Evaluation of biotreatability of ionic liquids in aerobic and anaerobic conditions

Andreja Žgajnar Gotvajn, Elizabeta Tratar-Pirc, Peter Bukovec and Polona Žnidaršič Plazl

ABSTRACT

The aim of our study was to set up an approach for reliable biotreatability assessment of ionic liquids (ILs). As a case study, two different ILs were selected: pyridinium-based 1-butyl-3-methylpyridinium dicyanamide ([bmpyr][dca]) and imidazolium-based 1-butyl-3-methylimidazole tetrafluoroborate ([bmim][BF_4]). Toxicity in aerobic conditions was determined by measurement of inhibition of oxygen consumption by activated sludge, while their biodegradability was calculated from measurements of oxygen consumption and dissolved organic carbon elimination. For their biotreatability in anaerobic conditions, the method with measurement of biogas production has been employed comparing flocculent and granular sludge. Both ILs were less toxic and more biodegradable in anaerobic conditions. IL [bmpyr][dca] was not toxic to granular sludge up to 742 mg L^{-1} and it even has been degraded at this concentration in the presence of easily degradable glucose. Flocculent sludge was completely inhibited at the lower concentration of 318 mg L^{-1}, but it degraded by 44% at 106 mg L^{-1} in the presence of glucose, indicating the appearance of cometabolism. IL [bmim][BF_4] was less toxic but also resistant to biodegradation in anaerobic conditions. It degraded via cometabolism 21% at 1,452 mg L^{-1}. It has been concluded that any assessment of biotreatability of ILs should include parallel determination in aerobic and anaerobic conditions.

Key words | activated sludge, anaerobic processes, biodegradability, biotreatability, ionic liquid, toxicity

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INTRODUCTION

Ionic liquids (ILs) have attached great interest lately in wide variety of small scale to industrial applications due to their low volatility, non-flammability, adjustable miscibility and reusability (Romero *et al.* 2008). Increasing application possibilities and usage of ILs also result in higher amount of final waste containing ILs and their potential appearance in wastewaters ended in aerobic or anaerobic biological wastewater treatment plant (Neumann *et al.* 2010). In the past years numerous studies on biodegradability, bioaccumulation and distribution of ILs in different environmental compartments were accomplished to overcome drawbacks for their wider industrial application (Zhao *et al.* 2007; Pham *et al.* 2010).

The studies of environmental fate and toxicity of ILs show that they are generally toxic, but their toxicity is strongly dependent upon their composition and it vary across organisms and trophic levels (Pham *et al.* 2010; Zagorc-Končan *et al.* 2010). Initial studies confirmed poor biodegradability of ILs and lately the biodegradable side chains were applied, mainly ester groups derived from C_2 acid or C_4 or higher alcohol to increase biodegradability of imidazole-based ILs (Gatherhood *et al.* 2004). Introduction of polar functional groups to the alkyl chain has been shown as a promising approach for increasing biodegradability of ILs to some extent, but many authors proved, that increase length of alkyl chain increased the rate of biodegradability but unfortunately also toxicity (Pham *et al.* 2010). However, there is still a lack of information about their treatability in anaerobic conditions (Markiewicz *et al.* 2013). The anaerobic biodegradation plays important role in different environmental compartments, such as eutrophic lakes, soils or sediments, while anaerobic digestion technology for waste and wastewater treatment as well as soil remediation is growing worldwide because of its economic and environmental

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benefits (Angelidaki *et al*. 2009). The stability of the process is dependent upon fragile balance between the symbiotic growth of the main groups of microorganisms: (i) acid forming bacteria, (ii) hydrogen producing acetogens and (iii) methanogens. Therefore, appropriate environmental conditions and presence of toxicants play a key role in efficiency of the process. Granular anaerobic sludge is often favored, allowing higher loading rates in comparison to conventional systems with flocculent sludge. In a granule, the most sensitive methanogenic microorganisms could be find in its central part, protected from toxins and other inhibitory components with acidogens and H_2 consuming/producing microorganisms (Hulshoff *et al*. 2004).

The focus of our study was to set up methodology for effective assessment of ILs and their treatability potential in aerobic and anaerobic process regarding also type of the sludge (flocculent, granular). For evaluation of methodological approach, two different ILs were selected, both with promising industrial applications and proven to be toxic to various organisms (Latala *et al*. 2005; Wang *et al*. 2009; Zagorc-Končan *et al*. 2010). The first investigated ionic liquid [bmpyr][dca] has been successfully used with *n*-heptane as a two-phase solvent system for lipase-catalyzed synthesis of isoamyl acetate in a continuously operated Ψ-shaped microreactor (Pohar *et al*. 2009) and in a micro droplet-based microfluidic system integrated with membrane separator enabling ionic liquid and enzyme recycle (Novak & Žnidaršič-Plazl 2013). The chosen solvent system with dissolved *Candida antarctica* lipase B (CaLB), which was attached to the ionic liquid/*n*-heptane interfacial area due to its amphiphilic properties, was proved to be highly efficient in contrast to using the investigated hydrophilic ionic liquid alone. The second investigated ionic liquid [bmim][BF_4] is one of the most widely used alternative solvents, especially in (bio)catalytic processes and in organic reactions promoted by ILs (Zhou & Malhotra 2002; Sheldon *et al*. 2002). Recently it was shown to be a promising component of an ionic-liquid-based aqueous two-phase system for a very efficient extraction of proteins (Novak *et al*. 2012) and also a good material for applications where conductivity and electrochemical stability are needed (Kareem & Kaliani 2013).

MATERIALS AND METHODS

Tested ILs

ILs [bmpyr][dca], obtained from Solvent Innovation GmbH (Cologne, Germany) and [bmim][BF_4], purchased from IoLiTec ILs Technologies GmbH (Heilbronn, Germany), were dried at moderate vacuum and temperature. The structures of ILs are shown in Figure 1, their physico-chemical characteristics are presented in Table 1 (Docherty & Kulpa 2005; Solvent Innovation 2009; Kamath *et al*. 2012; Kareem & Kaliani 2013).

Determination of toxicity and biodegradability

Anaerobic conditions

For determination of toxicity and biodegradability of ILs in anaerobic conditions method for the determination of ultimate biodegradability of organic compounds in digested sludge with measurement of biogas production was used with some minor modifications (ISO 11734 1995). Experiments were accomplished at $39 \pm 1\ ^{\circ}C$ because inoculum was obtained from treatment plant running at this temperature. It was digested prior use for 7 days to remove organics present. The concentrations of the ILs in the test were also higher as requested by standard procedure to achieve range of concentrations toxic to anaerobic sludge. They were selected on the basis of results of toxicity test in aerobic conditions. The increase of headspace pressure in test vessels (250 mL, containing 100 mL of liquid phase) resulting from the production of CH_4 and CO_2 (biogas) was measured by the OxiTop system (WTW GmbH, Germany,

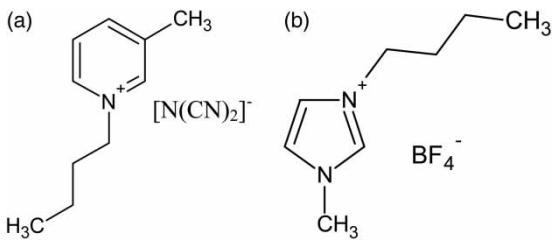

Figure 1 | Structure of 1-butyl-3-methylpyridinium dicyanamide (a) and 1-butyl-3-methylimidazole tetrafluoroborate (b).

Table 1 | Physical and chemical properties of the investigated ILs [bmpyr][dca] and ([bmim][BF_4])

Properties	[bmpyr][dca]	([bmim][BF_4])
CAS number	712355-12-9	174501-65-6
Color	Brown	Colorless
Density (g cm^{-3})	1.057	1.210
Molecular weight (g mol^{-1})	216.22	226.02
pH	7.4	7.3
Miscibility with water	High	Low
log P_{OW}	−2.4	−2.4

2008) in incubator. Two series of experiments with flocculent and granular sludge were accomplished each in three replicates and standard deviations evaluated (Žgajnar Gotvajn *et al*. 2011).

(i) Toxicity was determined by measurement of pressure increase due to the biogas production in the systems with different concentrations of the sample (106, 530 and 1,060 mg L^{-1} of [bmpyr][dca]; 847 and 1,452 mg L^{-1} of [bmim][BF_4]), reference compound glucose (60 mg L^{-1}) and anaerobic sludge (inoculum) in buffer solution. Blank test (inoculum with glucose) was run simultaneously to determine biogas production of the sludge alone.

(ii) Biodegradability was determined by measurement of pressure gain in the systems with the sample (106 IL mg L^{-1} of [bmpyr][dca]; 1,272 mg L^{-1} of [bmim][BF_4]), inoculum and buffer solution. The biodegradation experiments were conducted in the same way as experiments for determination of toxicity. Due to different concentrations of inoculum in experiments specific biogas production (hPa g^{-1}_{MLVSS}) was also calculated to make the amount of biogas evolved per mass unit of inoculum comparable. By injecting 2.5 mL of 6 M NaOH solution the ratio between methane and carbon dioxide in the gaseous phase was determined.

Aerobic conditions

Toxicity and biodegradability were also determined only with flocculent aerobic activated sludge at 21 ± 2 °C. Tests were run in duplicates.

(i) Toxicity. It was determined by the method for the determination of the toxicity to microorganisms of activated sludge by measurement of inhibition of oxygen consumption by activated sludge (1,500 mg_{MLVSS} L^{-1}) for carbonaceous and ammonium oxidation (ISO 8192 2007). Decrease of the oxygen consumption rate was measured as mg_{O2} L^{-1} min^{-1} by oxygen electrode. The inhibition in terms of EC (Effective Concentration) values was estimated. Experiments were conducted twice, with and without *N*-allylthiourea (ATU, a specific inhibitor of the nitrification) added, the inhibitory effect on oxygen uptake by total sludge microorganisms (without ATU added) as well as to heterotrophic microorganisms (with 11.4 mg L^{-1} of ATU) could be measured. Thus the inhibitory effect to nitrifying microorganisms was calculated. Tests were accomplished with 106, 212 and 530 mg L^{-1} of [bmpyr][dca] and 121, 241 and 604 mg L^{-1} of [bmim][BF_4] after 30 and 180 min.

(ii) Biodegradability. Non-adapted activated sludge from laboratory municipal wastewater treatment plant was used as inoculum (30 $mg_{MLVSS}.L^{-1}$). Biodegradability of [bmpyr][dca] was accomplished with concentration of 98 mg L^{-1} (initial COD = 77 mg L^{-1} and dissolved organic carbon (DOC) = 30 mg L^{-1}), while 116 mg L^{-1} of [bmim][BF_4] was tested. In this case COD was 81 mg L^{-1} and DOC was 37 mg L^{-1}. Biodegradability was monitored 28 days by two methods; the method by analysis of DOC (ISO 7827 2010) and the method by determining oxygen demand in a closed respirometer (ISO 9408 1999) (OxiTop®, WTW, Germany).

RESULTS AND DISCUSSION

Anaerobic toxicity and degradation

Ionic liquid 1-butyl-3-methylpyridinium dicyanamide

The biogas production curves expressed as pressure increase (hPa) versus time for a reference compound (glucose), a mixture of IL (106, 530 or 1,060 mg L^{-1}) and glucose (60 mg L^{-1}) and [bmpyr][dca] alone (106 mg L^{-1}) are presented in Figure 2(a). Flocculent anaerobic sludge in concentration of 5.46 g_{MLVSS} L^{-1} was used. At day 26, NaOH solution was added in the system to remove CO_2. In Figure 2(b), the biogas production curves for a reference compound (glucose), a mixture of IL (318, 742 mg L^{-1}) and glucose (60 mg L^{-1}) and [bmpyr][dca] alone (1,271 mg L^{-1}) in the presence of granular sludge (1.82 g_{MLVSS} L^{-1}) are presented. NaOH solution was added on day 5. The increase of pressure in the blank reached 70/85 hPa and it has been already subtracted from the presented measurements. Standard deviations of presented measurements were in the range of 4–10% indicating significant differences between the curves in Figure 2.

The increase of the pressure in the system with glucose reached 100 hPa in 26 days corresponding to 62% of glucose degradation (Figure 2(a)), calculated with comparison to theoretically expected pressure change due to addition of 60 mg L^{-1} of glucose. In the case of granular sludge (Figure 2(b), degradation of glucose reached 100% (170 hPa in 5 days). Measurements were considered valid (more than 60% of glucose degradation). In the first set of experiments, concentration of flocculent sludge was three times higher than in the second experiment (5.46/1.82 g_{MLVSS} L^{-1}).

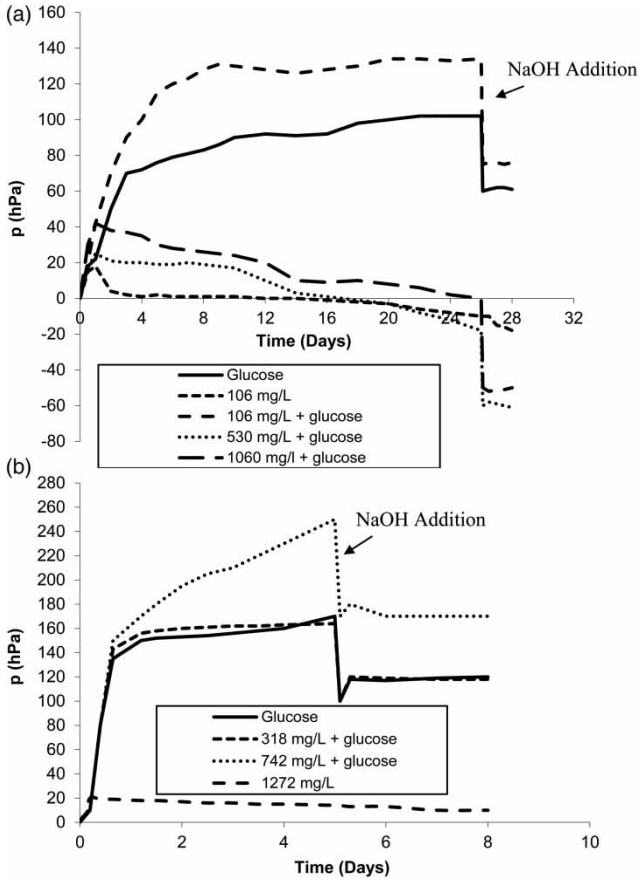

Figure 2 | Production of biogas (hPa) for reference compound (glucose), mixture of indicated concentrations of [bmpyr][dca] and glucose, and IL alone: (a) flocculent inoculum; and (b) granular inoculum.

Rapid degradation of glucose indicated much higher activity of granular sludge.

Hydrophilic [bmpyr][dca] alone did not degrade in the concentration of 106 mg L^{-1} in the presence of flocculent sludge (Figure 2(a)). It was even toxic to microorganisms at investigated concentrations. In concentration of 530 and 1,060 mg L^{-1} it inhibited degradation of glucose completely, so pressure decreased towards zero (Figure 2(a)). At the lowest concentration (106 mg L^{-1}) IL did not inhibit degradation of glucose, the pressure increase was even higher than in the system with glucose alone. The same phenomena was also shown by other authors regarding biodegradation of persistent PAHs with more than four rings, which degrade under methanogenic conditions only in combination with other metabolic routes, via cometabolism. These cometabolic routes can thus be stimulated by the addition of a readily biodegradable substrate, like glucose in the case of investigated IL (Chang *et al.* 2005; Dionisi *et al.* 2006). Data from Figure 2(a) were used to calculate specific biogas production as pressure increase per g of inoculum (hPa g^{-1}_{MLVSS}) for the blank, reference compound (glucose) and for the system with 106 mg L^{-1} of IL and glucose. In the blank specific biogas production reached 136 hPa g^{-1}_{MLVSS}, in the vessel with glucose it was 328 hPa g^{-1}_{MLVSS} while in the system with mixture of IL and glucose it was 426 hPa g^{-1}_{MLVSS}, confirming degradation of tested ionic liquid. Actual degradation of substrate in each system was also calculated and it reached negligible 4% in the systems with 106 mg $L^{-1}$of [bmpyr][dca], as well as it did not degrade more in the system with 530 and 1,060 mg L^{-1} of IL with glucose. In the mixture of glucose and IL in the lowest tested concentration of 106 mg L^{-1} IL degraded 44%. It was confirmed that at this concentration ionic liquid is not toxic to anaerobic sludge and it can degrade in the presence of easily biodegradable substrate. Addition of NaOH solution was used to determine the composition of the biogas evolved. In the case of glucose theoretically expected biogas composition is 50.0% of methane and 50.0% of carbon dioxide, while in the case of investigated ionic liquid alone its composition should be 44.8% of CO_2 and 55.2% of CH_4. Degradation of mixture (106 mg L^{-1} of IL + glucose) should result in 46.5% of CO_2 and 53.5% of CH_4. According to our measurements it was calculated, that in the headspace of the mixture of IL and glucose there is only 22% of CO_2, while the rest (78%) are other gases, but probably not only methane. If IL inhibits methanogenesis, volatile fatty acids, hydrogen and $NH_{3(g)}$ could appear in the biogas, because they are not transformed further to methane. Composition of the biogas should be further confirmed by gas chromatography–mass spectrometry (GC-MS) analyses. However, measurement of biogas composition confirmed only partial degradation of ionic liquid at the lowest concentration, as well as its toxic impact.

Ionic liquid [bmpyr][dca] was persistent in the presence of granular sludge (Figure 2(b), 1,272 mg/L) as in the experiment with flocculent sludge. But it was not toxic to microorganisms of granular sludge at investigated concentrations. Degradation of glucose in the presence of 318 mg L^{-1} of ionic liquid was not inhibited (Figure 2(b)), while in the presence of 742 mg L^{-1} of [bmpyr][dca] pressure increase was even higher (240 hPa). Because it has been confirmed that glucose degraded completely, the difference of 70 hPa represented degradation of ionic liquid. Again, cometabolic degradation of investigated IL could be confirmed. Specific biogas production in the blank reached 380 hPa g^{-1}_{MLVSS}, in the vessel with glucose it reached 1,255 hPa g^{-1}_{MLVSS}, while in the system with IL

and glucose it was 1,260 hPa g^{-1}_{MLVSS} (318 mg $L^{-1}$of IL) and 1,660 hPa g^{-1}_{MLVSS} (742 mg $L^{-1}$of IL), respectively. Specific biogas productions were much higher than in the first experiment with flocculent inoculum. Actual degradation of substrate in each system was also calculated and it reached 10% in the systems with 318 mg L^{-1} of [bmpyr][dca] and it degraded 34% in the system with 742 mg L^{-1} of IL with glucose. It was confirmed that at this concentration ionic liquid is not toxic to granular anaerobic sludge and that it can be degraded in the presence of easily biodegradable substrate. Addition of NaOH solution did not lead to reliable conclusions. Degradation of mixture (742 mg L^{-1} of IL + glucose) should result in 46.1% of CO_2 and 53.9% of CH_4, but our measurements have shown only 28% of CO_2, while the rest (62%) are other gases. The amount of methane produced could clearly not be determined only on the basis of NaOH addition.

Ionic liquid 1-butyl-3-methylimidazole tetrafluoroborate

The biogas production curves as pressure increase (hPa) versus time for reference compound, mixture of [bmim][BF_4] (847 and 1,452 mg L^{-1}) and glucose (60 mg L^{-1}) and [bmim][BF_4] alone (847 mg L^{-1}) are presented in Figure 3. As inoculum, flocculent anaerobic sludge in concentration of 13.65 g_{MLVSS} L^{-1} (Figure 3(a)) was used, while in the Figure 3(b) results obtained with 9.0 g_{MLVSS} L^{-1} of granulated sludge are presented. NaOH solution was added at day 6. The increase of pressure in the blank reached 60/100 hPa and it has been already subtracted from the presented measurements. Standard deviations of presented measurements were in the range of 2–8% indicating less significant differences between the curves in Figure 3.

In both sets of experiments (Figure 3), glucose degraded well (65/100%) confirming validity of the test and much higher activity of granular sludge. [bmim][BF_4] did not degrade in the concentration of 847 mg L^{-1} in the presence of flocculent or granular sludge. It inhibited degradation of glucose slightly in the presence of flocculent sludge (Figure 3(a)), because biogas production was reduced with higher concentration of investigated IL. In the case of granular sludge the pressure increase was even higher than in the system with glucose alone indicating no toxicity and cometabolism as for the first investigated ionic liquid [bmpyr][dca]. Neumann *et al.* (2010) measured biodegradability of nine different imidazole and pyridinium-based ILs in the presence of sodium acetate and they excluded occurrence of co-metabolism.

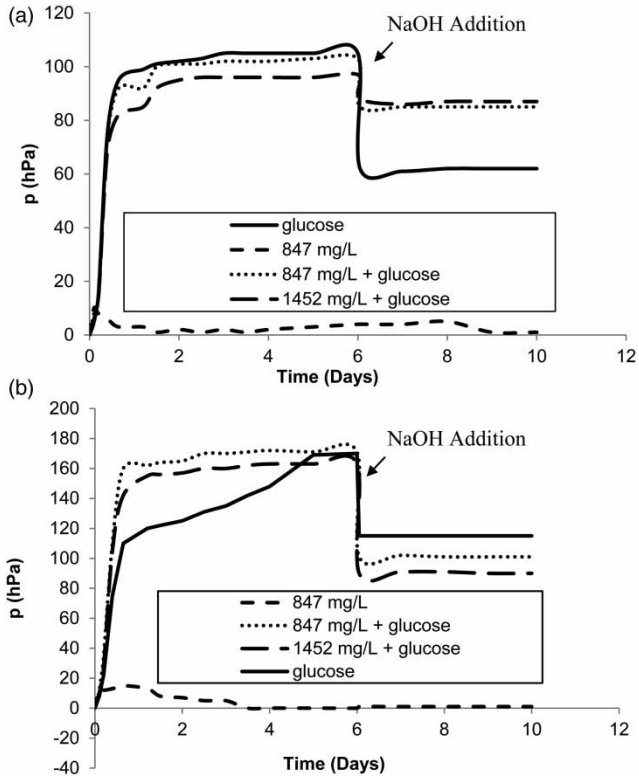

Figure 3 | Production of biogas (hPa) for reference compound (glucose), mixture of [bmim][BF_4] and glucose and IL alone: (a) flocculent inoculum; and (b) granular inoculum.

Specific biogas production for each system was calculated again. For blank with flocculent sludge it reached 145 hPa g^{-1}_{MLVSS}, while with flocculent sludge it was 450 hPa g^{-1}_{MLVSS}. In the vessel with glucose it was 333 and 1,342 hPa g^{-1}_{MLVSS}, respectively. In the system with mixture of IL, glucose and granular sludge it was 1,375 (847 mg L^{-1}) and 1,365 hPa g^{-1}_{MLVSS} (1,452 mg L^{-1}), confirming minimal degradation of tested ionic liquid. In the case of flocculent inoculum, specific biogas production in mixture of IL and glucose was lower than in the glucose alone, confirming low toxic impact. Degradation of tested substance in each system was also calculated for granular sludge and it reached 33% in the systems with 847 mg $L^{-1}$of and 21% in the mixture with 1,452 mg L^{-1}. There was no degradation in the test with flocculent inoculum.

In the case of [bmim][BF_4] composition of biogas should be 35.9% of CO_2 and 64.1% of CH_4. Complete degradation of 847 mg L^{-1} IL with 60 mg L^{-1} of glucose should result in 41.4% of CO_2 and 58.6% of CH_4. Measurements in the system with granular sludge indicated 33% of CO_2, while the rest (67%) are other gases. As for the [bmpyr][dca] it was assumed that beside methane other gases, specific for

Table 2 | The inhibitory effect of both ILs on total microorganisms of activated sludge as well as only on heterotrophic and only on nitrifying ones (30 and 180 min of incubation, $N = 3$)

Sample	EC_{50} values mg L^{-1}	Total microorganisms	Heterotrophic microorganisms	Nitrifying microorganisms
[bmpyr][dca]	30 min EC_{50}	307 ± 74	244 ± 21	488 ± 254
	180 min EC_{50}	68 ± 10	254 ± 32	3 ± 1
[bmim][BF_4]	30 min EC_{50}	386 ± 193	652 ± 193	640 ± 254
	180 min EC_{50}	169 ± 205	326 ± 36	5 ± 1

anaerobic stabilisation of organics, are also present. In any case, only partial anaerobic degradation of both ILs was confirmed. It was explained by lack of molecular oxygen to be inserted by monooxygenase to initialise the degradation, because it has been noticed, that initial biodegradation step involves oxygen as reactant (Neumann *et al*. 2010).

Aerobic toxicity and degradation

Inhibition of oxygen consumption of both ILs after 30 and 180 minutes is presented as EC_{50} values for different groups of microorganisms of activated sludge in Table 2.

Lower EC_{50} values of [bmpyr][dca] in comparison to [bmim][BF_4] indicated higher toxicity to aerobic activated sludge. For both ILs toxicity increased with time of exposure, affecting mainly nitrifying microorganisms. The antibacterial activity of various imidazolium-based tetrafluoroborates was also noticed by other authors (Pham *et al*. 2010; Markiewicz *et al*. 2013). Some authors suggested that toxicity of ionic liquid is mainly determined by cationic component, because the lipophilic part of the molecules can be intercalated into the cell membrane, whereas the ionic group is at least partially solvated in the aqueous medium (Austin *et al*. 1998). Data obtained with [bmim][BF_4] were much less repeatable probably due to its poor miscibility with water.

However, both ILs expressed higher toxicity to aerobic sludge than anaerobic one. If effect of 106 mg L^{-1} of [bmpyr][dca], which caused no inhibition in anaerobic system with flocculent sludge is calculated according to toxicity in aerobic conditions (Table 2, Total microorganisms, 30 min incubation) it would express 75% inhibition. The same is also true for the [bmim][BF_4]. There was only slight inhibition of biogas production in the concentration of 847 mg L^{-1}, while in the aerobic conditions it would cause complete inhibition of oxygen consumption (Table 2).

Biodegradability of both ILs in aerobic conditions was negligible. 1-butyl-3-methylpyridinium dicyanamide and 1-butyl-3-methylimidazole tetrafluoroborate degraded <3% in both biodegradability tests, based on oxygen consumption and DOC elimination. Abiotic elimination was also not detected. Other authors also determined poor biodegradability of cations incorporated short chains ($C \leq 4$) in aerobic conditions (Pham *et al*. 2010). It can be concluded that investigated ILs are not biodegradable in anaerobic and aerobic conditions via metabolic pathways.

CONCLUSIONS

Toxicity and biodegradability of two ILs were determined in anaerobic and aerobic conditions to illustrate appropriate approach for determination of biotreatability. High toxicity and poor biodegradability was observed in aerobic conditions. Anaerobic granular sludge was less sensitive to investigated ILs. [bmpyr][dca] was not toxic to it up to 742 mg L^{-1} and it even degraded at this concentration (34%) in the presence of glucose probably via cometabolism. On the other hand, flocculent sludge was completely inhibited at the concentration of 318 mg L^{-1}, while at the lower concentration cometabolism also occurred. But both sludges were unable to degrade [bmpyr][dca] without additional co-substrate, glucose. The same was also true for the second ionic liquid, 1-butyl-3-methylimidazole tetrafluoroborate, which degraded in the presence of glucose up to concentration of 1,452 mg L^{-1} (21%). To confirm cometabolic processes, primary degradation with measurement of IL concentration should be employed and biogas composition should be determined by specific GC-MS analyses. However, presented preliminary experiments indicate, that the structure and condition of the anaerobic sludge affect removal rate and also degradation in natural anaerobic compartments is expected to be also very site and time specific.

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