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BIODEGRADATION OF NEW IONIC LIQUID-BASED WOOD PRESERVATIVES IN SOIL AND WATER ENVIRONMENTS

The ability of structurally different ionic liquids with cocotrimethylammonium, didecyldimethyloammonium and bis-dimethyloctylammonium cations to degrade in water and soil environments were investigated. The test in water was conducted according to OECD guidelines 301 A and 302 B. Secondary effluent from a domestic sewage treatment plant in Zabrze, Poland was used as the inoculum. The ready biodegradability of the test compounds was monitored via DOC (dissolved organic carbon) for 28 days. Biodegradation by moulds in soil were carried out on Scots pine Pinus sylvestris L. using the soil-block method. The two-phase titration and HPLC chromatography methods were used to detect and determine the metabolites of the ionic liquids. All the tested compounds showed ready biodegradability in the water environment, as well as decomposition by the test fungi species in the treated wood exposed in the soil. The degree of decomposition of the ionic liquids by moulds during the 12-week soil test was dependent on their structure, reaching a maximum of 55% for the cocotrimethylammonium nitrate and 52% for the bis-dimethyloctylammonium nitrate.

Keywords: ionic liquids, biodegradation, moulds, water, soil, HPLC
Introduction

The use of impregnated wood in conditions where biotic and abiotic factors are involved is linked to the risk of migration and leaching of the components of chemical preparations into the environment, leading to the accumulation of toxic substances in the natural environment. The spectrum of biocidal action, the effectiveness of the protection of the lignocellulosic material, as well as possibilities of binding active ingredients with wood chemical substances, are among the main considerations when developing new wood protection preparations. However, overlooking the biodegradation problem of active substances during exploitation and, subsequently, in the course of utilisation, may have a detrimental impact on the environment. That is why chemical compounds, widely considered environmentally-friendly, have attracted the interest of scientists involved in research on wood protection against microbiological degradation. Ionic liquids (ILs), also referred to as green solvents and obtained from quaternary ammonium salts, are employed with increasing frequency in industry due to their softening, anti-electrostatic as well as bacterio- and fungicidal properties. The multifunctionality (the multi-task nature) of these organic compounds is the result of their ionic chemical structure and, practically speaking, their unrestricted synthetic possibilities. Growing interest in the investigation of ionic liquids has resulted in the development of manufacturing technologies for these compounds, characterised by novel functional properties, including technologies for application in wood protection [Takahashi et al. 1993; Thang and Ruddick 2000; Kartal et al. 2005; Pernak et al. 2006]. The toxicity of new ionic liquids has been thoroughly described in the literature [Pretti et al. 2005; Pernak et al. 2006; Garcia-Lorenzo et al. 2008; Latała et al. 2009, 2010; Grabińska-Sota 2010; Petrovič et al. 2011; Zhang et al. 2011]. Ecotoxicity studies conducted by Grabińska-Sota [2011], as well as investigations on mutagenicity, revealed that ammonium ionic liquids were not genotoxic and mutagenic, which is an important consideration from the point of view of ecological safety as well as protection of human health. The predicted possibility of the bio-elimination of ionic liquids in an aqueous environment has only been confirmed in a small number of investigations [Harjani et al. 2009; Ford et al. 2010]. Earlier studies on the biodegradation of quaternary ammonium chlorides by bacteria demonstrated some influence of the cation structure, especially of the quantity of long-chain alkyl substituents, on this process [Van Ginkel and Kolvenbach 1991; Van Ginkel et al. 1992; Doyle and Ruddick 1993; Nishiyama et al. 1995; Nashihara et al. 2000; Patrauchan and Oriel 2003]. Likewise, in investigations conducted by Bürgel et al. [1996], Zheng and Ruddick [1995] as well as Zabielska-Matejuk and Czaczyk [2006], possibilities for the degradation of quaternary ammonium chlorides, acetates and propionates by some species of mould fungi were reported.
Biodegradation of new ionic liquid-based wood preservatives...

Biological investigations of the structures of newly developed ionic liquids with a cation of natural origin obtained from plant fats, especially from coconut oil, and containing a mixture of alkyl substituents of C₈, C₁₀, C₁₂ and C₁₄ length and a nitrate or nitrite anion, revealed the high effectiveness of their action against fungi of brown and white degradation, as well as against fungi causing blue stained wood [Zabielska-Matejuk and Pernak 2009]. The fungicidal values of these ionic liquids with respect to Coniophora puteana ranged from 2.7 to 4.3 kg/m³. In the case of bis-quaternary ammonium-based ionic liquids, N,N-dimethyloctylamine derivatives with a nitrate anion, the effectiveness of the protective action was higher and ranged from 1.8 to 2.9 kg/m³.

This study presents research results concerning the sensitivity of new ionic liquid structures in aqueous solutions to biodegradation on the basis of OECD recommendations in accordance with the 92/32EEC directive [1992], as well as the migration and degradation of these compounds in treated wood by mould fungi in the soil environment. Quantities of degradation products were determined using high performance liquid chromatography (HPLC). Investigations were carried out on the above-mentioned ionic liquids with a cation obtained from: coconut oil, with a didecyldimethyl ammonium cation, as well as with a “gemini” ammonium cation containing two alkyl substituents with a chain length of C₈. Sodium benzoate was used as a reference substance in the biodegradation experiments performed in an aqueous environment.

Materials and methods

Chemicals

Five ammonium ionic liquids with nitrite or nitrate anions and different cation structures, tj didecyldimethylammonium, cocotrimethylammonium and N,N’-[1,10-(2,9-dioxadecane)] bis(dimethyloctylammonium) cations were selected for the biodegradation study. The structure of the examined compounds are presented in scheme 1. All the chemicals were prepared at the Faculty of Chemical Technology of Poznań University of Technology, while all the reagents for synthesis were purchased from commercial sources, Sigma-Aldrich and Akzo-Nobel. The chemical purity of the ILs was determined by a direct two-phase titration procedure [PN-EN ISO 2871-2:2000].

The prepared ILs were characterized by their ¹H NMR and ¹³C NMR spectra. ¹H NMR spectra were recorded on a Varian Model XL 300 spectrometer at 300 MHz with tetramethysilane as the standard. ¹³C NMR spectra were recorded on the same instrument at 75 MHz. NMR spectra and elemental analyses were performed at A. Mickiewicz University, Poznań.
\[
\begin{align*}
\text{R}^1 & \quad \text{N}^+ \quad \text{R}^2 \quad \text{X}^- \\
\text{X} & = \text{NO}_3, \text{NO}_2, \text{R}^1 & = \text{alkyl C}_{10}, \text{coco(}C_8-C_{14}\text{)}, \text{R}^2 & = \text{alkyl C}_1, \text{C}_{10}
\end{align*}
\]

\[
\begin{align*}
\text{N}^+ & \quad \text{O} \quad \text{O} \quad \text{N}^+ \\
\text{X} & = \text{NO}_3, n=6
\end{align*}
\]

Table 1. The studied ionic liquids

<table>
<thead>
<tr>
<th>ILs</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[DDA][NO₃]</td>
<td>97</td>
</tr>
<tr>
<td>[DDA][NO₂]</td>
<td>99</td>
</tr>
<tr>
<td>[ArqC₃₅][NO₃]</td>
<td>97</td>
</tr>
<tr>
<td>[ArqC₃₅][NO₂]</td>
<td>75</td>
</tr>
<tr>
<td>[bis(AmC₈)][NO₃]₂*</td>
<td>94</td>
</tr>
</tbody>
</table>

*Applied in ACQ preservative system (the amount of bis-IL in the preparation – 10% m/m)

Didecyldimethylammonium nitrate [DDA][NO₃]: \(1^H\) NMR (DMSO-\(d_6\)) \(\delta\) ppm = 3.24 (m, 4H), 2.99 (s, 6H), 1.62 (m, 4H), 1.26(m, 28H), 0.86 (t, \(J = 7\) Hz, 6H); \(1^3C\) NMR \(\delta\) ppm = 62.7, 49.9, 31.2, 28.8, 28.7, 28.6, 25.7, 22.0, 21.6, 13.9. Elemental analysis: Found: C 68.12 H 12.71 N 7.03. Calc. for C\(_{22}\) H\(_{48}\) N\(_2\) O\(_3\) (388.6): C67.99, H 12.45, N 7.21%.

N,N’-[1,10-(2,9-dioxadecane)]bis[dimethylctyloammonium nitrate]: \(1^H\) NMR (CDCl\(_3\)) \(\delta\) ppm =5.14 (s, 2H), 3.94 (m, 2H), 3.59 (m, 4H), 3.29 (s, 12H), 1.84( m, 2H), 1.39 (m, 28H), 0.9 (t, \(J = 7\) Hz, 6H); \(1^3C\) NMR (CDCl\(_3\)) \(\delta\) ppm = 90.2, 73.2, 60.9, 47.1, 31.4, 29.0, 28.8, 28.7, 26.2, 24.7, 22.3, 22.1, 13.8.

The ionic liquids under investigation were thermally stable. The decomposition temperatures of the [NO₃]-based compounds ranged between 215 and 230°C, and for [DDA][NO₂] they were between 160 and 190°C. Thermal analysis was performed by simultaneous thermal gravimetric and differential thermal analysis (TG/DTA) measurement using a Setsys 12 Setaram thermobalance, under air atmosphere.
Microorganisms

Fungal strains

*Gliocladium roseum* (Bainier) species No, 62726, obtained from Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH in Braunschweig, were used in the biodegradation studies of the ionic liquids by mould fungi in a soil environment, as well as mixtures of the following mould: *Penicillium brevicompactum* (Dierckx), *Penicillium funiculosum* (Thom), *Phialophora fastigiata* (Lagerb. & Melin) *Verticillium lecani* (Zimm.) Viégas, and *G. roseum*. The species were obtained from the collection at the Institute of Wood Technology, Poznań, Poland.

Methods

Biodegradation in a water environment

The tests for the susceptibility of the ionic liquids to biodegradation were performed in two stages (ready biodegradability and inherent biodegradability). The ready biodegradability test was conducted according to OECD guideline 301 A. The ready biodegradability of the test compounds was monitored via DOC (dissolved organic carbon) for 28 days. Secondary effluent from a domestic sewage treatment plant in Zabrze, Poland was used as the inoculum. A measured volume of inoculated mineral medium, containing a concentration of the test substance 40 mg DOC/L as the nominal sole source of organic carbon, was aerated in diffuse light at 22±2°C. The mineral medium was composed of 8.5 mg L\(^{-1}\) KH\(_2\)PO\(_4\), 21.75 mg L\(^{-1}\) K\(_2\)HPO\(_4\), 33.4 mg L\(^{-1}\) Na\(_2\)HPO\(_4\)-2H\(_2\)O, 0.5 mg L\(^{-1}\) NH\(_4\)Cl, 27.5 mg L\(^{-1}\) CaCl\(_2\), 22.5 mg L\(^{-1}\) MgSO\(_4\)-7H\(_2\)O, 0.25 mg L\(^{-1}\) FeCl\(_3\)-6H\(_2\)O (pH 7.4). The degree of biodegradation was calculated by expressing the concentration of DOC removed (corrected by the concentration in the blank inoculums control) as a percentage of the concentration initially present. As a reference compound, sodium benzoate was used.

The inherent biodegradability tested whether the test compound complied with the condition of “ready biodegradability”, i.e. that it was not removed in 70% (DOC) in a 28 day time period. At this level the test was conducted according to OECD guideline 302 B (Zahn-Wellens method) for assessing inherent biodegradability. The principle of the test was to aerate the mixture containing the tested compound (50 mgDOC/L), culture medium and relatively high amounts of activated sludge in the water medium for 28 days at a temperature of 20-25°C.

The test for the blank probe containing activated sludge and culture medium without the tested compound, while testing of sodium benzoate as the reference substance was conducted simultaneously. The biodegradation process was monitored via DOC measurement in the filtered probes collected at suitable time intervals. The ratio of the vanishing to primary DOC value, corrected by the value of the blank probe for the collected samples, was stated as the biodegradation percentage.
**Soil-block test – biodegradation in a soil environment**

A sample of Scots pine sapwood *Pinus sylvestris* L., measuring 22 × 17 × 12 mm (L × T × R) and saturated under vacuum conditions with studied ionic liquids with concentrations of 0.63 and 2.5%, was used for the degradation study of ionic liquids by moulds in treated wood. Twenty-four samples were saturated for each concentration of ionic liquid. After three weeks of conditioning in a closed container, as well as seasoning and sterilization by overheated steam, the wood samples were exposed to mould fungi for 12 weeks in a soil-block test. Garden substrate containing biohumus with a pH of 5.5 and relative humidity of 62% was applied. The procedure of the soil-block test were described in the earlier work of Zabielska-Matejuk and Czaczyk [2006] The quantity of ILs in the treated wood was determined, while at the same time a direct two-phase titration procedure [PN-EN ISO2871-2:2000] was carried out using sodium lauryl sulphate as the titrant, and a HPLC procedure [AWPA Standard A 16-93: 1993] was conducted. The ionic liquids were extracted from refined wood (particle size φ < 0.5 mm) using an ultrasound bath for three hours at 35 kHz frequency.

**HPLC analysis**

Determination of the ionic liquids was carried out on an Agilent Technologies 1200 series system consisting of an autosampler (model G1329B), a pump (model G1312B) and a diode array detector (model G1315C) set at 262nm. The analysis were performed on a Waters SCX Ion-Exchange Column (100 × 8 mm). Acetonitrile and water (70:30 for [DDA][NO₃], [DDA][NO₂] and 75:25 for [ArqC₃5][NO₃]) acidified with 1.2% acetic acid and containing 0.8g/l benzyltrimethylammonium chloride were used as the eluent [Bürgel et al. 1996]. The flow rate was 1.5 ml/min, and the injection volume – 60 μl. Standards were used to identify peaks in the chromatograms, and a peak area was used to determine the sample concentrations. This was carried out by computer integration (ChemStation for LC 3D Systems, Agilent) operated in external standard mode. The method was based upon the indirect UV analysis of the mobile phase after passing through the column (added benzyltrimethylammonium chloride was used to give a positive background). The chromatographic peaks appeared negative and their integration was made after computer transformation into positive.

**Results and discussion**

**Biodegradation in a water environment**

The results of the first phase of ready biodegradation testing showed that there was 69.54% removal of the didecyldimethylammonium nitrite ([DDA][NO₂] 99%) within 28 days. The reduction percentage of the compound marked as DOC was close to the required value of the temporary level for significant ready
Biodegradability of approx. 70%. This level was achieved within a 10 day period from the first day of the test, hence the compound was classified as readily biodegradable. However, two other ionic liquids did not comply to this condition, since 44.16% of [ArqC₃₅][NO₃] 97% was removed on the 28th day of the ready biodegradability test, and only 8.62% of [ArqC₃₅][NO₂] 75% (fig.1). In this situation, according to OECD guideline 302 B (Zahl-Wellens method), the two liquids underwent further testing to confirm these results. The results of the second phase of the biodegradation tests are presented in figures 2 and 3. The reference substance was completely biodegraded in 14 days from the beginning of the test, however the biological decomposition of ArqC₃₅[NO₂] 75% in the test for inherent biodegradation amounted to 93% in 28 days. [ArqC₃₅][NO₃] 97% underwent biodegradation in a similar manner. In the test for actual biodegradability, 96% was removed within 28 days.

Based on the tests performed, it was demonstrated that 99% of the didecyldimethylammonium nitrite ([DDA][NO₂]) ionic liquid was readily biodegradable, however the [ArqC₃₅][NO₂] 75% and [ArqC₃₅][NO₃] 97% ionic liquids were found to be prone to inherent biodegradation.

Fig. 1. The course of decomposition of ionic liquids and the reference substance in biodegradability study according to OECD 301 A

The mean degree of [DDA][NO₂] biodegradation in the treated wood (17.93 kg/m³) by Gliocladium roseum amounted to only 4.27% and by a mixture of mould fungi to 7.24% (tables 2 and 3). In the case of the lower level of wood treatment (4.58 kg/m³), the degree of biodegradation of this ionic liquid was
higher and amounted to approximately 20.5%. The didecyldimethyl nitrite migration from the treated wood to the sterile soil was less than 1% due to the insolubility of this compound in water. The ionic liquid with a nitrate anion and a [DDA] cation exhibited a slightly greater biodegradability (from 10.1% to 11.4%) in the case of higher wood retention. On the other hand, a change in the cation structure of the ionic liquid to cocotrimethylammonium made it possible to increase the sensitivity of the ionic liquid [Arq C35][NO3] to the degradation
caused, in particular, by a mixture of the test mould fungi (tables 2 and 3). The mean degree of degradation ranged from 52.17% to 55.33%. This indicates that the cation with the alkyl substituent from coconut oil, which was a mixture of hydrocarbons of C$_8$ to C$_{14}$ carbon lengths, underwent biodegradation more easily in comparison with the didecyldimethylammonium cation in the [DDA][NO$_3$] and [DDA][NO$_2$] ionic liquids. Likewise, in the case of the bis-ammonium nitrate with two alkyl C$_8$ substituents, the recorded biodegradability reached, depending on the wood retention, levels ranging from 33.15% to 51.98%. The migration of the water soluble ionic liquids [bis(AmC$_8$)][NO$_3$]$_2$ and [ArqC$_{35}$][NO$_3$] from the treated wood into the soil fluctuated from 9.73% to 17.62%.

Table 2. Changes in retention of ionic liquids in treated Scots pine wood (*Pinus sylvestris* L.) as a result of migration into the soil and degradation by mould fungi (soil-block test)

<table>
<thead>
<tr>
<th>Ionic liquid</th>
<th>Retention in the wood</th>
<th>Average content of ionic liquid in treated wood</th>
<th>Average content of ionic liquid in treated wood after 12-week soil test</th>
<th>Sterile soil</th>
<th>Gliocladium roseum</th>
<th>Mixture of mould fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/m$^3$</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ArqC$_{35}$] [NO$_3$]</td>
<td>4.43</td>
<td>1.0442</td>
<td>0.9426</td>
<td>0.6598</td>
<td>0.4978</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.68</td>
<td>3.9942</td>
<td>3.3212</td>
<td>2.5538</td>
<td>1.9102</td>
<td></td>
</tr>
<tr>
<td>[DDA][NO$_3$]</td>
<td>4.49</td>
<td>1.0959</td>
<td>0.9930</td>
<td>0.9055</td>
<td>0.8900</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.52</td>
<td>4.1350</td>
<td>4.0058</td>
<td>3.7173</td>
<td>3.6619</td>
<td></td>
</tr>
<tr>
<td>[DDA][NO$_2$]</td>
<td>4.58</td>
<td>1.0981</td>
<td>1.0972</td>
<td>0.8755</td>
<td>0.8696</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.93</td>
<td>4.0134</td>
<td>3.9883</td>
<td>3.8411</td>
<td>3.7228</td>
<td></td>
</tr>
<tr>
<td>[bis(AmC$_8$)][NO$_3$]$_2$</td>
<td>4.52*</td>
<td>0.1326</td>
<td>0.1115</td>
<td>0.0904</td>
<td>0.0654</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.37*</td>
<td>0.4155</td>
<td>0.3423</td>
<td>0.2861</td>
<td>0.2460</td>
<td></td>
</tr>
</tbody>
</table>

*The retention of ACQ preparation (the content of the ionic liquid – 10% m/m)*

Table 3. Average degree of biodegradation by mould fungi of ionic liquids in treated Scots pine wood (*Pinus sylvestris* L.) after 12-week soil test (soil-block test)

<table>
<thead>
<tr>
<th>Ionic liquid</th>
<th>Concentration of ionic liquids in impregnation solution</th>
<th>Retention in the wood</th>
<th>Average content of ionic liquid in treated wood</th>
<th>Average degree of biodegradation by mould fungi of ionic liquid in treated wood after 12-week soil test</th>
<th>Sterile soil</th>
<th>Gliocladium roseum</th>
<th>Mixture of mould fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>kg/m$^3$</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ArqC$_{35}$] [NO$_3$]</td>
<td>0.63</td>
<td>4.43</td>
<td>1.0442</td>
<td>36.81</td>
<td>55.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>17.68</td>
<td>3.9942</td>
<td>36.06</td>
<td>52.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[DDA][NO$_3$]</td>
<td>0.63</td>
<td>4.49</td>
<td>1.0959</td>
<td>17.37</td>
<td>18.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>17.52</td>
<td>4.1350</td>
<td>10.10</td>
<td>11.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[DDA][NO$_2$]</td>
<td>0.63</td>
<td>4.58</td>
<td>1.0981</td>
<td>20.21</td>
<td>20.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>17.93</td>
<td>4.0134</td>
<td>4.27</td>
<td>7.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[bis(AmC$_8$)][NO$_3$]$_2$</td>
<td>0.63</td>
<td>4.52</td>
<td>0.1326</td>
<td>33.15</td>
<td>51.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>18.37</td>
<td>0.4155</td>
<td>33.15</td>
<td>48.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3. HPLC chromatograms of extracts from wood samples treated with cocotrimethylammonium nitrate: a – standard, b – after test in sterile soil, c – after incubation with Gliocladium roseum strain no 62 726
The concentration values of the ionic liquids in the extract obtained from the treated wood presented in table 4 and determined with the assistance of high performance liquid chromatography (HPLC), confirmed a decline in the quantities of the examined compounds after the 12 week incubation period in the soil infected by the tested microorganisms. Moreover, the research results obtained also indicated a slight migration of ionic liquids into the soil. The HPLC chromatogram obtained of the nitrate with a cocotrimethylammonium (standard) cation showed a characteristic peak for the chain with a mixture of hydrocarbons C<sub>10</sub>, C<sub>12</sub> and C<sub>14</sub> and with a retention time of 16.797 min (fig. 3). The analysis performed of the extracts obtained from the wood after incubation with the *Gliocladium roseum* fungus and with the mixture of mould fungi showed peaks of similar retention times on the chromatograms (15.63 min. and 15.51 min, respectively.) as in the case of the extract from the treated wood (the initial sample had a retention time of 16.18 min.) but this peak did not reflect the chain with a mixture of C<sub>10</sub>, C<sub>12</sub> and C<sub>14</sub> hydrocarbons but only the C<sub>12</sub> hydrocarbon. This may indicate the uneven degradation of this alkyl substituent by mould fungi.

Table 4. Changes in concentration of ionic liquids in extract from treated Scots pine wood after 12-week soil test, determined using HPLC method

<table>
<thead>
<tr>
<th>Ionic liquid</th>
<th>Retention in the wood</th>
<th>Average concentration of ionic liquid in extract from treated wood</th>
<th>Average concentration of ionic liquid in extract from treated wood after 12-week soil test</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ArqC&lt;sub&gt;35&lt;/sub&gt;][NO&lt;sub&gt;3&lt;/sub&gt;]</td>
<td>4.43</td>
<td>0.564</td>
<td>0.5050</td>
</tr>
<tr>
<td>[DDA][NO&lt;sub&gt;3&lt;/sub&gt;]</td>
<td>4.49</td>
<td>0.4495</td>
<td>0.3044</td>
</tr>
<tr>
<td>[DDA][NO&lt;sub&gt;2&lt;/sub&gt;]</td>
<td>4.58</td>
<td>0.458</td>
<td>0.3964</td>
</tr>
<tr>
<td>[bis(AmC&lt;sub&gt;8&lt;/sub&gt;)][NO&lt;sub&gt;3&lt;/sub&gt;]</td>
<td>4.52</td>
<td>0.1685</td>
<td>–</td>
</tr>
</tbody>
</table>

In all the cases examined, no degradation metabolites with distinctly longer retention times (shorter alkyl chains in relation to the standard) were observed on the chromatographs, indicating the absence of defragmentation by mould fungi of the alkyl substituents from the examined compounds.

**Conclusions**

The examined ionic liquids characterised by different structures of anions and cations exhibited varying sensitivity to biodegradation in aqueous and soil environments, depending on the microorganisms activating these compounds. In conditions of limited opportunity for the biodegradation to occur (screening test), didecyldimethyl ammonium nitrite exhibited 69.54% degradation, whereas in soil conditions, its biological degradation in treated pine wood (at a retention
of 4.58 kg/m$^3$) as a result of the action of mould fungi amounted only to approximately 20%. Nitrate and nitrite with a cocotrimethyl ammonium cation proved to be sensitive to true biodegradation, amounting to 96% and 93%, respectively. Biodegradation of the cocotrimethyl ammonium nitrate in the wood caused by mould fungi ranged from 36.1% to 55.33% and was greater in comparison with the ionic liquids containing didecyldimethyl ammonium cations. The chromatogram runs of the extracts from wood after exposure to mould fungi were similar to the chromatograms of the examined compounds, with the exception of the ionic liquid containing a cocotrimethyl ammonium cation, developed from coconut oil, the alkyl substituents of which were a mixture of C$_{10}$, C$_{12}$ and C$_{14}$ hydrocarbons. This may indicate a disappearance of the compounds with C$_{10}$ and C$_{14}$ substituents.

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**List of standards**


**OECD guideline for the testing of chemicals. DOC Die-Away. Test Guideline 301A**

**OECD guideline for the testing of chemicals. Inherent Biodegradability: Zahn-Wellens/EMPA. Test Guideline 302B**

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