide ([C_2C_1py] [Tf_2N], > 99 wt%), 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide ([C_4C_1py][Tf_2N], > 99 wt%), N-butylpyridinium bis(trifluoromethylsulfonyl) imide ([C_4py][Tf_2N], > 99.5 wt%), 1-ethyl-3-methylimidazolium tricyanomethanide ([C_2C_1im][TCM], > 98 wt%), 1-butyl-3-methylimidazolium tricyanomethanide ([C_4C_1im][TCM], > 98 wt%), 1-butyl-4-methylpyridinium tricyanomethanide ([C_4C_1py][TCM], > 98 wt%), bis(1-ethyl-3-methylimidazolium)tetrathiocyanatocobaltate ([C_2C_1im]$_2$[Co(SCN)$_4$], > 99 wt%), and bis(1-butyl-3-methylimidazolium)tetrathiocyanatocobaltate ([C_4C_1im]$_2$[Co(SCN)$_4$], > 99 wt%), are shown in Table 1, along with their chemical structures. All the ionic liquids were supplied by Iolitec GmbH. Sulfolane (> 99 wt%) was purchased from Sigma-Aldrich, and acetone (> 99.5 wt%) from Fluka Analytical. All chemicals were used as received without any further purification. To avoid the hydration of ionic liquids, they were stored in a desiccator and handled in a glove box under dry nitrogen.

2.2 Microtox® test

The Microtox® toxicity test measures the inhibition in the bacterium *Vibrio fischeri* using the decrease in its bioluminescence, following the standard Microtox® test method based on ISO 11348-3 [26]. The Microtox® M500 analyzer, bioluminescent bacteria and reagents were supplied by Modern Water. In this test, a range of four consecutive diluted aqueous solutions of ionic liquid from 0 to 100 wt% was used. The concentration of 100 wt% corresponds to a known concentration of ionic liquid in water from 300 mg·L^{-1} for [C_4C_1im]$_2$[Co(SCN)$_4$] (the most toxic ionic liquid) to 37000 mg·L^{-1} for [C_2C_1im][SCN] (the least toxic ionic liquid).

The initial aqueous solution is composed by the diluted ionic liquid solution at 100 wt% concentration and 250 µL of the osmotic adjusting solution. The following aqueous solutions are serial dilutions consisting on 1 mL of pure water and 1 mL of the previous IL solution. Each solution is placed in one Microtox® cell, with a total of four per EC50 measurement, and the fifth cell is the reference cell with the standard sample of the bacterial suspension without ionic liquid. The standard and the four samples were used in 2% NaCl to adjust the osmotic pressure. Previous to the essays, the lyophilized bacteria are placed in a Microtox® cell at 278.5 K with a reconstitution and osmotic adjusting solutions. Each essay has been measured at 288.2 K and atmospheric pressure. After 5 and 15 min exposing the bacteria to the ionic liquid, the light output was measured and compared to the light output of a blank control sample. Toxicity results are obtained as a 50% reduction in luminescence, EC50 (mg·L^{-1}). Three replicate measurements were made for each ionic liquid.

3 Results and discussion

The study of the ecotoxicity is focused on the effect of the structure of the ionic liquids towards the *Vibrio fischeri* inhibition, such as anion core (thiocyanate [SCN], dicyanamide [DCA], tricyanomethanide [TCM], tetrathiocyanatocobaltate [Co(SCN)$_4$], and bis(trifuoromethylsulfonyl)imide [Tf_2N]), cation core (imidazolium and pyridinium), cation alkyl chain length ([C_1C_1im] to [C_6C_1im]), double bond in the cation alkyl chain (allyl group), and functional group in the cation alkyl chain (benzyl group). Table 2 shows the inhibition results for the studied ionic liquids after 5 and 15 min of exposure to the bacterium *Vibrio fischeri*, along with those for the organic solvents and from the literature. Ecotoxicity values (EC50) of the ionic liquids vary from 2 mg·L^{-1} for [C_4C_1im]$_2$[Co(SCN)$_4$] to 6310 mg·L^{-1} for [C_2C_1im][SCN] after 15 min of exposure. The experimental data are in good agreement with those from the literature.

As expected, the results show that the EC50 values decrease from 5 to 15 min. The necessary dose to cause the same inhibition effect on the bacterium *Vibrio fischeri* is lower at longer exposure time for most of the studied ionic liquids, and it is due to the time necessary for the toxic mechanism to occur [20]. However, this difference in the toxicity values is not observed for some of the ionic liquids or the organic chemicals because of their fast toxic action, that is those with high toxicity as sulfolane or those with low toxicity as acetone or [C_2im][Tf_2N].

Table 2 also shows the octanol-water partition coefficients estimated from the Eq. 1, proposed by Montalbán et al. [36]:

Table 1 Chemical structure of the ionic liquids

Abbreviation	Cation structure	Anion structure
[C$_2$C$_1$im][DCA]		
[AC$_1$im][DCA]		
[C$_4$C$_1$im][DCA]		
[BzC$_1$im][DCA]		
[C$_2$C$_1$im][SCN]		
[C$_4$C$_1$im][SCN]		
[C$_1$C$_1$im][Tf$_2$N]		
[C$_2$im][Tf$_2$N]		
[C$_2$C$_1$im][Tf$_2$N]		
[C$_3$C$_1$im][Tf$_2$N]		
[AC$_1$im][Tf$_2$N]		
[C$_4$C$_1$im][Tf$_2$N]		
[C$_6$C$_1$im][Tf$_2$N]		
[C$_2$-2,3C$_1$im][Tf$_2$N]		
[C$_3$-2,3C$_1$im][Tf$_2$N]		
[C$_4$-2,3C$_1$im][Tf$_2$N]		
[C$_2$C$_1$py][Tf$_2$N]		
[C$_4$C$_1$py][Tf$_2$N]		
[C$_4$py][Tf$_2$N]		
[C$_2$C$_1$im][TCM]		
[C$_4$C$_1$im][TCM]		
[C$_4$C$_1$py][TCM]		
[C$_2$C$_1$im]$_2$[Co(SCN)$_4$]		
[C$_4$C$_1$im]$_2$[Co(SCN)$_4$]		

Table 2 EC50 (mg·L^{-1}) results for the studied ionic liquids after 5 and 15 min of exposure to the bacterium *Vibrio fischeri* (95 percent confidence interval in brackets), along with literature data, and estimated values of K_{ow} using Eq. 1

Ionic liquid	The present work			Literature		
	EC50, 5 min (mg·L^{-1}) (95% c.i.)	EC50, 15 min (mg·L^{-1}) (95% c.i.)	K_{ow}	EC50, 5 min (mg·L^{-1})	EC50, 15 min (mg·L^{-1})	References
[C$_1$C$_1$im][Tf$_2$N]	2455 (1380–4266)	1549 (832–2884)	0.015	3631	2344	[27]
[C$_2$im][Tf$_2$N]	1288 (447–3467)	1300 (398–4169)	0.025	–	–	–
[C$_2$C$_1$im][Tf$_2$N]	1202 (832–1738)	575 (339–977)	0.027	437–1318	145–1622	[12, 19, 21, 27]
[C$_3$C$_1$im][Tf$_2$N]	577 (457–724)	407 (363–457)	0.049	479	291–282	[28, 29]
[AC$_1$im][Tf$_2$N]	479 (389–589)	355 (275–457)	0.058	–	–	–
[C$_4$C$_1$im][Tf$_2$N]	240 (200–282)	166 (141–200)	0.107	141–302	126–251	[13, 19, 21, 27, 29, 30]
[C$_6$C$_1$im][Tf$_2$N]	37 (36–39)	28 (22–35)	0.707	23	24–50	[21, 22, 27, 29]
[C$_2$-2,3C$_1$im][Tf$_2$N]	759 (427–1445)	501 (324–851)	0.039	–	–	–
[C$_3$-2,3C$_1$im][Tf$_2$N]	282 (263–339)	195 (182–229)	0.093	–	–	–
[C$_4$-2,3C$_1$im][Tf$_2$N]	141 (132–155)	100 (87–115)	0.177	132	87–324	[21, 27]
[C$_2$C$_1$py][Tf$_2$N]	288 (234–347)	214 (151–309)	0.091	–	–	–
[C$_4$C$_1$py][Tf$_2$N]	65 (62–69)	47 (41–52)	0.385	–	–	–
[C$_4$py][Tf$_2$N]	162 (141–186)	115 (98–138)	0.155	–	–	–
[AC$_1$im][DCA]	6918 (5754–8511)	3890 (3236–4786)	0.007	–	–	–
[C$_2$C$_1$im][DCA]	9333 (6457–13,183)	5495 (4467–6761)	0.006	–	–	–
[C$_4$C$_1$im][DCA]	1023 (977–1047)	562 (513–617)	0.031	977	933–989	[29–31]
[BzC$_1$im][DCA]	275 (229–324)	166 (117–234)	0.095	–	–	–
[C$_2$C$_1$im][SCN]	11,482 (8128–16,596)	6310 (5129–7943)	0.005	–	2399	[29]
[C$_4$C$_1$im][SCN]	724 (589–891)	513 (282–933)	0.041	–	–	–
[C$_2$C$_1$im][TCM]	4677 (1995–10,965)	3467 (2455–5012)	0.009	–	–	–
[C$_4$C$_1$im][TCM]	794 (457–1380)	513 (282–933)	0.038	–	–	–
[C$_4$C$_1$py][TCM]	200 (191–214)	115 (110–117)	0.127	–	–	–
[C$_2$C$_1$im]$_2$[Co(SCN)$_4$]	3467 (1820–6607)	851 (479–1549)	0.012	–	–	–
[C$_4$C$_1$im]$_2$[Co(SCN)$_4$]	5 (2–14)	2 (1–3)	9.891	–	–	–
Acetone	13,804 (5888–32,359)	14,791 (5890–42,658)	0.004	19,953	7943–19,498	[13, 31–34]
Sulfolane	50 (32–81)	56 (42–91)	0.508	–	30–59	[35]

$$\log \left(\frac{1}{EC50} \right) = -1.436 + 0.859 \cdot \log \left(K_{ow} \right) - 0.119 \cdot \left[\log \left(K_{ow} \right) \right]^2 \tag{1}$$

in which the *Vibrio fischeri* toxicity at 15 min of exposure [expressed as log(1/EC50)] is correlated with the hydrophobicity of the solvent (expressed as log(K_{ow}). This comparison is useful as the binary system water saturated-octanol closely represents the physico-chemical environment of the organisms; the higher the value of the K_{ow} parameter, the higher the hydrophobicity and the lipophilic character of the solvent and also the interaction with the bacteria. Most of the values of the octanol-water

partition coefficients for the ionic liquids are much lower than those for the sulfolane, except for two highly hydrophobic ionic liquids: [C$_6$C$_1$][Tf$_2$N] and [C$_4$C$_1$im]$_2$[Co(SCN)$_4$].

3.1 Anion effect on ionic liquids ecotoxicity

Figure 1 depicts the EC50 values of the Microtox test for [C$_2$C$_1$im]- and [C$_4$C$_1$im]-based ionic liquids. Focusing on the toxicity related to the anion structure, a comparison between ionic liquids with the same cation in their structure leads to an ecotoxicity trend with the following order: [Tf$_2$N]>[TCM]>[DCA]>[SCN]. The biggest and most
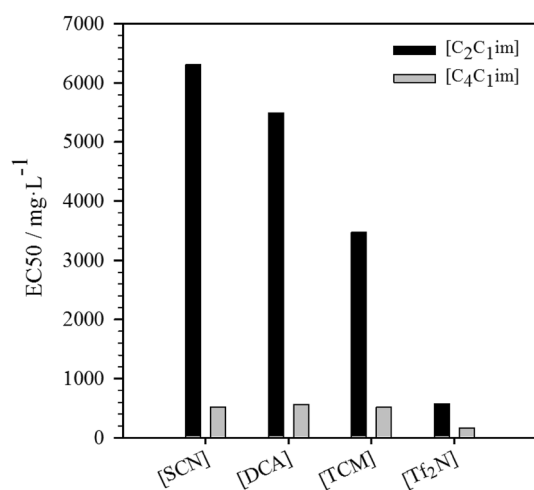
Fig. 1 Effect of the anion on the *Vibrio fischeri* inhibition, as EC50 (mg·L^{-1})

Fig. 2 Effect of the cation on the *Vibrio fischeri* inhibition, as EC50 (mg·L^{-1})

hydrophobic anion, [Tf$_2$N], presents the highest toxicity on the bacterium *Vibrio fischeri* while the smallest and more hydrophobic anion, [SCN], has the lowest toxicity among all the studied ILs, which is in good agreement with previous studies [37, 38].

It is interesting to highlight that the effect of the anion on the toxicity decreases with longer cation alkyl chains. Cations play a more important role than anions in the ionic liquid toxicity and the effect of cations overcomes the effect of anions for long alkyl chains; for the [C$_2$C$_1$im] cation the toxicity follows the order [TCM]>[DCA]>[SCN], meanwhile for the [C$_4$C$_1$im] cation, the toxicity for [TCM], [DCA], and [SCN] anions shows a similar value.

Couling et al. [39] reported that the toxicity of ionic liquids decreases with the increase of localized charges on cations or anions and increases with the presence of positively charged atoms on anions. The localization of the positive charge on the cyano-based ionic liquids depends on the number of nitrogen atoms or cyano-groups in the structure; the [SCN] anion has the highest electron density and one cyano-group while the [TCM] anion the lowest electron density as the negative charge of the [TCM] anion is shared with three –CN groups [40]. Therefore, the more delocalized charge on [TCM] anions and the more numerous regions of positive charge increase its toxicity, compared to [DCA] and [SCN] anions. Regarding the [Tf$_2$N] anion, its highest toxicity is due to the fluorine atoms in its structure which highly distribute and delocalize the anionic charge of the imide and to the highest positive charge because of the two bistriflimide groups. These results are also in agreement with the previous study of Costa et al.; perfluorated ions can be hydrolyzed to hydrofluoric acid, so the double bistriflimide group in the [Tf$_2$N] anion highly
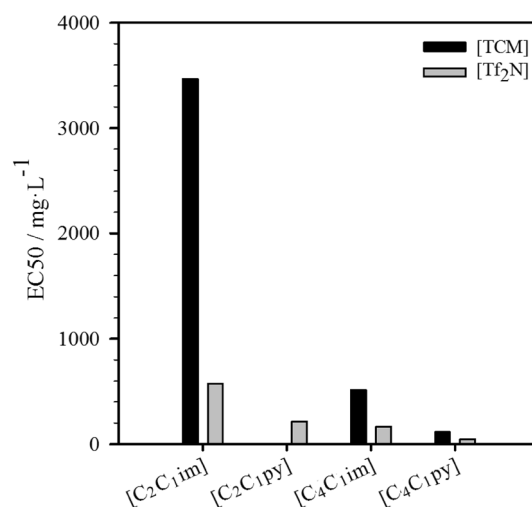
contributes to the IL toxicity and is potentially dangerous for the environment [12].

According to the values of the K_{ow} parameter in Table 2, the order of the hydrophobic and lipophilic character of the anions follows the same order than its ecotoxicity: [Tf$_2$N] > [TCM] > [DCA] > [SCN].

3.2 Cation effect on ionic liquids ecotoxicity

As seen in Fig. 2, pyridinium-based ionic liquids show a higher toxicity than imidazolium-based ionic liquids; [C$_2$C$_1$py] and [C$_4$C$_1$py] cations have lower EC50 values than [C$_2$C$_1$im] and [C$_4$C$_1$im] cations, respectively. These results are in good agreement with the experimental results from literature [12, 21, 27], but not with the model proposed by Couling et al. [38] in which imidazolium cations are predicted to be more toxic than pyridinium cations. Ventura et al. [27] determined that the aromatic structure of the imidazolium and pyridinium cations increased the toxicity, compared to those ionic liquids based on non-aromatic cations, such as piperidinium or pyrrolidinium cations. In the present work, the more remarkable aromatic nature of the pyridinium cations leads to higher toxicity than that of the imidazolium cations, as the former presents a high-density electron cloud similar to that of the aromatic hydrocarbons meanwhile the aromatic structure of the latter is mainly due to the lone pair of the nitrogen [40].

Figure 3 represents the effect of the cation alkyl chain length on the ecotoxicity. The results show that, for a common anion, longer alkyl chain length leads to higher toxicity of the ionic liquid. An increase in the alkyl chain length leads to higher toxicity, as studied previously by
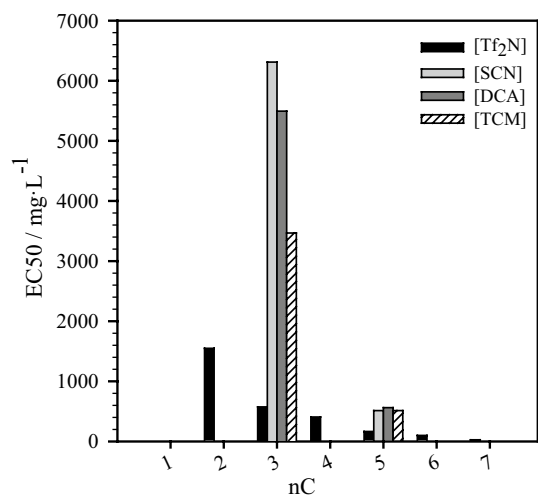
Fig. 3 Effect of the total number of carbon atoms (nC) bound to the imidazolium cation core on the *Vibrio fischeri* inhibition, as EC50 (mg·L^{-1})
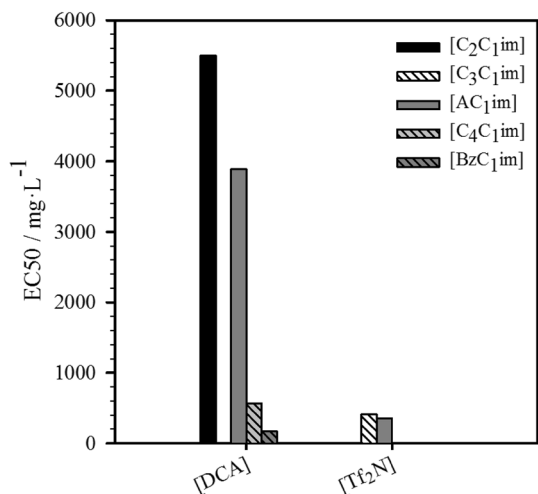

Fig. 4 Effect of the functional group bound to the cation on the *Vibrio fischeri* inhibition, as EC50 (mg·L^{-1})

different authors [10, 21, 31, 41, 42]. This trend could be due to the decrease in the polarity of the cation which leads to a higher hydrophobicity nature when the length of the alkyl chains is increased [20].

Various functional groups can be bound to the cation core, such as allyl and benzyl, modifying the toxicity of ionic liquids (Fig. 4). The double bond in the functional group scarcely affects the inhibition of the bacterium *Vibrio fischeri*; the value of EC50 for [C$_3$C$_1$im][Tf$_2$N] is 407 mg·L^{-1} meanwhile for [AC$_1$im][Tf$_2$N] is 355 mg·L^{-1}. The toxicity of the cation with a benzyl group in their structure, [BzC$_1$im][DCA], is lower than expected when it is compared to that of cations with alkyl chains, [C$_2$C$_1$im][DCA] and [C$_4$C$_1$im][DCA].

These results are also in agreement with the hydrophobicity of the ionic liquids shown in Table 2; and increase in the hydrophobicity leads to a higher lipophilicity and cell permeability. Therefore, the toxicity of the ionic liquids is increased.

3.3 Toxicity scale

To describe the toxicity of the ionic liquids, we have followed the scale proposed by Passino and Smith for EC50 in mg·L^{-1} [43]. So, all the studied ionic liquids and chemicals are classified in Table 3. Most of the [DCA]- and [SCN]-based ionic liquids present harmless toxicity, similar to acetone's one, which is a widely used solvent in the industry. Most of the [Tf$_2$N]-based ionic liquids, longer cation alkyl chain [SCN]-, [DCA]-, and [TCM]-based ionic liquids, and [C$_2$C$_1$im]$_2$[Co(SCN)$_4$] ionic liquid have toxicity considered as practically harmless. Only four ionic liquids show a slightly or moderately toxicity, the [Tf$_2$N]-based ionic liquids with the longest cation alkyl chains and [C$_4$C$_1$im]$_2$[Co(SCN)$_4$]. The latter has the lower EC50 value and presents moderate toxicity, mainly due to its structure with two cations and the butyl group. It is essential to highlight that all the studied ionic liquids have similar or even lower toxicity than that of sulfolane, except for [C$_4$C$_1$im]$_2$[Co(SCN)$_4$].

The data reported would be of practical use as a guideline for manufacturers to properly develop and regulate the use of ionic liquids as alternative solvents. Bioaccumulation and biodegradability of these ionic liquids should be also studied as they are important indicators to evaluate the environmental impact of the ionic liquid in the aquatic media. But the EC50 values obtained in the present study suggest that the use of ionic liquids would not only lead to a drastic decrease in atmospheric emissions but a lower aquatic impact.

4 Conclusions

The ecotoxicity of 24 imidazolium- and pyridinium-based ionic liquids towards the bacterium *Vibrio fischeri* has been measured by means of the ecotoxicity values, EC50 (mg·L^{-1}). The results report that pyridinium-based ionic liquids and longer alkyl chains bound to the cation lead to higher toxicity, as well as [Tf$_2$N] anion, compared to [SCN], [DCA], and [TCM] anions. It has been seen that the cation plays a main role in the ecotoxicity, and the anion also has an influence on it in ionic liquids with shorter cation alkyl chains. When the alkyl chain has a functional group, such as double bond or benzyl group, the toxicity is similar to that of a non-functional alkyl chain. The similar or lower toxicity of ionic liquids with respect to that of DESs makes

Table 3 Toxicity scale (EC50, mg·L^{-1}) of the studied ionic liquids according to Passino and Smith classification [43]

Harmless > 1000 mg·L^{-1}	Practically harmless 100–1000 mg·L^{-1}	Slightly toxic 10–100 mg·L^{-1}	Moderately toxic 1–10 mg·L^{-1}
[C$_2$C$_1$im][DCA]	[C$_4$C$_1$im][DCA]	[C$_6$C$_1$im][Tf$_2$N]	[C$_4$C$_1$im]$_2$[Co(SCN)$_4$]
[AC$_1$im][DCA]	[BzC$_1$im][DCA]	[C$_4$-2,3C$_1$im][Tf$_2$N]	
[C$_2$C$_1$im][SCN]	[C$_4$C$_1$im][SCN]	[C$_4$C$_1$py][Tf$_2$N]	
[C$_1$C$_1$im][Tf$_2$N]	[C$_2$C$_1$im][Tf$_2$N]	Sulfolane	
[C$_2$im][Tf$_2$N]	[C$_3$C$_1$im][Tf$_2$N]		
[C$_2$C$_1$im][TCM]	[AC$_1$im][Tf$_2$N]		
Acetone	[C$_4$C$_1$im][Tf$_2$N]		
	[C$_2$-2,3C$_1$im][Tf$_2$N]		
	[C$_3$-2,3C$_1$im][Tf$_2$N]		
	[C$_2$C$_1$py][Tf$_2$N]		
	[C$_4$py][Tf$_2$N]		
	[C$_4$C$_1$im][TCM]		
	[C$_4$C$_1$py][TCM]		
	[C$_2$C$_1$im]$_2$[Co(SCN)$_4$]		

the ionic liquids more attractive to be used as new solvents. Finally, the present study suggests that the ionic liquids would drastically reduce the aquatic impact if they were used as an alternative to the current organic solvent, sulfolane, in the aromatic/aliphatic separation process. Further work is still needed, however, in different living organisms and environments as well as in other factors related to the ecotoxicity, such as biodegradation and bioaccumulation, in the search for ionic liquids with optimal properties in the development of alternative solvents for environmental and sustainable technologies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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