Comparison of imidazolium ionic liquids and traditional organic solvents: effect on activated sludge processes

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ABSTRACT

Data concerning the biodegradability and ecotoxicity of ionic liquids (ILs) obtained so far are insufficient in the context of IL removal from wastewater in activated sludge systems. Thus, in this work the selected imidazolium ionic liquids and two organic solvents (methanol and acetone) were tested with respect to their influence on activated sludge processes, particularly on the morphology of sludge flocs. The presence of ionic liquids with the chemical structure of 1-alkyl-3-methyl imidazolium bromide in wastewater did not deteriorate biological wastewater treatment processes if their concentration was not higher than 5 mg l^{-1}. Regarding the structure of the ILs studied, the longer the alkyl substituent was, the stronger the effect on sludge flocs. The highest decrease in activated sludge floc area and biomass concentration was exerted by the ionic liquid with the longest alkyl chain, i.e. 1-decyl-3-methylimidazolium bromide. The action of both methanol and acetone on floc size, activated sludge concentration and efficiency of organic pollutants removal was weaker compared to all tested 1-alkyl-3-methyl imidazolium bromides.

Key words | activated sludge, floc morphology, imidazolium ionic liquids, removal

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INTRODUCTION

Ionic liquids (ILs) are a class of technologically advanced solvents consisting of a large organic cation with alkyl side chain(s) and an organic or inorganic anion. They have many favourable physicochemical properties, including negligible volatility, non-flammability, high polarity, remarkable chemical and thermal stability and solvent miscibility behaviour. What is more interesting is that all these properties can be easily changed by using various cation/anion combinations in the molecule of ILs (Pham *et al.* 2010). For this reason, in some publications these compounds are called 'designed solvents' (Luis *et al.* 2007; Romero *et al.* 2008). The 1-alkyl-3-methylimidazolium salts are one of the most often used ILs, particularly as solvents for a wide range of inorganic and organic materials. They have been applied in various industrial processes such as biocatalysis, organic synthesis or sequestration of carbon dioxide (Gordon 2001; Bernot *et al.* 2005).

Apart from their physicochemical properties, the biological impact of ILs is also very important. For this reason, several studies on the biodegradability and ecotoxicity of ILs have been carried out in the last decade. The biodegradability of ILs has usually been evaluated on the basis of the results of standard OECD tests. Imidazolium ionic liquids are poorly or very poorly biodegraded compounds (Gatherhood *et al.* 2004, 2006; Garcia *et al.* 2005). No ionic liquids could be classified as 'readily biodegradable' corresponding to OECD standards, for which 60–70% or greater biodegradation is required within a 10-day window in a 28-day period. Moreover, Stolte *et al.* (2008) found that activated sludge communities were not able to metabolise most of the imidazolium ionic liquids, in particular the imidazolium salts with short alkyl side chains (<C6).

The ecotoxicity of ILs has been tested with the use of many species of organisms representing different trophic levels. In the context of this study, the toxicity of ILs towards microorganisms is the issue of particular importance. Microtox bioassay based on the bioluminescence inhibition method to assess the bacterium *Vibrio fischeri* has been commonly applied to evaluate the antimicrobial properties of ILs (Ranke *et al.* 2004; Romero *et al.* 2008; Ventura *et al.* 2010, 2012). Tests on other microorganisms, including *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* has also been performed (Docherty & Kulpa 2005; Matsumoto *et al.* 2004; Pham *et al.* 2010). A trend of increasing

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toxicity with an increase in the alkyl chain length substituent in the pyridinium, imidazolium and quaternary ammonium salts to various bacteria has been observed. At the same time, the results of tests on bioluminescent bacteria *V. fischeri* as well as those obtained in tests on other microorganisms showed that varying the anion has almost no effect on antimicrobial activity in the presence of imidazolium salts (Pham *et al.* 2010). In addition, the antimicrobial properties of ionic liquids and traditional organic solvents have been compared (Ranke *et al.* 2004; Luis *et al.* 2007). The results of tests on *V. fischeri* have shown that imidazolium ionic liquids are more toxic than conventional solvents such as methanol, acetone and acetonitrile (Luis *et al.* 2007). Analysing literature data concerning the biodegradability and ecotoxicity of ILs, it is easy to see that few ILs concern such mixed cultures as activated sludge microorganisms. This makes the information available about the characteristics of the biological impact of ILs on the environment incomplete.

Therefore, in this work the selected imidazolium ionic liquids and two organic solvents (methanol and acetone) were tested with respect to their influence on the activated sludge process. The main objective of this study was to compare the effect of the imidazolium ionic liquids and traditional organic solvents on the morphology of activated sludge flocs.

MATERIALS AND METHODS

Chemicals tested

Three ionic liquids were tested: 1-ethyl-3-methylimidazolium bromide, denoted as [C2mim][Br], 1-hexyl-3-methylimidazolium bromide, denoted as [C6mim][Br] and 1-decyl-3-methylimidazolium bromide, denoted as [C10mim][Br]. They were purchased from Ionic Liquids Technologies GmbH (Denzlingen, Germany). The purity of [C2mim][Br] and [C6mim][Br] was 99%, while the purity of [C10mim][Br] was 98%. For the purposes of comparison, two commonly used volatile solvents were selected, i.e. the shortest aliphatic alcohol – methanol (CH_3OH) and the simplest aliphatic ketone – acetone (CH_3COCH_3). Both were purchased from POCh (Poland).

Activated sludge

The activated sludge taken from the aeration chamber at the Combined Wastewater Treatment Plant (WWTP) in Lodz (Poland) was used as the inoculum in all experiments. The Combined WWTP in Lodz treats municipal wastewater from the city of Lodz, several communities located near the city and industrial wastewater originating mainly from small and medium enterprises. The average pollutant load to the plant corresponds to approximately $1 \cdot 10^6$ PE, while the average inflow of wastewater was 150, 430–194, 240 $m^3\,d^{-1}$ when the sludge for the experiments was taken. The contribution of industrial wastewater was usually 10–15% in this plant. The biological stages consist of seven lines of three-zone bioreactors and secondary clarifiers run in the UCT process configuration. Average sludge loading rate was 0.04–0.07 g BOD g $TSS^{-1}\,d^{-1}$, while sludge retention time (SRT) was 7.5–11 days when the sludge for the experiments was taken.

Inoculation always occurred within 2 hours of sampling the activated sludge. Activated sludge had typical properties, i.e. the total solids (TS) were 3.5–5.6 g l^{-1} and volatile solids (VS) were 2.1–4.1 g l^{-1}. Sludge volume index (SVI) was 84–130 ml g TSS^{-1}. Flocs were small on average (mean diameter 73–82 μm), irregular (circularity 0.327–0.358) and with the average number of filaments corresponding to category 2 according to the Eikelboom & van Buijsen (1992) classification.

Tests

All experiments were conducted in shake flasks under aerobic conditions. A 40 ml measure of activated sludge (inoculum) was transferred into a 300 ml Erlenmeyer flask containing 160 ml fresh synthetic wastewater with or without imidazolium ionic liquid or traditional solvent. The composition of the synthetic wastewater was as follows: 300 mg peptone, 100 mg sodium acetate, 50 mg potassium monophosphate, 50 mg sodium bicarbonate, 50 mg ammonium hydrophosphate, 5 mg magnesium sulphate and 5 mg sodium chloride per litre.

The following concentrations of [C2mim][Br], [C6mim][Br] and [C10mim][Br] were tested: 1, 5, 25 and 50 mg l^{-1}. The initial activated sludge biomass concentration was 645 ± 45 mg VSS l^{-1}. The flasks were incubated at 20 ± 0.5 °C in a thermostated rotary shaker Certomat® IS at 130 min^{-1} for 24 hours. The experimental conditions were set to minimize the evaporation of the volatile organic solvents (methanol and acetone) tested. All experiments, i.e. ionic liquid or traditional solvent runs as well as control runs (without addition of these compounds) were conducted according to this procedure. A similar procedure was applied and described elsewhere (Zhang *et al.* 1999;

Liwarska-Bizukojc *et al*. 2008). For each ionic liquid, methanol and acetone the experiments were carried out in triplicate, with five replicates for the control runs.

Physicochemical analyses

At the beginning and at the end of each test the following analyses were carried out: concentration of ionic liquid, soluble chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), TS, total suspended solids (TSS), VS and volatile suspended solids (VSS). Soluble COD, TS, TSS, VS, VSS and BOD_5 were carried out according to the standard procedures (APHA-AWWA 1995). Concentrations of ILs were determined with the use of UPLC® (Waters, USA). A Waters Symmetry Shield RP18 column was used at the various CH_3CN–water (v/v) isocratic elutions (both eluents modified with 1% formic acid): 2:98, 15:85 and 40:60 for [C2mim][Br] [C6mim][Br] and [C10mim][Br] respectively the temperature of the column was 40 °C. All ionic liquids were detected with a Synapt G2 MS-MS detector at the positive electrospray ionisation using M+ ion masses of each ionic liquid: 111.0945, 167.1554, and 223.2154 for [C2mim][Br], [C6mim][Br] and [C10mim][Br] respectively.

Image analysis of the flocs

The vital unstained slides of activated sludge samples were prepared for the purpose of image analysis. The activated sludge flocs were observed under a light microscope Nikon Eclipse N*i* with the magnification of the objective lens at 4×. The RGB (red, green, blue model of colour) images were taken, processed and analysed with the help of NIS-Elements AR software (Nikon, Japan). At least 40 images from each sample were processed and analysed with the help of the special automated procedure to measure the basic morphological parameters of the flocs, i.e. projected area, perimeter, diameter, convexity and circularity. The projected area is the basic image analysis parameter and easily found by pixel count and then multiplication by scaling factor. Diameter was measured as the mean value of lines between two points on the boundary of the individual object going through its centroid. The perimeter is simply the length of the boundary of the object, while the convex perimeter is the length of the boundary obtained after filling of all concavities of the object. The convexity is the ratio of perimeter to convex perimeter. Circularity is the shape factor that indicates to what extent the measured object is similar to the true circle. If it is equal to 1, the object is a true circle. The lower the value, the less circular the object. A description of these morphological parameters of activated sludge flocs have also been presented elsewhere (Liwarska-Bizukojc 2005).

Calculation of the relative change in floc area

The relative change in floc area (A_C) was calculated according to the following equation:

$$A_C = \frac{(A_{24} - A_{24,\text{control}})}{A_{24,\text{control}}} \cdot 100$$

where A_{24} is the mean projected area of the flocs exposed to the studied ionic liquid or organic solvent after 24 hours of testing and $A_{24,\text{control}}$ is the mean projected area of the flocs not exposed to the ionic liquid or organic solvent after 24 hours of testing.

The relative change of floc area (A_C) was expressed as a percentage. Positive values of A_C showed an increase in the floc size, while negative values, showed a decrease in floc size.

RESULTS AND DISCUSSION

Analysing the values of morphological parameters obtained in the experiments, it was found that the imidazolium ionic liquids studied affected the activated sludge floc morphology particularly with regard to floc size. Therefore the relative change in mean projected floc area was calculated and presented in Table 1. These data allow for the comparison of the influence of the ionic liquids and traditional organic solvents on floc size.

At low initial concentrations of the ILs in wastewater (1 and 5 mg l^{-1}) the flocs were bigger than in the control tests, whereas at high initial concentrations of the ILs in wastewater (25 and 50 mg l^{-1}) the mean projected area of flocs

Table 1 | Comparison of the relative change in the area of flocs

	Relative change in floc area (%)				
Concentration (mg l^{-1})	**[C2mim][Br]**	**[C6mim][Br]**	**[C10mim][Br]**	**Methanol**	**Acetone**
1	28.3	31.1	24.4	16.2	12.3
5	11.6	10.7	3.9	23.5	26.1
25	26.5	−1.2	−41.7	32.5	19.6
50	6.4	−21.8	−53.6	22.2	18.4

decreased in comparison to the control run, excluding the flocs exposed to [C2mim][Br]. The smallest flocs were found in the tests with [C10mim][Br], when the initial concentration of this ionic liquid was 50 mg l^{-1}. The mean projected area of these flocs was, on average, 53.6% lower than in the control tests (Table 1). Generally, the longer the alkyl chain of the studied ionic liquids, the stronger the decrease in floc area. The decrease of the floc size in the tests with the ionic liquids was most probably caused by the decay of extracellular polymeric substances and the decomposition of bacterial agglomerates. It is known that ionic compounds, particularly monovalent cations, contribute to the release of biopolymers in activated sludge suspension (Bitton 2010).

At the same time it was found that the flocs exposed to acetone and methanol were bigger than in the control tests and their size was independent of the initial concentration of the organic solvents in wastewater (Table 1). This meant that the presence of these solvents in wastewater did not contribute to the decomposition of sludge flocs and, as a result, did not decrease their mean projected area. These observations agree with previous findings concerning comparisons between ionic liquids and traditional organic solvents with respect to their influence on activated sludge microorganisms (Ranke *et al.* 2004).

To provide a closer look at the effect exerted by the imidazolium ionic liquids on the size of activated sludge flocs, the diameters of activated sludge flocs exposed and not exposed to the tested ILs are depicted in Figure 1. The changes in floc diameter in the tests gave similar results to the changes in mean projected area described above. First of all, the mean diameter of activated sludge flocs depended on the chemical structure of the ILs tested. The diameters of flocs exposed to [C2mim][Br] were 114.8–121.5 μm and were higher than in the control run (on average 113 μm) after 24 hours of testing. What is important is that the changes in diameter did not depend on the initial concentration of this ionic liquid in wastewater. At the same time the mean diameters of flocs exposed to [C6mim][Br] and [C10mim][Br] decreased with an increase in the initial concentration of these ionic liquids. In case of the tests with [C6mim][Br], the mean diameter decreased from 126.9 to 98.8 μm with an increase of the initial IL concentration from 1 to 50 mg l^{-1}, whereas in the tests with [C10mim][Br], the decrease of mean diameter was from 118.8 to 78.1 μm. According to Eikelboom & van Buijsen (1992) classification, activated sludge flocs of diameter below 100 μm are classified as small. However, according to other classifications they can be rated as flocs of medium size (Eikelboom 2000; Mielczarek *et al.* 2012). The mean diameters of

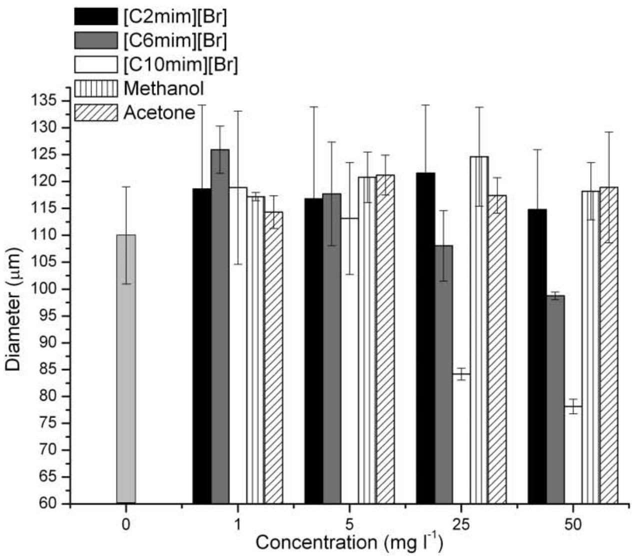

Figure 1 | Comparison of the mean diameter of activated sludge flocs exposed and not exposed to ILs or traditional solvents after 24 hours of testing.

sludge flocs exposed to the traditional solvents were usually higher than those in the control tests and did not correlate with the initial concentration of these chemicals in wastewater. In the tests with methanol, the mean diameters of flocs were 117.2–124.6 μm, while in the tests with acetone they were 114.3–121.2 μm (Figure 1). It confirmed that these compounds did not influence floc morphology, particularly floc size, even at a concentration of 50 mg l^{-1}.

Beside the parameters representing floc size, the parameters describing their shape were also measured. The circularity of activated sludge flocs exposed and not exposed to the ILs or traditional solvents is shown in Figure 2. In the tests carried out, the circularity of activated sludge flocs did not vary significantly and remained in the 0.269–0.353 μm range, dependent on the chemicals tested and their initial concentrations. Sludge flocs were far from circular in shape. According to the formula implemented in NIS–Elements AR, a circularity of 1 corresponds to a perfect circle. The convexity of flocs, which expresses the protrusions from the edges of sludge flocs, varied slightly from 0.701 and 0.734 in the tests. These values showed that the number of protrusions was moderate and did not change with on the chemicals studied or their initial concentrations. It indicated that neither the initial concentration of the chemicals studied nor their structure influenced floc shape to any great extent. Both the circularity and convexity of sludge flocs were slightly higher at the higher initial concentrations (25 and 50 mg l^{-1}) of two ILs, i.e. [C6mim][Br] and [C10mim][Br]. It can be claimed that the most hydrophobic

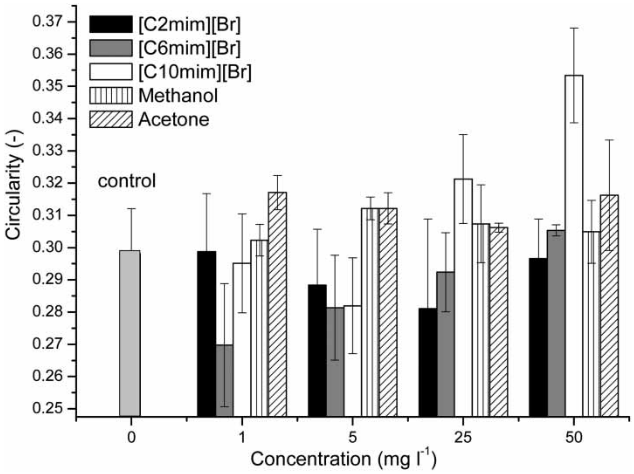

Figure 2 | Comparison of the mean circularity of activated sludge flocs exposed to the tested ILs or traditional organic solvents and not exposed to these chemicals after 24 hours of testing.

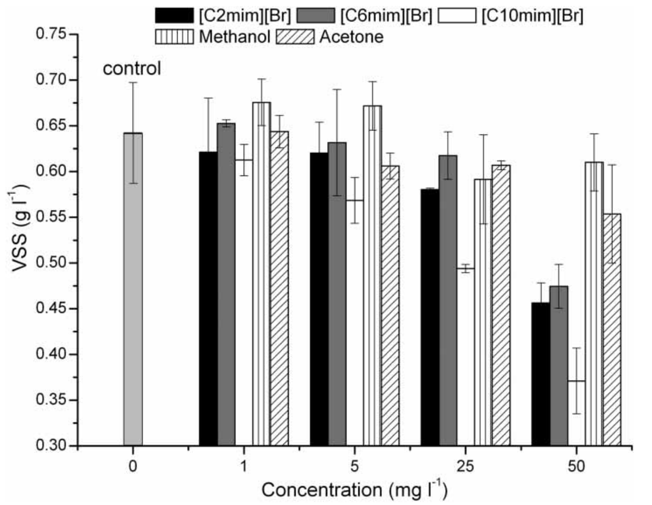

Figure 3 | Comparison of biomass concentration in the experiments with ionic liquids and traditional organic solvents added to wastewater after 24 hours of testing.

ILs of those studied at the higher concentrations caused a decrease of activated sludge floc size and an increase in their circularity. A similar phenomenon has been observed with respect to synthetic surfactants (Liwarska-Bizukojc & Bizukojc 2006).

The presence of the ILs in wastewater at the higher concentrations (25 and 50 mg l^{-1}) caused a decrease of activated sludge biomass concentration (Figure 3). The decrease was highest in the tests with [C10mim][Br], which is in agreement with the aforementioned observations concerning the morphology of activated sludge flocs. Neither methanol nor acetone contributed much to the decrease of activated sludge biomass concentration (Figure 3). At the highest tested concentration of 50 mg l^{-1}, the mean value of VSS in the tests with methanol or acetone was 6–15% lower than in the control runs, while in the tests with the ILs this decrease was higher and was 26–42% (Figure 3). It was once again confirmed that the organic solvents had a weaker effect on activated sludge than the imidazolium ionic liquids tested.

The values for the degree of COD removal obtained in the tests performed are compared in Figure 4. It was found that the presence of the tested ILs in wastewater did not decrease the removal of organic contaminants in the activated sludge system, if the initial concentrations of the ILs was low (1 or 5 mg l^{-1}). In these conditions the degree of COD removal in the tests with the ILs was at the same level (about 94%) as in the control runs. At the higher initial concentrations of the ILs (25 and 50 mg l^{-1}) the degree of COD removal decreased and was 64–89%, dependent on the ionic liquid added (Figure 4). The presence of methanol in wastewater

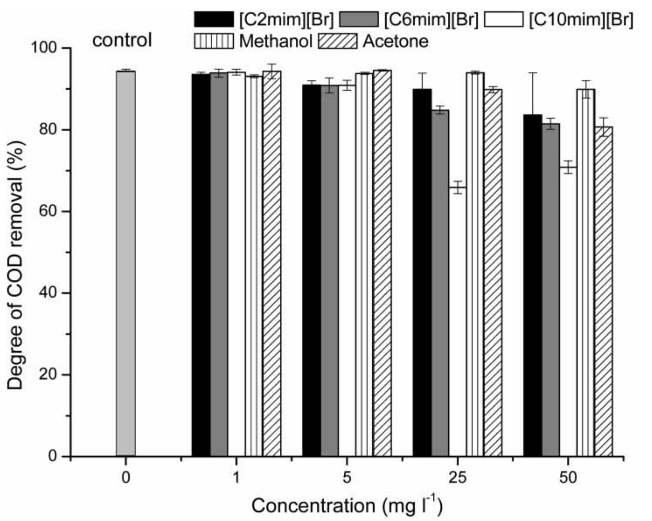

Figure 4 | Comparison of the degree of COD removal after 24 hours of testing.

even at the highest concentration of 50 mg l^{-1} did not cause any decrease in the degree of COD removal, while the presence of acetone did. The mean value of the degree of COD removal in the tests with methanol at the initial concentration of 50 mg l^{-1} was 90%, whereas in the tests with acetone it was 80%. Methanol is often added to wastewater as an external carbon source to support the processes of denitrification, and thus it was expected that its presence in wastewater would not act negatively on biological wastewater treatment (Henze *et al.* 2002). It was also observed in this work. Of all chemicals tested, the presence of the ionic liquid [C10mim][Br] in wastewater exerted the strongest effect on wastewater treatment in the activated sludge system. The values of COD removal were the lowest in the tests with this ionic liquid and were equal to 66% and 70% at concentrations 25 and 50 mg l^{-1} respectively.

Regarding the degree of BOD_5 removal, at the lower initial concentrations of ILs it was at a similar level as in the control runs, i.e. about 95%. At the higher initial concentrations of the ILs (25 or 50 mg l^{-1}) it was two to three times lower compared to the control tests. At the same time the values of BOD_5 removal were 90–96% and 83–94% in the tests with methanol and acetone, respectively.

The degree of ionic liquid removal was 15–99.8%. Its value increased with the elongation of the alkyl chain length and decreased with the increase of the initial concentration of the ILs in wastewater (Figure 5). It was found that the ionic liquid [C10mim][Br] was completely (the degree above 99%) removed from wastewater, if its initial concentration was equal to 1 or 5 mg l^{-1}. At the same time the removal of [C2mim][Br] was 15–40%, while this value for [C6mim][Br] was 20–63%, dependent on its initial concentration. These results confirmed that ionic liquids with longer alkyl substituent were more susceptible to biodegradation (Garcia *et al*. 2005; Pham *et al*. 2010), although their removal from wastewater depended on the initial concentration. Stolte *et al*. (2008) noted that activated sludge communities are not able to metabolise most of the imidazolium ionic liquids, in particular the imidazolium salts with short alkyl side chains consisting of less than six carbon atoms. At the same time it was found that the imidazolium-based ILs with hexyl or octyl substituents were able to achieve a certain degree of mineralization (Stolte *et al*. 2008). The degree of ionic liquid removal with a hexyl substituent [C6mim][Br] was higher than this with ethyl, although it remained relatively low level (Figure 5). The results obtained in this work showed that the removal of ionic liquids from wastewater also depended on their initial concentration.

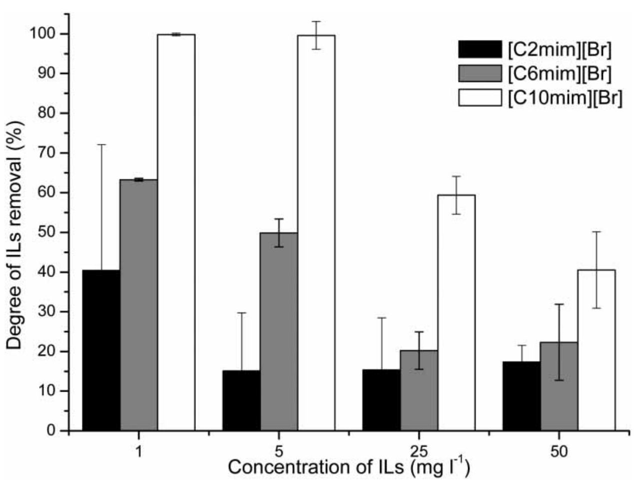

Figure 5 | Comparison of the degree of ILs removal from wastewater after 24 hours of the tests.

CONCLUSIONS

The influence of imidazolium ionic liquids on activated sludge depends on their chemical structure, particularly the length of their alkyl chain substituents and the concentration of ILs in wastewater. Generally, the presence of ionic liquids with the chemical structure of 1-alkyl-3-methyl imidazolium bromide in wastewater does not decrease biological wastewater treatment processes if the concentration of ILs does not exceed 5 mg l^{-1}. At higher concentrations (25 and 50 mg l^{-1}) the presence of ionic liquids causes the decrease of activated sludge floc area, biomass concentration and removal of organic pollutants from wastewater. The strongest effect on activated sludge, including floc morphology, is exerted by the ionic liquid with the longest alkyl chain, i.e. 1-decyl-3-methylimidazolium bromide. The presence of the traditional organic solvents methanol and acetone in wastewater does not contribute to a decrease in activated sludge floc area and biomass concentration. Their influence on floc size, activated sludge concentration and efficiency of organic pollutant removal is weaker than that of 1-alkyl-3-methyl imidazolium bromides.

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