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Jadwiga ZABIELSKA-MATEJUK, Juliusz PERNAK

MYCOLOGICAL STUDY OF AMMONIUM IONIC LIQUIDS

The paper presents results of investigations of the effectiveness action of innovative structures of ionic liquids – quaternary ammonium derivatives – against three species of fungi decaying softwood and a species causing blue stain of softwood[1]. *The experiments were carried out using the culture medium method and inhibition of mycelium colony growth on agar-maltose substrate made toxic with ionic liquids was determined.*

Keywords: ionic liquids, fungi, biocidal activity

Introduction

Enhancement of durability of wood and wood-based materials in the aspect of environmental protection requirements and counteracting the environment degradation requires verification of technology of protection with agents harmful to living organisms. The requirement of obligatory tests of biocidal products, including wood preservatives, in terms of their emission to the environment contained in Directive 98/8/EC eliminated from the market compounds of chromium, fluorine, arsenic, pentachlorophenol, tin organic compounds (TBTO) and other biologically active substances of similar toxicity that accumulate in the environment. Together with dynamic development of building industry there was an increase in demand for impregnated building wood, especially impregnated in pressure preserving works, as well as for particleboards and plywood of

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Jadwiga ZABIELSKA-MATEJUK, Wood Technology Institute, Poznań, Poland
Juliusz PERNAK, Poznan University of Technology, Poland

higher resistance to microorganism action. It is anticipated that in the nearest future in Poland the demand for impregnated wood will be approximately 1.2-1.5 M m^3/year, which implicates an increase in demand for new, safe preservatives. The ecology aspect of management of impregnated wood and its post-consumer waste is one of the ideas of sustainable development, which is the overriding goal of economic and social activity. An innovative look at the issue of safe preservation of lignocellulosic materials and the importance of subject matter connected with development of new biologically active substances drew the researchers' attention to novel organic compounds, i.e. ionic liquids which are often called "green" solvents. Multifunctionality of ionic liquids, compounds made of organic cation and inorganic or organic anion, stems from huge number of possible combinations of the cation-anion structure. The state of the art knowledge in the field of organic technology makes it possible to design structures and synthesis of ionic liquids of strong biocidal activity, as well as structures of non-volatile ionic liquids of affinity for cellulose.

The innovative concept of application of ionic liquids to cellulose dissolution (with the use of enzymes) may contribute to development of new biotechnological processes for obtaining cellulose from renewable wood raw materials, and in the next stage, obtaining many chemical substances (that are important from pharmacologic, antiseptic, and cosmetic point of view) as well as additions to biofuels. The search after structures of ionic liquids which intensively dissolve natural and the most popular on Earth polymer, i.e. cellulose, as well as selection of enzymes facilitating this process constitute a research issue faced by the researchers of the 21st century. The latest research indicates that 1-butyl-3-methylimidazolium chloride and 1-allil-3-methylimidazolium chloride [Remsing et al. 2006; Moulthrop et al. 2005; Stolte et al. 2008] are good cellulose solvents, and the process may be accelerated by heating with microwaves. The research of Binder et al. [2009] and Pu et al. [2007] describes a possibility of depolymerisation of model lignin compounds in ionic liquids, which in the future may contribute to obtainment of a series of aromatic chemical structures from biomass rich in lignocellulosic complex. Apart from numerous applications of task specific ionic liquids (TSILs) in catalysis, organic synthesis, extraction processes [Lee 2005], nanomaterial technology (nano-sized materials; metal nanoparticles, silica nanostructure modification), and electrochemistry (as solutions of electrolytes in lithium batteries), the third generation ionic liquids of therapeutic properties were developed, e.g. lidocaine [Hough et al. 2007]. The multifaceted action of ionic liquids, when at the same time they are susceptible to bio-elimination in the environment, are the features that make the application of these novel organic compounds at the industrial scale preferred in the conditions of sharpened environmental protection requirements.

A result of cooperation between the Wood Technology Institute in Poznan and the Institute of Chemical Technology and Engineering of the Poznan

University of Technology was development of ionic liquids, quaternary ammonium derivatives, characterised by high fungitoxicity against wood-decaying fungi, microbes, and pathogenic fungi, as well as demonstrating good tissue preserving properties [Pernak et al. 2006]. Those substances demonstrated strong penetration properties in contact with wood and their hydrophobicity ensured limitation (or lack) of emission from impregnated wood to the environment. Those positive effects of research on ionic liquids in the aspect of lignocellulosic material protection persuaded the authors to take up research on design and development of novel structures with function natural anion and cation obtained from vegetable or animal fats or other market products as well. The Wood Technology Institute in Poznan took out financial support for carrying out of that research in the form of a three-year development project entitled "Ionic liquids in innovative technologies connected with processing of lignocellulosic raw materials" executed within the framework of Operational Programme Innovative Economy. The executor of the project, in cooperation with the Institute of Chemical Technology and Engineering of the Poznan University of Technology and the Institute of Papermaking and Printing of the Technical University of Lodz, is working to achieve two partial goals, i.e. enhancement of durability of wood, particleboards and plywood using novel and biologically active ionic liquids, and development of structural modification of ionic liquids of affinity for cellulose with the view of using them in the processes of cellulose technology.

In this publication the first effects of investigations of the fungicidal activity of designed, novel structures of ionic liquids obtained as a result of developed methods for syntheses and product isolation, purification, and identification. In the first phase of the project syntheses of thirty ionic liquids of biologically active properties were developed and carried out. The structures of the ionic liquids were confirmed by analysis of proton and carbon spectra of magnetic nuclear resonance (Varian 300 VT type spectroscope) or by the method of CHN elementary analysis. As a result of determination of the syntheses parameters, ionic liquids with nitrate(V) anion, with environmentally friendly vegetable and animal cation, and with modified function anion were obtained from market products. Other obtained compounds were the so-called ciechowskie ionic liquids and ionic liquids from market products by PCC Rokita S.A. Company. The applied methods of isolation and purification of products of the reactions of quarternation and ionic exchange made it possible to obtain ionic liquids of active substance content > 95%.

Mycological tests

The identification of fungistatic and fungitoxic properties of designed structures of ionic liquids was carried out using the culture medium method and brown rot fungi *Coniophora puteana* (Schum: Fr.) Karst. strain BAM 15, white rot fungi *Trametes versicolor* (L.: Fr.) Pilát- strain CTB 863 A, soft rot fungi *Chaetomium globosum* Kunze strain ATCC 6275, and *Sclerophoma pithyophila* (Corda) van Höhn fungi, strain S 231, causing blue stain. The examined compounds were dissolved in the culture medium so as to obtain the following concentrations: 10, 25, 50, 100, 250, 500, 750, 1000, 2500, and 5000 ppm. The colony diameter was measured and standard deviation from the average from six measurements was calculated. The inhibitions of the growth of test fungus colonies on culture media containing ionic liquids were determined. In addition, toxic values ED_{50} and ED_{100}, i.e. concentrations effectively inhibiting mycelium growth in 50 and 100% in relation to fungus on the culture medium without fungicide, as well as LD value, i.e. minimum concentration causing mycelium death, were determined. All experiments were performed in three replications.

Toxic values of developed ionic liquids were compared with the results obtained for commercial didecyldimethylammonium chloride.

Test results

Fig. 1 presents the growth of a colony of *Coniophora puteana* mycelium on culture media made toxic using five ionic liquids obtained from market products by Ciech S.A. Company ([Ciech][1], [Ciech][2], and [Ciech][3]) and PCC Rokita S.A. Company ([Rok][1] and [Rok][2]). That growth confirms very effective fungistatic action of all the above-mentioned ionic liquids. The inhibition of the growth of *C. puteana* colony when the concentration in the culture medium was 50 ppm (compared to the colony growth on non-toxic culture medium which was 87 mm on average) was the greatest in the case of compounds with the symbols [Ciech][1] and [Ciech][3]. The ionic liquid obtained from products by PCC Rokita Company with the symbol [Rok][1] containing in its structure strongly bioactive ammonium cation demonstrated strong fungistatic properties as well. The most effective fungicidal action was demonstrated by a compound with the symbol [Ciech][3]. That action was confirmed by threshold values ED_{100} and LD that were 1000 ppm for *C. puteana*. Those values are comparable to commercial didecyldimethylammonium chloride (ED_{100}, LD = 1000 ppm) and five times lower than the values for benzalkonium chloride. The ionic liquids with the symbol [Ciech][2] demonstrated the weakest fungicidal action against the test brown rot fungus. The threshold values ED_{100} and LD were more than 5000 ppm.

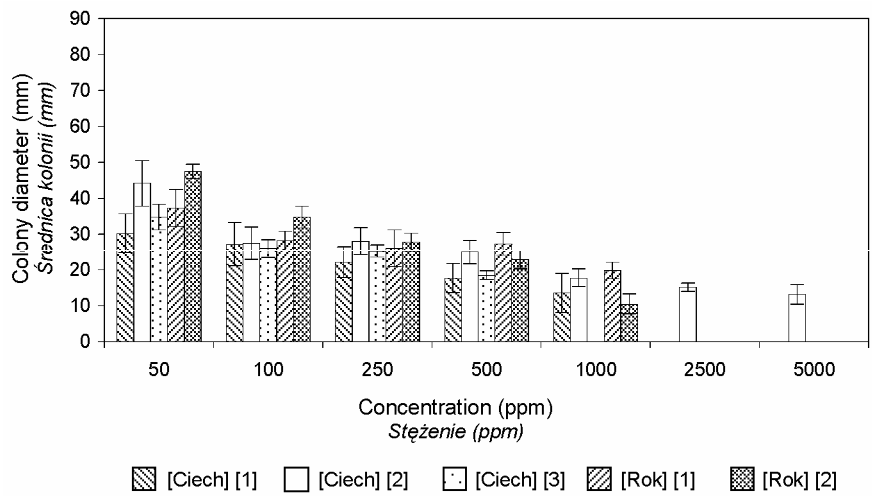

Fig. 1. The growth of *Coniophora puteana* fungus colony on culture medium containing ionic liquids after 11 days of incubation

Ryc. 1. Wzrost kolonii grzyba Coniophora puteana na pożywce zawierającej ciecze jonowe po 11 dniach inkubacji

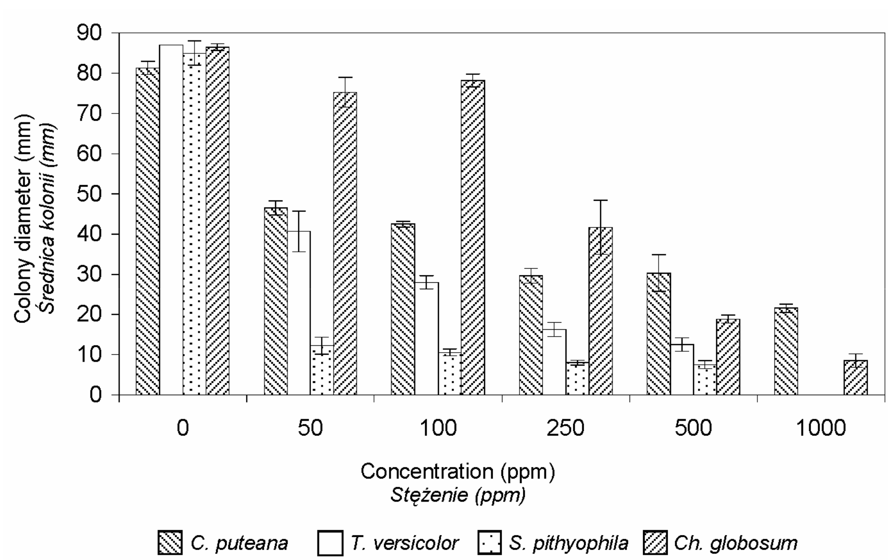

Fig. 2. The growth of test fungus colony on culture medium containing ionic liquid – a coconut oil derivative after 11 days of incubation (*T. versicolor*, *Ch. globosum* after 7 days of incubation)

Ryc. 2. Wzrost kolonii grzyba testowego na pożywce zawierającej ciecz jonową – pochodną oleju kokosowego – po 11 dniach inkubacji (T. versicolor, Ch. globosum po 7 dniach inkubacji)

Fig. 2 shows the results of biocidal activity tests of nitrate(V) containing an environmentally friendly natural cation obtained from coconut oil (symbol [Eth C/12][NO_3]) against four species of test fungi. The developed ionic liquid demonstrated the highest activity against *S. pithyophila* fungus causing blue stain of wood: ED_{50} was 25 ppm, ED_{100} and LD was 1000 ppm. The soft rot fungus *Ch. globosum* was the most resistant to the action of that compound: ED_{50} was 250 ppm, ED_{100} – 2500 ppm, LD > 5000 ppm. The activity of that compound against white and brown rot fungi was higher than the activity of commercial didecyldimethylammonium chloride.

Conclusions

The developed structural modifications of ionic liquids, obtained from market products by Ciech S.A. Company and PCC Rokita S.A. Company and natural vegetable products, are characterised by strong fungistatic and fungitoxic properties against wood-decaying species. The threshold toxic values of the synthesised ionic liquids are comparable to, and in some cases lower than, the values of commercial didecyldimethylammonium chloride.

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BADANIA MIKOLOGICZNE AMONIOWYCH CIECZY JONOWYCH

Streszczenie

Opracowano nowe struktury amoniowych cieczy jonowych o właściwościach bioczynnych z anionem azotanowym (V), z kationem pochodzenia naturalnego, uzyskanym z tłuszczów roślinnych i zwierzęcych, jak również z innych produktów rynkowych i funkcyjnym anionem. Określono ich aktywność biobójczą w stosunku do grzybów niszczących drewno: *Coniophora puteana*, *Trametes vesicolor, Chaetomium globosum i Sclerophoma pithiophila.* Nowe modyfikacje cieczy jonowych są silnymi grzybostatykami, a ich progowe wartości toksyczne są porównywalne z komercyjnym chlorkiem didecylodimetyloamoniowych.

Słowa kluczowe: ciecze jonowe, grzyby, aktywność biobójcza