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# Antifungal Activity of Paper Modified with Ionic Liquids

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## Abstract

Paper is a material that is easily biodegradable. The most active group of microorganisms responsible for biodegradation processes affecting paper are moulds. In order to prevent this unfavorable impact, various methods are used, including biocides, which added to paper during the production process to protect the final product. In this study, the following ionic liquids were applied: benzalkonium nitrate(V), benzalkonium DL-lactate, and didecyldimethylammonium DL-lactate. They were added (in a concentration of 3% and 5%) to paper samples made of the following pulp grades: pine bleached kraft pulp (pine BKP), birch bleached kraft pulp (birch BKP), recovered fibres and CTMP. The antifungal activity of paper was assessed using a qualitative and a quantitative method with strains *A. niger*, *A. versicolor*, *P. chrysogenum* and *P. aurantiogriseum*. The most resistant strain to the ionic liquid activity was *Aspergillus niger*. The best antimicrobial effect was shown by paper samples made of pine BKP with biocides. The weakest effect was obtained for the CTMP-based paper.

**Key words:** paper biodegradation, filamentous fungi, biocides, ionic liquids.

involved in the aerobic degradation of paper through producing outer cell cellulases. Moulds from the genus of *Aspergillus*, *Penicillium*, and *Trichoderma* are mainly responsible for the first stage of this process. They use components contained in paper as nutrient substances and prepare a base for fungi requiring higher humidity: *Chaetomium*, *Alternaria* or *Stachybotrys*. These fungi, in turn, can hydrolyse resistant cellulose fibres [2, 3]. However, it should be remembered that there are many paper grades of different composition and quality, manufactured for different purposes, from ordinary wrapping paper to the most expensive grades used for printing works of arts. The extent to which paper is susceptible to the effect of microorganisms depends, among other factors, on the type of fibrous material used, its chemical composition, biochemical reactivity as well as various excipients (adhesives, fillers, dyes, protective coatings) added to it throughout the technological process. Paper durability is therefore influenced by technological factors; however, an important role is also played by environmental factors such as temperature, humidity as well as the intensity and type of light [4 - 6].

Paper is characterised by very low resistance to the activity of microorganisms. For this reason, over the last few years, cellulose products have been more and more often modified in order to make them resistant to the destructive effect of microorganisms. Thus, already at the production stage, chemical compounds are added, which build into the structure

of the paper. Paper stored in different forms (books, photos, paintings) in libraries, archives and museums is also protected [4, 7, 8].

The new law (including the *Directive 98/8/EC of the European Parliament and the Council concerning the placing of biocidal products on the market and Regulation (EU) No. 528/2012 of the European Parliament and the Council concerning the making available on the market and use of biocidal products*) limits the application of substances considered toxic for human health and the environment, which are often contained in many widely used biocides [9, 10]. That is why efforts have been made to obtain compounds that would be safe for the environment. Ionic liquids seem to be such safe substances. They are characterised by the ability to dissolve organic and inorganic compounds, they moisten the surface of metals, polymers and minerals, and they can be both hydrophobic and hydrophilic. They are also good conductors. Chemically, electromechanically and thermally stable, they do not decompose in high temperatures. Due to their negligible volatility, they are considered as non-hazardous to the environment. They are more and more commonly used in various fields of science and the economy, for instance as compounds with antimicrobial activity [11 - 14]. In addition to this, they are used in such areas as histopathological diagnostics, paper protection [15, 16], wood conservation [17, 18], biotechnology [19, 20] and are efficient food deterrents [21, 22], herbicides [23 - 26] and herbicide-plant regu-

## ■ Introduction

Despite the growing popularity of electronic media, paper is still considered the most durable carrier of information used in everyday life. Manufactured from renewable plant resources, it is practically inexhaustible. It can be recycled, easily burned or biodegraded. On the one hand, the biodegradation of paper waste unsuitable for processing is a beneficial process, but on the other, its low resistance to the influence of microorganisms makes it prone to irreversible damage (when inadequately protected), and may cause the loss of information recorded on it [1]. The microorganisms that play the most significant role in paper biodegradation are filamentous fungi. They are

lators [27, 28]. In this study, ionic liquids were added to paper as biocides. The aim of the study was to examine the antifungal effect of three compounds contained in paper samples of four different grades.

## Materials and methods

### Materials

The ionic liquids used were prepared according to the methods previously described [29, 30].

The material tested at the Institute of Papermaking and Printing of the Lodz University of Technology was paper made of birch BKP, pine BKP, recovered fibres and CTMP, containing 3% and 5% of ionic liquids: benzalkonium nitrate, benzalkonium DL-lactate, and didecylodimethylammonium DL-lactate.

### Microorganisms

Microorganisms from the American Type Culture Collection: *Aspergillus niger* ATCC 16404, *Aspergillus versicolor* ATCC 9577, *Penicillium chrysogenum* ATCC 60739 and *Penicillium aurantiogriseum* ATCC 18382 were used for the purpose of the study tests.

### Tests of the antimicrobial effect of the paper with ionic liquids

The antifungal activity of the types of paper tested was assessed with a qualitative and a quantitative method. Qualitative analysis allowing for determination of the antimicrobial activity was carried out with the parallel streak method based on AATCC Test Method 147, which was described in an earlier article [31, 32]. A spore suspension ( $10^6$  conidia/ml) was placed on Petri dishes containing Malt Extract Agar (Merck). Then paper stripes soaked with the bioactive substances tested were placed there one by one, followed by the placing of samples without these substances (reference samples). The dishes were incubated at 28 °C for 24 - 72 h. When the incubation was completed, the inhibition zones of microorganism growth were measured in mm. The effectiveness was determined for each sample as presented in **Table 1**.

In the next stage of the study, the survival rate of mould conidia under the effect of ionic liquids was determined. For the purpose of the tests, pine-based paper and cellulose pulp were selected, and *Aspergillus niger* was applied as the biological factor. Next the amount of 100 µl of the

**Table 1.** Interpretation of results of the antimicrobial activity of papers examined.

| Growth inhibition zone, mm | Description of the growth of microorganisms compared to the control sample without an active agent | Result              |
|----------------------------|--|---------------------|
| >1                         | Growth inhibition zone bigger than 1 mm, no growth under the sample                                | Good effect         |
| 0 - 1                      | Growth inhibition zone up to 1 mm, no growth under the sample                                      |                     |
| 0                          | No growth inhibition zone, no growth under the sample  | Boundary of effect  |
| 0                          | No growth inhibition zone, almost no growth under the sample                                       |                     |
| 0                          | No growth inhibition zone, growth under the sample reduced by 50%                                  | Insufficient effect |
| 0                          | No growth inhibition zone, slightly reduced or normal growth under the sample                      |                     |

**Table 2.** Sizes of growth inhibition zones [mm] observed for filamentous fungi under the influence of ionic liquids contained in the paper samples examined.

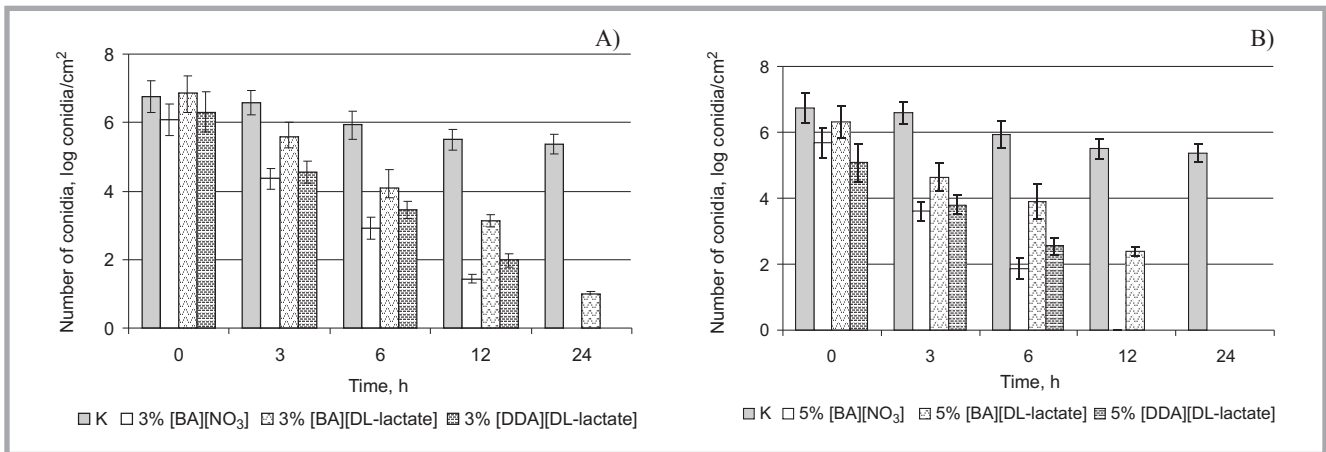
| Mold strain               | Kind of paper    | Kind of ionic liquid   |    |                   |    |                    |    |
|---------------------------|------------------|------------------------|----|-------------------|----|--------------------|----|
|                           |                  | [BA][NO <sub>3</sub> ] |    | [BA] [DL-lactate] |    | [DDA] [DL-lactate] |    |
|                           |                  | 3%                     | 5% | 3%                | 5% | 3%                 | 5% |
| <i>A. niger</i>           | Pine BKP         | 3                      | 4  | 1                 | 2  | 3                  | 5  |
|                           | Birch BKP        | 2                      | 3  | 1                 | 2  | 2                  | 4  |
|                           | Recovered fibres | 2                      | 3  | 1                 | 2  | 2                  | 3  |
|                           | CTMP             | 0                      | 2  | 0                 | 2  | 0                  | 2  |
| <i>A. versicolor</i>      | Pine BKP         | 10                     | 11 | 7                 | 10 | 4                  | 6  |
|                           | Birch BKP        | 7                      | 9  | 7                 | 9  | 5                  | 5  |
|                           | Recovered fibres | 7                      | 9  | 7                 | 8  | 4                  | 5  |
|                           | CTMP             | 7                      | 10 | 4                 | 7  | 3                  | 5  |
| <i>P. chrysogenum</i>     | Pine BKP         | 5                      | 6  | 4                 | 5  | 3                  | 4  |
|                           | Birch BKP        | 5                      | 6  | 4                 | 5  | 2                  | 3  |
|                           | Recovered fibres | 4                      | 5  | 4                 | 5  | 2                  | 3  |
|                           | CTMP             | 3                      | 5  | 3                 | 4  | 2                  | 3  |
| <i>P. aurantiogriseum</i> | Pine BKP         | 3                      | 5  | 3                 | 4  | 4                  | 4  |
|                           | Birch BKP        | 3                      | 4  | 3                 | 3  | 3                  | 3  |
|                           | Recovered fibres | 4                      | 4  | 1                 | 3  | 3                  | 3  |
|                           | CTMP             | 2                      | 3  | 0                 | 3  | 0                  | 2  |

conidia suspension ( $10^8$  conidia/ml) was placed on each paper sample and dishes with the samples were incubated for 24 h at 28 °C and relative humidity of 80%. Paper stripes without biocides were used as reference samples. After 0, 3, 6, 12 and 24 h, the paper samples were shaken in a saline solution with the addition of a neutraliser in order to leach microorganisms from the material tested. Then, following appropriate dilution, the suspension was placed on Petri dishes and poured over by Malt Extract Broth (Merck). The dishes were incubated for 72 h at 28 °C and relative humidity of 80%. Taking into account the dilutions, the conidia that survived on the surface of the stripes were calculated and the results given in cfu/10 cm<sup>2</sup> of the paper sample.

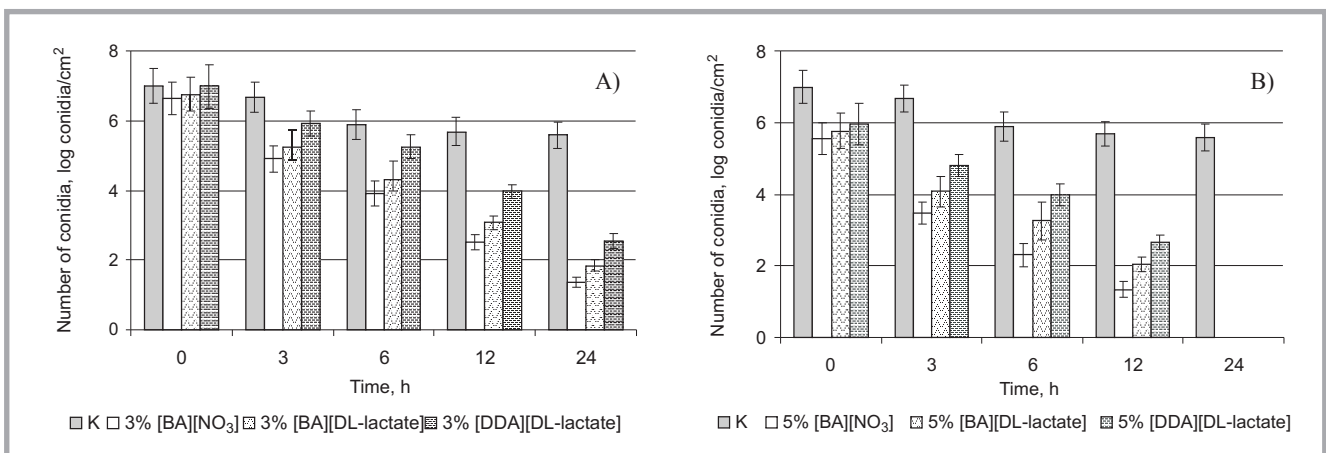
## Results

According to **Table 1** presenting the interpretation of the results, all test paper samples containing ionic liquids are characterised by good antifungal activity.

Only for *Aspergillus niger* and *Penicillium aurantiogriseum* in the sample with CTMP, no inhibition zone was registered. Moreover it was not recorded under the sample, which indicates lower antifungal activity of the compounds applied (**Table 2**). Taking mould species into account, the largest growth inhibition zones (3 - 11 mm) were observed for *Aspergillus versicolor*, followed by *Penicillium chrysogenum* (3 - 6 mm), *Penicillium aurantiogriseum*, and *Aspergillus niger* (0 - 5 mm each). A comparative analysis of the antimicrobial effect of the ionic liquids used shows that benzalkonium nitrate [BA][NO<sub>3</sub>] was the most efficient compound (growth inhibition zones: 0 - 11 mm), followed by benzalkonium DL-lactate [BA][DL-lactate] (0 - 10 mm) and didecylodimethylammonium DL-lactate [DDA][DL-lactate] (0 - 6 mm). Another factor whose significance cannot be ignored is the grade of the paper tested. The largest growth inhibition zones were registered for the paper made of pine BKP treated with ionic liquids (1 - 11 mm), and for that made of CTMP



**Figure 1.** Changes over time in the number of conidia on the surface of the test paper stripes (pine bleached kraft pulp) modified with ionic liquids; A – concentration of ionic liquids in the samples - 3%, B – concentration of ionic liquids in the samples - 5%.



**Figure 2.** Changes over time in the number of conidia on the surface of the test paper stripes with CTMP modified with ionic liquids; A – concentration of ionic liquids in the samples - 3%, B – concentration of ionic liquids in the samples - 5%.

their size equalled 0 - 7 mm (only in one case was it 10 mm). The ionic liquids contained in the paper samples made of birch BKP and recovered fibres had a similar effect with respect to the mould strains applied.

The most resistant strain of *Aspergillus niger* was selected for analysis of the survival rate of conidia. The paper (pine BKP) examined demonstrated a gradual decline in the number of spores after using all three types of ionic liquids (Figures 1.a, 1.b). In the case of the paper samples containing 3% of compounds, the most significant fungicidal effect was observed for benzalkonium nitrate. After 12 h, the number of conidia decreased by over 4 log, and after 24 h no active spores were observed, which was manifested by the lack of growth on the medium. A similar decreasing effect was noted under the influence of didecylodimethylammonium DL-lactate. In the case of benzalkonium DL-lactate, after 24 h only single conidia were still observed

on the samples; but compared to the initial count, their number had decreased by almost 6 log, proving the fungicidal effectiveness of the compound. Adding ionic liquids to the paper examined in the amount of 5% accelerated the loss of activity of the conidia. After 12 h, no conidia of *Aspergillus niger* were observed on the paper samples protected with benzalkonium nitrate and didecylodimethylammonium DL-lactate. As regards the paper samples, with the addition of benzalkonium DL-lactate, the number of spores was at that time at the level of 2.4 log. However, compared to the initial count, it had decreased by 4 log. After 24 h, no conidia were observed in this sample either. Less prominent fungicidal activity against the conidia of *Aspergillus niger* was observed for the paper made of CTMP with the addition of ionic liquids, compared to that made of pine BKP (Figures 2.a, 2.b). In samples containing 3% of biocidal compounds, benzalkonium nitrate and benzalkonium DL-lactate turned out to be the most efficient. After

24 h, active conidia were still observed on these papers in the amount of 1.4 and 1.8 log, respectively, which was a result lower by over 5 log compared to the initial count at t = 0. In the case of the samples with didecylodimethylammonium DL-lactate, the number of conidia decreased by 4.5 log compared to the initial number, but still, after 24 h, it was at the level of 2.5 log. For this reason, ionic liquids in the amount of 5% were added to the next samples. The compounds did not work as effectively as on the paper made of softwood BKP. Nevertheless, after 12 h the number of conidia had decreased by 4.2 log for benzalkonium nitrate, 3.7 log for benzalkonium DL-lactate and 3.3 log for didecylodimethylammonium DL-lactate, compared to their count at the beginning of the experiment. After 24 h, no conidia were observed on any of the paper samples, except the reference sample (without any biocidal compounds).

## Conclusions

The microbiological tests confirmed very good fungistatic and fungicidal activity of the ionic liquids tested. Some of them are consistent with results of the analysis previously obtained [32]. Taking three variables into account: the type of ionic liquid, the paper grade and the type of filamentous fungi, the largest growth inhibition zones (10 - 11 mm) and, in turn, the most effective activity were observed for benzalkonium nitrate, the paper made of pine BKP and *Aspergillus versicolor*.

The quantitative analyses showed that the ionic liquids tested, added to the paper made of pine BKP (concentration of 3% and 5%) totally inhibited conidia growth after 24 h (apart from benzalkonium DL-lactate, with a concentration of 3%). In the case of the paper made of CTMP, all three compounds with a concentration of 3% effectively reduced conidia growth; however, they did not eliminate their presence entirely. Conidia with the number of 2 log (10<sup>2</sup> cfu/sample) in the paper with increased humidity may develop and grow into mycelium, thereby causing material destruction. The addition of benzalkonium nitrate, benzalkonium DL-lactate and didecylodimethylammonium DL-lactate at a concentration of 5% effectively protects the paper against conidia growth on its surface. The tests presented in this paper demonstrate new possible applications of ionic liquids as substances protecting paper products against the growth of filamentous fungi.

## References

1. Wandelt P. The evolution of graphic paper over the centuries and their durability (in Polish). *Paper Industry Review* 2010; 9: 21-44.
2. Zyska B. Fungi isolated from library materials: A review of the literature. *International Biodeterioration and Biodegradation* 1997; 40(1): 43-51.
3. Stobińska H, Zyska B. Paper - manufacturing, paper products, materials in library collections. In: Zyska B, Żakowska Z. (Ed.) *Microbiology of materials* (in Polish). Ed. Publisher Lodz University of Technology, 2005: 137-180.
4. Fabbri AA, Ricelli A, Brasini S, Fanelli C. Effect of different antifungals on the control of paper biodeterioration caused by fungi. *International Biodeterioration and Biodegradation* 1997; 39: 61-65.
5. Pinzari F, Pasquariello G, De Mico A. Biodeterioration of paper: A SEM study of fungal spoilage reproduced under controlled conditions. In: *Macromolecular Symposia* 2006; 238: 57-66.
6. Araujo Reis-Menezes A, Gambale W, Giudice MC, Shirakawa MA. Accelerated testing of mold growth on traditional and recycled book paper. *International Biodeterioration and Biodegradation* 2011; 65: 423-428.
7. Neves ER, Schäfer S, Philips A, Canejo J, Macedo MF. Antifungal effect of different methyl and propyl paraben mixtures on the treatment of paper biodeterioration. *International Biodeterioration and Biodegradation* 2009; 63: 267-272.
8. Sequeira S, Cabrita EJ, Macedo MF. Antifungals on paper conservation: An overview. *International Biodeterioration and Biodegradation* 2012; 74: 67-86.
9. Directive 98/8/EC of the European Parliament and of the Council of 16 February, Concerning the Placing of Biocidal Products on the Market, 1998.
10. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May, 2012. Concerning the Making Available on the Market and Used of Biocidal Products.
11. Demberelnyamba D, Kim K, Choi S, Park S, Lee H, Kim C, Yoo I. Synthesis and antimicrobial properties of imidazolium and pyrrolidinium salts. *Bioorganic & Medicinal Chemistry* 2004; 12: 853-857.
12. Carson L, Chau PKW, Earle MJ, Gilea MA, Gilmore BF, Gorman SP, McCann MT, Seddon KR. Antibiofilm activities of 1-alkyl-3-methylimidazolium chloride ionic liquids. *Green Chemistry* 2009; 11: 492-497.
13. Busetti A, Crawford DE, Earle MJ, Gilea MA, Gilmore BF, Gorman SP, Lavery G, Lowry AF, McLaughlin M, Seddon KR. Antimicrobial and antibiofilm activities of 1-alkyl-3-methylimidazolium bromide ionic liquids. *Green Chemistry* 2010; 12: 420-425.
14. Gilmore BF. Antimicrobial Ionic Liquides. In: Kokorin A. Ed. *Ionic Liquides: Application and Perspectives*. *InTech* 2011: 587-604.
15. Pernak A, Iwanik K, Majewski P, Grzymisławski M, Pernak J. Ionic liquids as an alternative to formalin in histopathological diagnosis. *Acta Histochemica* 2005; 107: 149-156.
16. Przybysz K, Drzewińska E, Stanisławska A, Wysocka-Robak A, Cieniecka-Rosłonkiewicz A, Foksowicz-Flaczyk J, Pernak J. Ionic liquids and paper. *Industrial & Engineering Chemistry Research* 2005; 44: 4599-4604.
17. Zabielska-Matejuk J, Urbanik E, Pernak J. New bis-quaternary ammonium and bis-imidazolium chloride wood preservatives. *Holzforschung* 2004; 58: 292-299.
18. Pernak J, Goc I, Fojutowski A. Protic ionic liquids with organic anion as wood preservative. *Holzforschung* 2005; 59: 473-475.
19. Van Rantwijk F, Lau R, Sheldon RA. Biocatalytic transformations in ionic liquids. *Trends Biotechnology* 2003; 21: 131-138.
20. Erbeltinger M, Mesiano AJ, Russel AJ. Enzymatic catalysis of formation of Z-aspartame in ionic liquid - an alternative to enzymatic catalysis in organic solvents. *Biotechnology Progress* 2000; 16: 1129-1131.
21. Markiewicz B, Sznajdrowska A, Chrzanowski Ł, Ławniczak Ł, Zgoła-Grześkowiak A, Kubiak K, Nawrot J, Pernak J. Ionic liquids with a theophyllinate anion. *New Journal of Chemistry* 2014; 38: 3146-3153.
22. Pernak J, Nawrot J, Kot M, Markiewicz B, Niemczak M. Ionic liquids based stored product insect antifeedants. *RSC Advances* 2013; 3: 25019-25029.
23. Kordala-Markiewicz R, Rodak H, Markiewicz B, Walkiewicz F, Sznajdrowska A, Materna K, Praczyk T, Pernak J. Phenoxy herbicidal ammonium ionic liquids. *Tetrahedron* 2014; 70: 4784-4789.
24. Cojocar O, Shamshina JL, Gurau G, Syguda A, Praczyk T, Pernak J, Rogers RD. Ionic liquids forms of the herbicide dicamba with increased efficacy and reduced volatility. *Green Chemistry* 2013; 15: 2110-2120.
25. Praczyk T, Kardasz P, Jakubiak E, Syguda A, Materna K, Pernak J. Herbicidal ionic liquids with 2,4-D. *Weed Science* 2012; 60: 189-192.
26. Pernak J, Syguda A, Janiszewska D, Materna K, Praczyk T. Ionic liquids with herbicidal anions. *Tetrahedron* 2011; 67: 4838-4844.
27. Pernak J, Niemczak M, Materna K, Marcinkowska K, Praczyk T. Ionic liquids as herbicides and plant growth regulators. *Tetrahedron* 2013; 69: 4665-4669.
28. Pernak J, Niemczak M, Zakrocka K, Praczyk T. Herbicidal ionic liquid with dual-function. *Tetrahedron* 2013; 69: 8132-8136.
29. Pernak J, Smiglak M, Griffin ST, Hough WL, Wilson TB, Pernak A, Zabielska-Matejuk J, Rogers RD. Long alkyl chain quaternary ammonium-based ionic liquids and potential applications. *Green Chemistry* 2006; 8: 798-806.
30. Cybulski J, Pernak J, Wiśniewska A, Kulig-Adamiak A, Lewicka L, Cieniecka-Rosłonkiewicz A, Kita K, Materna K. Long-alkyl-chain quaternary ammonium lactate based ionic liquids. *Chemistry - A European Journal* 2008; 14: 9305-9311.
31. AATCC Test Method 147-2011. Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method.
32. Koziróg A, Wysocka - Robak A, Przybysz K, Żakowska Z, Jeziórska R. Technical materials modified biocides and their resistance to the growth of filamentous fungi (in Polish). *Corrosion Protection* 2012; 9s/A: 331-335.

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