ECOLOGICAL BIOCIDE SYSTEMS BASED ON UNMODIFIED AND EPOXYDATION LIGNINS, FURAN RESIN AND COPPER

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The paper presents the results obtained in the characterization of some products based on lignin, its epoxy derivative and furan resins as potential biocides. These compounds were studied comparatively with inorganic copper ions, using samples of fir and beech wood as a substrate. With this end in view, the samples of fir and beech wood were impregnated with the solutions of the above-mentioned compounds with a 5% concentration. The efficiency of the treatment has been established *versus* the inhibition of the development of some fungi (*Alternaria geophola*, *Chaetonium funicola*, *Chaetonium olivaceum*, *Fusidium viride*, *Humicola grisea*, *Stachybotrys alternans*, *Penicillium brevi-compactum*, *Penicillium funiculosum*). The results of these tests evidenced that lignin, its derivative and the furan resins, although having a lower inhibition capacity for fungi development – compared with copper compounds – could be used to develop environmentally friendly biocides to be applied in wood preservation.

Keywords: lignin, epoxy lignin, furan resin, copper ions, biocide systems, fir and beech wood, fungi

INTRODUCTION

The lignocellulose materials are mainly made of cellulose, lignin and hemicelluloses – natural polymers that present more reactive groups capable of taking part in reactions of modification. Research studies point out the fact that lignocellulose can be modified by means of microorganisms and the enzymatic systems involved in biodegradation.[1-3]

Wood is a biological composite material and thus it is inevitably exposed to deterioration, in some cases its biodegradability being a shortcoming, various possibilities of biostability are currently being studied.

The components of wood (polysaccharides and lignin) can be characterized by different biological stability. Thus, among these, lignin is the material that is resistant to the action of microorganisms, whereas polysaccharides are characterized by reduced stability.[3,4.]

Nowadays, in order to protect wood, people use products that are toxic to microorganisms and insects, and are incompatible with the environment. Besides, some of the extensively used products have copper ions in their composition, which, as reported, penetrate the less ordered areas of cellulose and hemicelluloses.

When copper ions are included in the composition of some ammonia systems, the ammonia is considered to evaporate totally or partially, resulting in the precipitation of the insoluble copper hydroxide. Also, part of the copper ions can be bound to the woody substrate by means of hydroxyl or carboxyl groups from the wood composition, which are in ionized state under high pH conditions.[5-8] In such a situation, the binding of the copper ammonia complexes to the individual components of the wood is manifested in the following order: lignin, hemicelluloses and cellulose, and the pH growth intensifies the interaction with lignin.[4,8]

If considering the different biological stability of the wood components and the existing information on the action of enzymatic systems, there have been suggested and developed a series of chemical treatments, to act efficiently against the attack of microorganisms (bacteria and fungi) and insects. Considering the toxicity of classic biocide agents, today there is a trend of seeking resistance to the attack of microorganisms by systems based on the properties of some natural products, such as for example lignins and aromatic products.[9-11]

In previous papers,[3-5,10-12] the results obtained in the preservation of lignocellulose materials (paper filter) using lignin or its derivatives, furan resins and compounds based on copper salts have been presented. Based on the favorable results obtained, experiments with the mentioned compounds have been carried out, with samples of fir and beech wood as a substrate. The substrate impregnated with compounds with biocide potential was tested as to its biostability against different fungi. The results here presented evidence the possibility of obtaining environmentally friendly biocides.

EXPERIMENTAL

Materials

The following materials have been used in the study:

- 7.5x3.0x1.5 cm fir test pieces, with equilibrium relative humidity, U1 = 13.63%, and 8.5x4.3x1.5 cm beech test pieces with equilibrium relative humidity, U1 = 14.74%;
- unmodified straw lignin (L1) from Granit Recherche Développement S.A. Company (Table 1), and straw lignin modified (L1E) by epoxydation[13-15] (Table 2);
- Biorez furan resin (R2) from Trans Furans Chemicals Company (Table 3);
- copper chloride ($CuCl_2$);
- tetraminocopper hydroxide ($[Cu(NH_3\ OH)_2)_4]$);
- 0.1N ammonia solution (NH_4OH 0.1 N).

Methods

The epoxydation reaction

Epoxydation of lignin was performed as follows: 10 g of lignin (L1) were dissolved at room temperature in a solution of 20% NaOH in L1:NaOH (w/w) ratios of 1:3 (sample 1) and 1:6 (sample 2) by mechanical stirring, and the solution was maintained for one hour at 40 °C. Then epichlorhydrin (EPC) was added using an L1: EPC ratio of 1:10 (w/w) and the stirring process was continued for 3 hours at 50 °C, respectively 70 °C, for sample 1 and sample 2, in order to establish the optimal reaction conditions.

Finally, a 20% solution of NaH_2PO_4 was added until pH 7 was reached to precipitate the epoxydated lignin. The resulted two phases (solid phase noted – ph. sol. and liquid phase noted – ph. liq.) were separated by centrifugation at 2500 rpm for 10 min. The reaction products obtained after centrifugation were washed twice with distilled water, dried and characterized. The influence of different factors, such as mass ratio of L1:NaOH (1:6 and 1:3 (w/w)) and temperature was observed, while the reaction time was kept constant for 3 hours.

Optimal reaction conditions were established as a function of epoxydation number, at an L1:NaOH ratio of 1:3, temperature of 70 °C and reaction time of 3 hours. Thus, for sample 1 a number of epoxydation of 1.76 was obtained, and for sample 2 – 1.18. Since epoxydation number had a higher value for sample 1, it was selected for testing.

Table 1
Characteristics of unmodified lignin (L1)

Characteristics	(L1)
Relative humidity, %	5.00
Ash, %	2.30
pH in suspension	2.70
Carboxyl groups	3.80
Mn, %	0.7
N, %	1.00
Solubility in acids, %	1.00

Table 2
Characteristics of modified (L1E) straw lignin[17]

Samples	T, °C	L:NaOH (w/w)	t, h	CE, %		η, %	U, %	Ash, %
				ph. sol.	ph. liq.			
L1E	70	1:6	3	1.18	0.19	85	6.55	4.18
	50	1:6	3	1.45	0.39	78	5.56	4.62
	70	1:3	3	1.76	0.60	77	3.95	1.31
	50	1:3	3	1.84	0.54	98	9.51	8.79

CE – Number of epoxydation, U – humidity, ph. sol. – solid phase, ph. liq. – liquid phase, η – yield

Table 3
Characteristics of furan resin (R_2)

Characteristics	(R_2)
Relative humidity, %	36
Viscosity (25 C), CP	<100
pH in suspension	6.9
Formaldehyde released, wt%	<0.1
Furfuryl alcohol, wt%	0.6
Solid product, wt%	47.8

Treatment samples and development of microorganisms

The fir and beech test pieces were impregnated through immersion for 1 min in unmodified/modified (epoxydation) straw lignin solutions, furan resin, copper chloride and tetraminocopper hydroxide with a concentration of 5%. After the treatment, the samples were dried for 24 h under ambient environmental conditions.[9-11] The conditions for testing the biocide activity were established by the Romanian standard 80022/91 (equivalent to ISO 4833/93 or T 487 pm-85). The microorganisms, kept and grown on potato dextrose agar, were obtained from the collection of "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi.

The samples were placed on Petri dishes, using an agar cultivation medium, containing 40 g $NaNO_3$ (sodium nitrate), 20 g K_2HPO_4 (potassium acid phosphate), 10 g $MgSO_4$ (magnesium sulphate), 10 g KCl (potassium chloride), 0.2 g Fe_2SO_4 (iron sulphate), 30 g sucrose, 20 g agar, and were finally dissolved in 1000 mL distilled water under sterile conditions. The samples were inoculated with the above-mentioned microorganisms. All cultures were incubated in a dark cultivation chamber for 28 days, at a temperature of 25 ± 2 °C and relative air humidity of 80 ± 2%.

RESULTS AND DISCUSSION

As a result of the sample analysis, the development of the following micromycetes has been noticed: *Fusidium viride*, *Humicola grisea*, *Stachybotrys alternans*, *Alternaria geophala*, *Penicillium brevy-compactum*, *Chaetomium olivaceum*, *Penicillium funciculosus* and *Chaetomium funicola*.

Fungal colony growth scale was visually estimated, as a function of the extent of microorganism inhibition or development: **0** – almost no growth; **1** – poor growth – small and flat colonies; **2** – moderate growth – larger and thicker colonies; **3** – good growth – bigger and thicker colonies; elevated folds on the colony surface (Table 4).

Table 4
Microorganism development on fir and beech wood treated with different compounds with potential biocide action

Variant	Fungi	Macroscopic observations on mycelium development	Fungi development	Protection efficiency
Untreated fir and beech wood (control sample)	*Fusidium viride*, *Humicola grisea*, *Stachybotrys alternans*, *Alternaria geophola*, *Penicillium brevi-compactum*, *Chaetomium olivaceum*, *Penicillium funiculosum*, *Chaetomium funicola*	The surface of the untreated test pieces is 90% covered	3	No inhibition of fungi development
Treatment with 5% L_1	*Fusidium viride*, *Humicola grisea*, *Stachybotrys alternans*	The surface of the test pieces is 60% covered. The emergence of the mycelium can be noticed on the edge and at the ends of samples	2	Moderate
Treatment with 5% L_1 E	*Fusidium viride*, *Humicola grisea*, *Stachybotrys alternans*	Moderate development of the mycelium, the level or the musty surface being of maximum 30%	1-2	Moderate

Treatment with 5% R_2	*Chaetomium olivaceum*, *Humicola grisea*, *Penicillium funiculosum*, *Stachybotrys alternans*,	A slight development of fine mycelium and isolated spores covering about 20%	1-2	Good
Treatment with 5% $CuCl_2$	*Alternaria geophola*, *Chaetomium olivaceum*, *Humicola grisea*, *Penicillium brevi-compactum*, *Penicillium funiculosum*	The fungus mycelium is developed at the edges, on the lateral and superior sideways, the level of coverage being of 15%	0-1	Good
Treatment with 5% $[Cu(NH_3)_4](OH)_2$	*Chaetomium olivaceum*, *Chaetomium funicola*, *Humicola grisea*, *Penicillium funiculosum*, *Stachybotrys alternans*	On the surface of the test pieces, there can be noticed a slight development of fine grey mycelium, which covers almost 10% of the surfaces	0	Very good

L1 – unmodified lignin; L1E –lignin modified by epoxydation; R2 – furan resin; $CuCl_2$ – copper chloride; $[Cu(NH_3 OH)_2)_4]$ – tetraminocopper hydroxide

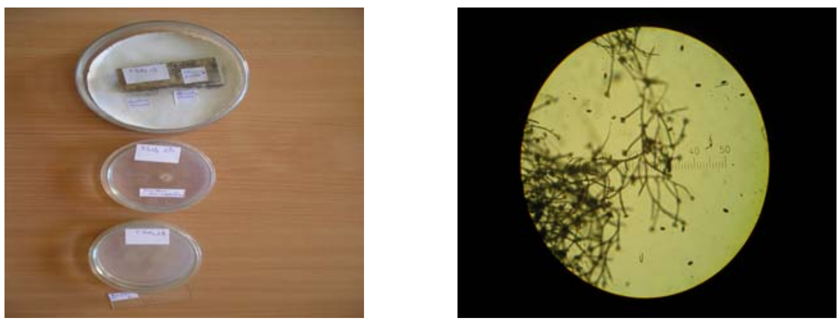

Figure 1: Macro- (left) and micro-images (right) of *Stachybotrys alternans* on the surface of beech wood treated with 5% L1E

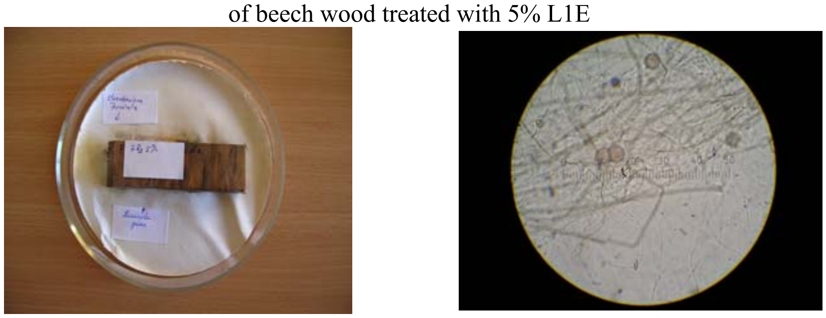

Figure 2: Macro- (left) and micro-images (right) of *Humicola grisea* on the surface of fir wood treated with 5% L1E

The microscopic images evidence the action of the agents studied and the development of microorganisms. On the one hand, it is thus possible to point out the characteristics of the fungi able to biosynthesize enzymes, which might destroy the wood, and on the other hand, these results allow an explanation regarding the inhibition of the development of microorganisms.

It was noticed that the fungus species, which developed on the lignocellulose material, depended on the nature of the chemical and its concentration. *Fagus sylvatica L*. and *Abies alba Mill* being new hosts for our country, the analysis showed that they presented new types of microorganisms (*Alternaria geophila Dasz.*). Also, more micromycetes were found on the beech test pieces than on the fir ones. The macroscopic and microscopic images (Figs. 1, 2) illustrate the action of the studied compounds on microorganism development.

The process can be correlated with the capacity of fungi to biosynthesize the cellulase enzymes involved in lignocellulose material biodegradation. Also, the capacity of the studied products to inhibit the enzymatic action could be correlated with the morphological characteristics of the fungi. Mention should be made here of species that tolerate the presence of compounds with a biocide potential action.

As to their efficiency, the chemical compounds used meet the conditions required for a biocide, as the lignin and the furan resin offer an average up to good efficiency, whereas the copper solutions have a good to very good efficiency.

These effects were speculated upon in our recent investigations (data not published), by combining the use of natural products with copper ions. Consequently, the systems based on compounds from renewable sources (lignin, furan resins, tannins) could provide new opportunities for achieving and developing environmentally friendly treatments for wood biostabilization.

CONCLUSION

The efficiency of chemical substances in the treatment of some beech and fir wood samples has been studied from the point of view of their resistance to various biological agents.

The potentially biocide action of lignin, its epoxy derivative, furan resin and copper ions (copper chloride and tetraminocopper hydroxide) on the development of *Alternaria geophola*, *Chaetonium funicola*, *Chaetonium olivaceum*, *Fusidium viride*, *Humicola grisea*, *Stachybotrys alternans*, *Penicillium brevi-compactum*, *Penicillium funiculosum* micromycetes was studied.

The morphological aspects of some fungi cultivated on both untreated and treated wood samples were also observed microscopically.

The chemical modification of lignin by epoxydation assures a higher capacity of biostabilization for the protection of wood samples, compared to the unmodified product.

The experimental data evidence the effects of biostabilization of the tested products, found to depend on their type and concentration, as well as on the wood species.

The biocide systems based on products obtained from renewable resources (types of lignin and furan resins) represent new opportunities for achieving new, environmentally compatible biostabilization treatments. More than that, there exists the possibility of obtaining ecological effects by using products of natural origin along with copper ions, a research direction to be followed.

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