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ScienceDirect
Journal of Hazardous Materials 151 (2008) 268–273
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Short communication

# Toxicity and biodegradability of imidazolium ionic liquids

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Received 26 July 2007; received in revised form 15 October 2007; accepted 18 October 2007
Available online 30 October 2007

**Abstract**

Several bioassays have been carried out to analyze the toxicity and biodegradability of several imidazolium ionic liquids (ILs) in aqueous phase. The synthetized compounds consist of an imidazolium cation with two alkyl substituents in positions 3 ($R_1$) and 1 ($R_2$) and a counter-ion. The alkyl substituent $R_1$ has been fixed as a methyl group and the effect of the alkyl chain length ($C_1$–$C_8$) of the other substituent ($R_2$) has been tested. Moreover, the influence of diverse counter-ions $A^-$ ($Cl^-$, $PF_6$, $XSO_4^-$) has been analyzed. Acute toxicity and $EC_{50}$ values of each compound in the aqueous solution have been determined by using the Microtox® standard procedure. Biodegradability of IL has been determined by measuring $BOD_5$ of aqueous samples containing IL and/or D-glucose and the IL residual content and/or D-glucose concentration after this assay. The viability of the microorganisms used in the $BOD_5$ has been related to the ATP in the samples, measured by a bioluminescence assay. All the ILs tested were not biodegradable in the considered conditions. Besides, it was found that the shorter the chain length of side chain $R_2$, the lower the toxic effect is. On the contrary, the anion has a little effect on the IL toxicity.


*Keywords:* Ionic liquid; Biodegradability; Microtox; Imidazolium

## 1. Introduction

Ionic Liquids, ILs, are low-melting-point salts that have become increasingly attractive as green solvents for industrial applications [1–10]. This green adjective is mainly attributed to their negligible vapor pressure, which avoids the loss of solvent to the atmosphere and decreases the worker exposure risk. Thus, room-temperature ionic liquids could provide environmentally friendly solvents for the chemical and pharmaceutical industries [1,5,6,11]. Typical ILs consist of an organic cation with delocalized charges and a small inorganic anion, most often halogen anions weakly coordinating such as Cl, $BF_4$ or $PF_6$ [12]. An ionic liquid can be thought of as "designer" solvent [13] so it should be possible to design, or tailor, a solvent for a certain reaction. Therefore, many cation and anion combinations are possible, changing properties as polarity, hydrophobicity and solvent miscibility behavior. Among these possibilities, the 1-alkyl-3-methylimidazolium is one of the most used because it is non-volatile, non-flammable, presents high thermal stability and is an excellent solvent for a wide range of inorganic and organic materials [6,7,9].

Although, the low vapor pressure of ILs may reduce the air pollution with respect to the typical volatile organic solvents, this is not enough to justify calling a "green" process. It must be considered that a release of ILs from industrial processes into aquatic environments may lead to water pollution, because of their high solubilities in water. Moreover, because of the high stability of ionic liquids in water these compounds could become as persistent pollutants in wastewaters. For this reason it is prioritary to determine the further consequences and the environmental risk of the presence of ILs in wastewaters.

Most employed methods to evaluate the environmental risk of a substance in an aqueous media are those measuring their toxicity by using an inhibition assay. Different microorganism or enzymes have been used in this inhibition measurements, the acute toxicity test which uses the *V. fischeri* (formerly *Photobacterium phosphoreum*) bioluminiscence inhibition assay being one of the most applied [14,15]. This is a standard ecotoxicological bioassay in Europe (DIN EN ISO 11348). It is very rapid, cost-effective, and it is a widely accepted method for toxicity determination used extensively in the literature focusing on environmental issues. The experimental procedure Microtox

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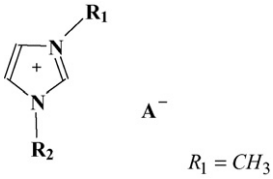
doi:10.1016/j.jhazmat.2007.10.079

Table 1
Ionic liquids synthesized and tested




| Acronym | $R_2$ | $A^-$ |
|---|---|---|
| [$C_1$mim][$CH_3SO_4$] | $CH_3$ | $CH_3SO_4^-$ |
| $C_2$mim][$C_2$ $H_5SO_4$] | $CH_2$–$CH_3$ | $CH_3$–$CH_2SO_4^-$ |
| [$C_4$mim][Cl] | $(CH_2)_3$–$CH_3$ | $Cl^-$ |
| [$C_6$mim][Cl] | $(CH_2)_5$–$CH_3$ | $Cl^-$ |
| [$C_8$mim][Cl] | $(CH_2)_7$–$CH_3$ | $Cl^-$ |
| [$C_6$mim][$PF_6$] | $(CH_2)_5$–$CH_3$ | $PF_6^-$ |
| [$C_8$mim][$PF_6$] | $(CH_2)_7$–$CH_3$ | $PF_6^-$ |

has been adopted from the official standards of several countries including USA (ASTM method D5660-1995), Germany (DIN 38412-1990), France (AFNOR T90-320-1991) and Spain (ISO 11348-3-1998).

By using the Microtox assay it has been observed an increasing toxicity trend when the alkyl chain length substituent in imidazolium ionic liquids [16–18] increases. On the other hand, minimal effects on toxicity are observed varying the anion of the methyl imidazolium salts. Consequently, toxicity of the ILs was mainly attributed to the alkyl chain.

Similar results were found using various bacteria for the inhibition test [19–22], with IPC-81 and $C_6$ glioma cells [17], or using higher organisms, including *Caenorhabditis elegans* and *Daphnia magna* [23,24]. Few studies have been carried out analyzing the biodegradability of ILs in the aqueous media. García et al. [16] using the closed bottle test, have measured the 28 days biochemical oxygen demand (BOD) in series of butylmethylimidazolium (bmimX, X = Br, $BF_4$, $PF_6$, $NTf_2$, $N(CN)_2$ and octyl$OSO_3$) and methyl-(propoxycarbonyl)-imidazolium ionic liquids. They found that ILs generally proved to be poorly biodegradable. The corresponding 3-methyl-1-(propoxymethylcarbonyl)-imidazolium series showed higher levels of biodegradability but none of the compounds that were tested could be classified as "readily biodegradable". Therefore, the ILs could become persistent pollutants and break through classical treatment systems into natural waters.

In this work, the toxicity and biodegradability of several imidazolium ILs in aqueous phase have been determined. Solutions of these compounds, summarized in Table 1, have been prepared in redistillated water and they were synthesized in our laboratory. A methyl group is at position $R_1$ and different alkyl chain substituents (chain length from $C_1$–$C_8$) are at position $R_2$. Diverse counter-ions $A^-$ ($Cl^-$, $PF_6$, $XSO_4^-$) have been tested. Acute toxicity and $EC_{50}$ values of each compound in the aqueous solution have been determined by using the Microtox® standard procedure. To analyze the biodegradability of IL, the biochemical oxygen demand for 5 days, $BOD_5$ of several aqueous samples containing known initial amounts of IL and/or D-glucose have been determined. Furthermore, the residual IL content and/or D-glucose concentration in these aqueous samples have been measured after 5 and 10 days. Finally, the viability of the microorganisms used in the $BOD_5$ has been related to the APT in the samples, measured by a bioluminescence assay.

## 2. Experimental

ILs were prepared according to slightly modified literature procedures [25–29] in our laboratory. The progress of the reaction was monitored by thin layer chromatography using aluminium sheets silica gel 60 GF-254, $CH_2Cl_2$–10% MeOH as eluent. H NMR analysis was made at 400 MHz and in $D_2O$. The maximum water contents of the liquids were determined using a 756 Karl Fisher coulometer.

### 2.1. Luminiscent bacteria acute toxicity test

The toxicity of the aqueous samples with the different ILs was determined by means of a bioassay following the standard Microtox test procedure (ISO 11348-3, 1998) based on the decrease of light emission by *P. phosphoreum* resulting from its exposure to a toxicant. The more toxic the sample is, the greater is the percent light loss from the test suspension of luminescent bacteria. Bacterial bioluminescence has proved to be a convenient measure of cellular metabolism and consequently, a reliable sensor for measuring the presence of toxic chemicals in aquatic samples. Strain 11177 was originally chosen for the acute and chronic tests because it displays a high sensitivity to a broad range of chemicals.

A Microtox® M500 Analyzer (Azur Environmental) was used. The inhibition of the light emitted by the bacteria was measured after 15 min contact time. The $IC_{50}$ is defined as the ratio of the initial volume of sample ($V_S$) to the one yielding, after the required dilution, a 50% reduction of the light emitted by the microorganisms ($V_F$). Therefore the $IC_{50}$ is related to the dilution of the sample required to achieve a 50% of light emission reduction. From $IC_{50}$, the toxicity units of the wastewater are calculated as:

$$TU_{50} = \frac{100}{IC_{50}} \quad (1)$$

The $EC_{50}$ is defined as the effective nominal concentration of the toxic chemical (in mg/L) that reduces the intensity of light emission by 50%. Therefore, the $IC_{50}$ corresponding to a sample containing only one toxicant $i$, in concentration $C_i$, will correspond to:

$$IC_{50} = \frac{100C_i}{EC_{50}} \quad (2)$$

By using Eq. (2), the $EC_{50}$ of each ionic liquid is obtained from the $IC_{50}$ value of the aqueous sample containing the ionic liquid in a known amount $C_i$. A pH readjustment in order to prevent the pH effect before measuring the toxicity was not necessary because the pH of the samples ranged between 6.5 and 7. All the chemicals used in the toxicity test were purchased from

Sigma–Aldrich and the microorganisms were Microtox® Acute Reagent supplied by I.O. Analytical.

### 2.2. Biochemical oxygen demand

The biochemical oxygen demand of the aqueous sample containing the ionic liquid and/or other carbon source (glucose) was evaluated using a respirometric BOD measurement system (OxiDirect® by Lovibond). Biochemical oxygen demand is usually defined as the amount of oxygen (mg/L) required by bacteria while stabilizing decomposable organic matter under aerobic conditions. The term "decomposable" may be interpreted as meaning that the organic matter can serve as food for the bacteria, and energy is derived from its oxidation.

Solutions containing 100 mg/L of ionic liquid and/or 100 mg/L of glucose were prepared, in aerated media, pH of the liquid samples being in the range of 6.5–7.

A volume of 244 mL of each solution was then inoculated with 1 mL of an effluent collected from a biological reactor of a wastewater treatment plant and each well-mixed solution was dispensed into a series of BOD bottles (500 mL of volume) and incubated at $20 \pm 1\ ^{\circ}C$ in the dark for 5 days, obtaining the $BOD_5$ value.

### 2.3. Glucose and ionic liquid measurement

D-Glucose was measured by UV-method using an enzymatic kit (Cat. No. 10 716 251 035 BOEHRINGER MANNHEIM / R-BIOPHARM). D-Glucose is phosphorylated to D-glucose-6-phosphate (G-6-P) in the presence of the enzyme hexokinase (HK) and adenosine-5′-triphosphate (ATP) with the simultaneous formation of adenosine-5′-diphosphate (ADP). In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidized by nicotinamide adenine dinucleotide phosphate (NADP) to D-gluconate-6-phosphate with the formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The amount of NADPH formed in this reaction is stoichiometric to the amount of D-glucose. The increase in NADPH is measured by means of its light absorbance at 334 nm. The test combination contains a bottle A, with approx. 7.2 g powder mixture, consisting of triethanolamine buffer, pH approx. 7.6; NADP, approx. 110 mg; ATP, approx. 260 mg; magnesium sulfate; a bottle B, with approx. 1.1 mL suspension, consisting of hexokinase, approx. 320 U; glucose-6-phosphate dehydrogenase, approx. 160 U. Bottle A content is dissolved with 45 mL redistillated water. Into the cuvettes, 1 mL of solution A with 2 mL of redistillated water and 0.020 mL of solution B is pipetted for the blank. For measurement of glucose in samples, 0.9 mL of solution A + 0.100 mL of sample is pipetted in the cuvette with 2 mL of redistillated water and 0.020 mL of solution B. Calibration of the absorbance measurements was made by using glucose standard solution from 10 to 100 mg/L.

Ionic liquid concentration in the aqueous solution was measured by UV at 210 nm. All the ILs tested showed a maximum at this wavelength. Calibration for each IL was made by preparing IL standard aqueous solution from 10 to 100 mg/L.

### 2.4. ATP determination

ATP in living cells was measured by a bioluminiscence method using the ATP Biomass Kit (Biothema). All cells contain ATP, which plays the role of energy currency between different cellular processes. When cells die of natural causes, ATP is normally degraded. The intracellular concentration of ATP is carefully regulated to similar levels in all types of cells. ATP is therefore a good estimate of the total intracellular volume. Before the assay, ATP is released from the cell using the extractant B/S included in the commercial Kit cited. ATP is assayed using ATP reagent HS (highly sensitive) having a detection limit of $10^{-17}$ mol corresponding to five bacterial cells. The following reaction takes place:

$$\mathrm{ATP} + \text{D-luciferin} + \mathrm{O_2} \xrightarrow{\text{luciferase}} \mathrm{AMP} + \mathrm{PPi} + \text{oxyluciferin} + \mathrm{CO_2} + \text{light} \quad (3)$$

The intensity of the light is proportional to the amount of ATP and it is measured in a luminometer (Optocomp I, MGM Instruments).

## 3. Results and discussion

### 3.1. Toxicity assessment

For all substances tested concentration–response curves were obtained and $EC_{50}$ values (μmol/L) were calculated. A typical plot of inhibition of the luminescence vs. the IL concentration (as dilution ratio $V_S/V_F \times 100$) is shown in Fig. 1.

The obtained $EC_{50}$ values at 15 min are given in Table 2, expressed as the corresponding logarithm, the confidence interval of the lognormal regression also being included. Literature $EC_{50}$ values for imidazolium ionic liquids are also given in Table 2 as well as the $EC_{50}$ values for typical volatile organic compounds. Notice that the $EC_{50}$ values obtained in this work are similar to the values found in literature for butyl, hexyl and octyl $R_2$ substituents. The methyl ($C_1$mim$MSO_4$) and ethyl ($C_2$mim$ESO_4$) $R_2$ substituent were not previously analyzed in literature, the results obtained being consistent with the rest of the $EC_{50}$ values.

The data in Table 2 shows that the measured $EC_{50}$ values vary between 58,000 and 5 μM (corresponding logarithm 4.76 and 0.70, respectively) depending on their chemical structure. On the other hand, the influence of the anion on the $EC_{50}$ value is minimal taking into account the slight differences found for $C_j$mim ($j = 6, 8$) when counter ions $Cl^-$ or $PF_6^-$ are tested (log values of $EC_{50}$ being 2.18 and 2.11 for $C_6$mimCl and $C_6$mim$PF_6$, respectively; log values of $EC_{50}$ being 0.94 and 0.70 for $C_8$mimCl and $C_8$mim$PF_6$, respectively). The small effect of the anion on toxicity of imidazolium compounds has also been noticed by other authors [16–18] as can be deduced from the results in Table 2.

On the other hand, a longer chain length of the alkyl substituent $R_2$ results in a remarkable increase of the acute toxicity, obtaining a lower $EC_{50}$ value. The linear regression analysis of the log $EC_{50}$ vs. the alkyl chain length of $R_2$ has been carried

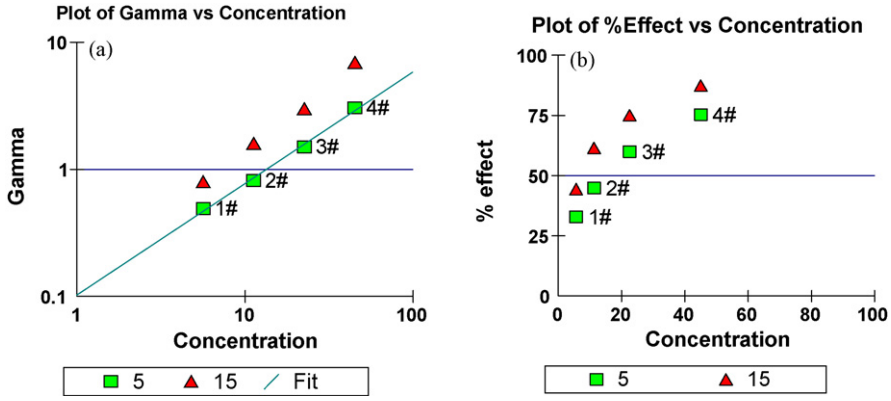



Fig. 1. Example of an inhibition plot of the luminescence vs. the concentration of the ionic liquid. The IL used was $ClC_6$ min at $C_i = 430$ mg/L. (a) Gamma vs. concentration, as $(V_S/V_F) \times 100$ and (b) % effect vs. concentration.

Table 2
$EC_{50}$ values and confidence intervals for the tested ionic liquids

| IL | log $EC_{50}$ (μM), this work | log $EC_{50}$ (μM) [18] | log $EC_{50}$ (μM) [16] | log $EC_{50}$ (μM) [17] | Common VOCs | log $EC_{50}$ (μM) [14] |
|---|---|---|---|---|---|---|
| $C_1$mimMSO$_4$ | >4.76 | | | | Methanol | 5.55–7.00 |
| $C_2$mimESO$_4$ | 4.02 ± 0.14 | | | | Ethanol | 5.70–6.08 |
| $C_4$mimCl | 3.39 ± 0.15 | 3.71 ± 0.13 | 3.34 ± 0.13 | | 2-Propanol | 5.77–5.84 |
| $C_4$mimPF$_6$ | | | 3.07 ± 0.29 | | Acetonitrile | 5.77 |
| $C_4$mimBr | | 4.01 ± 0.05 | 3.27 ± 0.09 | 3.07 ± 0.03 | Acetone | 5.35–5.70 |
| $C_4$mimBF$_4$ | | | 3.10 ± 0.17 | 3.55 ± 0.04 | Dicloromethane | 4.07–4.53 |
| $C_6$mimCl | 2.18 ± 0.09 | | 2.32 ± 0.16 | | Chloroform | 3.55–4.32 |
| $C_6$mimPF$_6$ | 2.11 ± 0.11 | | 2.17 ± 0.06 | | Benzene | 1.41–3.12 |
| $C_6$mimBr | | 1.42 ± 0.10 | | | Phenol | 2.35– |
| $C_6$mimBF$_4$ | | | | 3.18 ± 0.03 | Toluene | 2.29–2.64 |
| $C_8$mimCl | 0.94 ± 0.14 | | 1.19 ± 0.11 | | | |
| $C_8$mimPF$_6$ | 0.70 ± 0.16 | | 0.95 ± 0.12 | | | |
| $C_8$mimBr | | 0.63 ± 0.06 | | | | |
| $C_8$mimBF$_4$ | | | | 1.41 ± 0.07 | | |

out and the following equation has been obtained in this work:

$$\log EC_{50} = 5.33 - 0.549 nC_{R_2} \quad (4)$$

This relationship has a similar slope with the number of carbons of the alkyl substituent in $R_2$ $(nC_{R_2})$ as the one obtained by Ranke et al. [17] analyzing $nC_{R_2} \geq 4$.

$$\log_{10} EC_{50} = +6.65 - 0.66 nC_{R_1} - 0.57 nC_{R_2} \quad (5)$$

$nC_{R_1}$ being the number of carbons of the alkyl substituent in $R_1$.

In Fig. 2, the log $EC_{50}$ values are plotted vs. the alkyl chain length of $R_2$. The figure shows that a linear relationship is obtained for the interval $1 \leq nC_{R_2} \leq 8$. The validity of this relationship does not depend on the anion, and thus can be used to predict the toxicity of the $R_2$mim ILs.

### 3.2. Biodegradability

The 5 days BOD was measured for six samples containing 100 mg/L of D-glucose. An amount of 100 mg/L of IL was added in four of these D-glucose solutions. Tested ILs for this analysis have been $C_2$mim$CH_3CH_2SO_4$, $C_4$mimCl, $C_6$mimCl, $C_8$mimCl. They were selected in order to analyze the influence of the alkyl chain length in the $BOD_5$. The experiments carried out for $BOD_5$ measurements as well as the obtained $BOD_5$ values are shown in Table 3. All the samples yield similar values of $BOD_5$, the average value being about 100 mg $O_2$/L. At the experimental conditions used, the expected $BOD_5$ of a sample

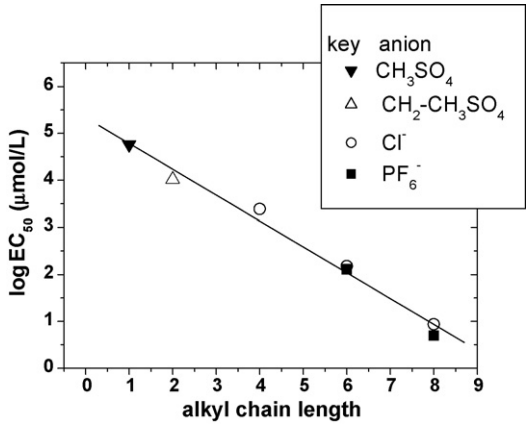



Fig. 2. Effect of the anion and alkyl chain length on the acute toxicity (Microtox®) for 1-alkyl-3-methylimidazolium ionic liquids.

Table 3
Biodegradability and microorganism inhibition for ionic liquid-D-glucose aqueous solutions (bioluminescence assay used for ATP measurements)

| Sample | $t = 0$ days | | | $t = 5$ days | | | | $t = 10$ days |
|---|---|---|---|---|---|---|---|---|
| | D-Glucose (mg/L) | IL type | IL (mg/L) | $BOD_5$ (mg $O_2$/L) | D-Glucose (mg/L) | IL (mg/L) | Light emission | IL (mg/L) |
| 1 | 100 | – | – | 98 | ≈0 | – | $2.8 \times 10^7$ | – |
| 2 | 100 | – | – | 106 | ≈0 | – | $2.7 \times 10^7$ | – |
| 3 | 100 | $C_2mimC_2H_5SO_4$ | 100 | 93 | ≈0 | 89 | $2.1 \times 10^7$ | 90 |
| 4 | 100 | $C_4mimCl$ | 100 | 101 | ≈0 | 98 | $2.2 \times 10^7$ | 97 |
| 5 | 100 | $C_6mimCl$ | 100 | 100 | ≈0 | 99 | $1.3 \times 10^7$ | 98 |
| 6 | 100 | $C_8mimCl$ | 100 | 102 | ≈0 | 95 | $1.2 \times 10^7$ | 96 |

containing 100 mg/L of D-glucose as carbon source was calculated as 106 mg $O_2$/L. Therefore, a higher value of $BOD_5$ than 100 mg $O_2$/L would be expected from samples containing IL as additional carbon source.

Final concentration of D-glucose and IL in the samples were analyzed, using an enzymatic kit or by UV-spectrometry, respectively. Values obtained are also given in Table 3. D-Glucose has been almost totally consumed in all the samples while the ionic liquid remains always in a concentration close to the initial one. Thus, all the ionic liquids tested were poorly biodegradable in presence of glucose. As the living organisms inoculated in this assay consumed only this monosacharide and not the ionic liquid, the almost constant $BOD_5$ (100 mg/L) obtained is consistent.

Since this preference for the glucose was unsurprising, it was analyzed if the microorganisms would consume the IL in absence of another source of carbon. For this, the aqueous samples after the $BOD_5$ measurements, free of glucose, containing only the microorganism and the ionic liquids, were stirred in aerobic atmosphere for 5 days more at 20 °C in the dark. Remaining concentration of the ionic liquid at this final time of 10 days in contact with the bacteria was determined and values obtained are summarized in Table 3. The amounts of ionic liquid in the solutions after 10 days were almost the same as the initial ones and those obtained after 5 days. In conclusion, the poor biodegradability of ILs in presence and absence of other carbon source is confirmed.

### 3.3. Influence on the biological activity

To examine if the presence of an ionic liquid produce cellular stress on the microorganism in the aqueous phase, a bioluminescence assay have been used to measure the ATP in the samples after $BOD_5$ experience. The light emitted (proportional to the ATP in the samples) is given in Table 3. Control samples are set like those containing only glucose in the $BOD_5$ measurements. In Table 3, the light emission values obtained in the samples that contained ILs were lower than those obtained in the control samples. Therefore, the aqueous samples with ionic liquid added yield a lower living cells concentration than the control samples. Because all the samples were inoculated with the same type and amount of microorganisms, it can be deduced that a lower microorganism growth takes place if the microorganism is inoculated in a medium containing an ionic liquid. Again, the shorter the chain length of side $R_2$, the lower the toxic effect.

The main limitation of the Microtox test is that the toxic effects noticed against the microorganism *V. fischeri* cannot be directly extrapolated to predict the toxicity effect against other organisms, as those living in the biological reactor of a wastewater treatment plant.

Nevertheless, it has been found that the IL compounds are not biodegradable and cause cellular stress, these results being in agreement with the high ecotoxicity values obtained for some ILs by using the Microtox test. We consider that the non-biodegradability related to the cellular stress of the microorganisms in the activated sludge is an important finding not previously described in literature.

Moreover, the same trend is noticed for the effect of the chain length of the alkyl substituent $R_2$ on both cellular stress and ecotoxicity, thus the utility of the Microtox test to elucidate the potential toxicity of a compound can be validated.

## 4. Conclusions

It has been shown that imidazolium based ionic liquids have a wide range of toxicities in short bioassay used (Microtox®). In general, their toxicity ($EC_{50}$ value) correlates directly with the length of the *n*-alkyl substituent in the methyl imidazolium cation while the anion has a low effect on this $EC_{50}$ value. These new solvents can be more toxic towards cells than conventional solvents and this must be taken into consideration with regard to their fate and persistence in the environment. In fact, the ILs tested were poorly biodegradable and the microorganisms do not consume them as carbon source. If the aqueous sample contains an ionic liquid it is certain that the microorganisms used in the $BOD_5$ determination suffer a cellular stress: a lower growth of the microorganism inoculated at the same operational conditions occurs when there is an ionic liquid in the media.

The relatively high solubilities of the ILs in the water phase, the low $EC_{50}$ values obtained for some of them and their poor biodegradability (that makes them persistent pollutants) have important environmental consequences and should be taken into account for the design of processes that use ILs. To avoid the potential contamination of the aqueous phase with ILs, several strategies should be planned. Firstly, it is important to improve the processes, minimizing the IL leaches to the aquatic media. Furthermore, downstream separation step must be required at the end of these processes to remove the ILs from wastewater streams.

## Acknowledgement

The authors acknowledge financial support for this research from the Spanish MCYT (contract no. CTM2006-00317).

## References

[1] J.D. Holbrey, K.R. Seddon, Room-temperature ionic liquids as replacements for organic solvents in multiphase bioprocess operations, Clean Prod. Process. 1 (1999) 223–236.
[2] T. Welton, Room-temperature ionic liquids. Solvents for synthesis and catalysis, Chem. Rev. 99 (1999) 2071–2084.
[3] M. Freemantle, Ionic liquids prove increasingly versatile, Chem. Eng. News 77 (1999) 23–24.
[4] M.J. Earle, P.B. McCormac, K.R. Seddon, Diels–Alder reactions in ionic liquids, Green Chem. 1 (1999) 23–25.
[5] D.W. Rooney, K.R. Seddon, in: G. Wypych (Ed.), Handbook of Solvents, ChemTech Publishing, Toronto, 2001, pp. 1459–1484.
[6] C.M. Gordon, New developments in catalysis using ionic liquids, Appl. Catal. A 222 (2001) 101–117.
[7] R. Sheldon, Catalytic reactions in ionic liquids, Chem. Commun. 23 (2001) 2399–2407.
[8] J. Dupont, R.F. de Souza, P.A. Suarez, Ionic liquid (molten salt) phase organometallic catalysis, Chem. Rev. 102 (2002) 3667–3692.
[9] F. Rantwijk, R.M. Lau, R.A. Sheldon, Biocatalytic transformations in ionic liquids, Trends Biotechnol. 21 (2003) 131–138.
[10] R.A. Sheldom, Green solvents for sustainable organic synthesis: state of the art, Green Chem. 7 (2005) 267–278.
[11] C.F. Poole, Chromatographic and spectroscopic methods for the determination of solvent properties of room temperature ionic liquids, J. Chromatogr. A 1037 (2004) 49–82.
[12] P.A.Z. Suarez, S. Einloft, J.E.L. Dullius, R.F. de Souza, J. Dupont, Synthesis and physical–chemical properties of ionic liquids based on 1-*n*-butyl-3-methylimidazolium cation, J. Chim. Phys. 95 (1998) 1626–1639.
[13] M. Freemantle, Designer solvents – ionic liquids may boost clean technology development, Chem. Eng. News 76 (March) (1998) 32–37.
[14] K.L.E. Kaiser, V.S. Palabrica, Photobactrium phosporeum, toxicity data index, Water Poll. Res. J. Can. 26 (1991) 361–431.
[15] S.M. Steinberg, E.J. Poziomek, W.H. Engelmann, K.R. Rogers, A review of environmental applications of bioluminescence measurements, Chemosphere 30 (1995) 2155–2197.
[16] T. García, N. Gathergood, N.J. Scammells, Biodegradable ionic liquids. Part II. Effect of the anion and toxicology, Green Chem. 7 (2005) 9–14.
[17] J. Ranke, K. Molter, F. Stock, U. Bottin-Weber, J. Poczobutt, J. Hoffman, B. Ondruschka, J. Filser, B. Jastorff, Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays, Ecotoxicol. Environ. Saf. 58 (2004) 396–404.
[18] K.M. Docherty, C.F. Kulpa, Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids, Green Chem. 7 (2005) 185–189.
[19] J. Pernak, J. Kalewska, H. Ksycinska, J. Cybulski, Synthesis and anti-microbial activities of some pyridinium salts with alkoxymethyl hydrophobic group, Eur. J. Med. Chem. 36 (2001) 899–907.
[20] J. Pernak, J. Rogoza, I. Mirska, Synthesis and antimicrobial activities of new pyridinium and benzimidazolium chlorides, Eur. J. Med. Chem. 36 (2001) 313–320.
[21] J. Pernak, P. Chwala, Synthesis and anti-microbial activities of choline-like quaternary ammonium chlorides, Eur. J. Med. Chem. 38 (2003) 1035–1042.
[22] J. Pernak, I. Goc, I. Mirska, Anti-microbial activities of protic ionic liquids with lactate anion, Green Chem. 6 (2004) 323–329.
[23] R.P. Swatloski, J.D. Holbrey, S.B. Memon, G.A. Caldwell, K.A. Caldwell, R.D. Rogers, Using Caenorhabditis elegans to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids, Chem. Commun. 6 (2004) 668–699.
[24] R.J. Bernot, M.A. Brueseke, M.A. Evans-White, G.A. Lamberti, Acute and chronic toxicity of imidazolium-based ionic liquids on *Daphnia magna*, Environ. Toxicol. Chem. 24 (2005) 87–92.
[25] J.D. Holbrey, W.M. Reichert, R.P. Swatloski, G.A. Broker, W.R. Pitner, K.R. Seddon, R. Rogers, Green Chem. 4 (2002) 407–413.
[26] A.B. Pereiro, E. Tojo, A. Rodríguez, J. Canosa, J. Tojo, Properties of ionic liquid HMIMPF6 with carbonates, ketones and alkyl acetates, J. Chem. Thermodyn. 38 (2006) 651–661.
[27] A.B. Pereiro, E. Tojo, A. Rodríguez, J. Canosa, J. Tojo, HMImPF(6) ionic liquid that separates the azeotropic mixture ethanol plus heptane, Green Chem. 8 (2006) 307–310.
[28] A.B. Pereiro, F. Santamarta, E. Tojo, A. Rodríguez, J. Tojo, Temperature dependence of physical properties of ionic liquid 1,3-dimethylimidazolium methyl sulphate, J. Chem. Eng. Data 51 (2006) 952–954.
[29] E. Gómez, B. González, A. Domínguez, E. Tojo, J. Tojo, Physical properties of pure 1-ethyl-3-methylimidazolium ethylsulfate and its binary mixtures with ethanol and water at several temperatures, J. Chem. Eng. Data 51 (2006) 2096–2102.