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Brown-rot decay of copper-impregnated wood

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Abstract

Copper-based preservatives will be the predominant solution for wood protection in future decades. The composition of those preservatives will likely change from copper–chromium to copper–ethanolamine, due to environmental demands. The most important drawback of copper-impregnated wood is the presence of tolerant fungal organisms that have developed an ability to degrade such preserved wood. In order to elucidate these processes, specimens ($0.5 \times 1.0 \times 15$ cm) made of Norway spruce (*Picea abies*) were vacuum impregnated with copper-, chromium-, and copper–ethanolamine-based aqueous solutions ($c_{Cu} = 0.5\%$), and afterwards exposed to copper-sensitive *Gloeophyllum trabeum* and copper-tolerant *Antrodia vaillantii* for various times, between 1 and 8 weeks. After incubation, specimens were isolated, and modulus of elasticity (MOE) losses determined using a nondestructive technique. Mass losses, FTIR spectra, and color changes were measured as well. The results showed that there is significant difference between brown-rot decay caused by *G. trabeum* and *A. vaillantii*. Decay caused by *A. vaillantii* is more selective than that caused by *G. trabeum*. Additionally, it was proven that copper effectively protected spruce from *G. trabeum,* but not completely against *A. vaillantii*. Decay of copper-impregnated wood by copper-tolerant fungi is similar to decay of control, unimpregnated wood, whereas decay of copper-impregnated specimens by *G. trabeum*, was effectively stopped in its initial stage.

Keywords: *Antrodia vaillantii*; *Gloeophyllum trabeum*; Wood decay fungi; *Picea abies*; Modulus of elasticity; FTIR; Nondestructive testing; Copper tolerance; Wood preservatives

1. Introduction

Copper biocides have been applied successfully for more than two centuries. They were combined with chromium to enable fixation and arsenic to improve performance against copper-tolerant fungi and insects (Richardson, 1997). Due to arsenic toxicity, the use of copper-based preservatives is no longer desired in most European countries (Humar et al., 2006), thus they were generally replaced with boron. However, the situation is currently changing even more with the introduction of the Biocidal Products Directive (BPD, 98) (Anonymous, 1998). This directive will likely ban or drastically limit use of chromium in wood preservatives. Thus, new solutions to enable copper fixation need to be developed. Similar trends are foreseen in North America (Green and Clausen, 2005). Amines seem to be the most appropriate replacement for chromium. Ethanolamine is reported as the most suitable amine source in numerous research studies, and it is used already for several emerging preservative systems, including alkaline copper quat (ACQ), copper dimethyl-dithio-carbamate (CDDC), Cu-HDO ((Bis-(N-cyclohexyldiazeniumdioxy)-copper) and copper azole (CA) (Cao and Kamdem, 2004).

However, the other issue related to copper-treated wood is the presence of copper-tolerant wood-decay fungi. These fungi express an ability to degrade copper-treated wood. This ability is associated primarily with oxalic acid excretion (Green and Clausen, 2005). Oxalic acid produced by copper-tolerant fungi reacts with copper in wood to form insoluble, and therefore bio-unavailable, inert forms (Steenkjær Hastrup et al., 2005). Additionally it has been shown that lowering of pH with oxalic or any other acid has more to do with copper tolerance than low solubility of copper oxalate (Humar et al., 2005).

Acids excreted by wood-decay fungi react with copper, but these organisms also de-polymerize cellulose, hemicellulose, and lignin. Therefore the question arises of how

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acids influence the mechanical properties of copper-impregnated wood that is decayed by copper-tolerant wood-decay fungi. There is a question of whether oxalic acid reacts predominately with copper or if it depolymerizes wood components directly. In previous research (Pohleven et al., 2002), it was noticed that the mass of the copper-treated specimens exposed to copper-tolerant fungi actually increased, particularly during initial stages of decay. The cell lumina of those specimens were filled with copious amounts of copper oxalate and oxalic acid crystals. Therefore, it is possible, that due to significant amounts of oxalic acid in cell lumina, mass loss in the early stages of decay cannot be detected. In order to elucidate changes that appear in copper-treated wood during brown-rot decay, nondestructive measurements of Young's modulus were compared to mass losses and FTIR spectra of decayed and non decayed wood.

Decay of copper-impregnated wood by copper-tolerant fungi is also interesting from the bioremediation point of view. In most bioremediation studies, the main effort is focused on elucidating the changes in structure and composition of active biocide ingredients in wood or on the responses of the copper-tolerant organisms (Clausen and Green, 2003; Humar et al., 2004). There is almost no report on properties of impregnated wood during bioremediation with brown-rot decay fungi. Therefore, it is of significant importance to consider changes in chemical composition and mechanical properties of wood exposed to brown-rot fungi to maximize possible use of remediate wood fibers.

2. Material and methods

2.1. Preparation of the specimens

Norway spruce (*Picea abies*) samples ($15 \times 10 \times 5$ mm) were vacuum impregnated with two different copper-based waterborne solutions according to the EN 113 procedure (European Committee for Standardization, 1989). The first solution (CuCr) was made of copper(II) sulfate ($CuSO_4 \cdot 5H2O$) and potassium dichromate(VI) ($K_2Cr_2O_7$). The other one (CuE) consistent of copper(II) sulphate and ethanolamine (C_2H_7NO). The concentration of copper in both solutions was equal to 0.5%. The treatment resulted in retention of active ingredients of about 10 kg m^{-3} for specimens impregnated with CuCr solution and 19 kg m^{-3} for those impregnated with CuE. The samples were then conditioned for 4 weeks: the first 2 weeks in closed chambers, the third week in half closed chambers, and the fourth week in open ones. Following conditioning, the samples were leached according to the EN 84 procedure for 7 days (European Committee for Standardization, 1994). Afterwards, the samples were oven dried (103 °C), and their initial masses were determined. For comparison, control, unimpregnated specimens were prepared.

Conditioned and steam-sterilized specimens were then exposed to two different brown-rot fungi: a copper-tolerant strain of *Antrodia vaillantii* (ZIM L037) and a copper-sensitive *Gloeophyllum trabeum* (ZIM L018). Cultures were grown and maintained on 3.9% potato dextrose agar medium (PDA, Difco). Kolle flasks with PDA were inoculated with small pieces of fungal mycelia. After 1 week of fungal growth, four wood samples were placed on a sterilized holder made of stainless steel in each inoculated Kolle flask and exposed to fungal decay for 1, 2, 4 or 8 weeks in the growth chamber ($T = 25$ °C, RH = 75%). After respective incubation periods, specimens were isolated and their oven dry mass were determined and mass loss calculated. The experiment was performed with five parallel samples for each condition (treatment, fungus used, week of exposure). However mass losses, MOE, FTIR spectra and color were determined on all of the samples used.

2.2. FTIR spectroscopy

Infrared spectra of exposed and unexposed samples were collected using horizontal attenuated total reflection (HATR) technique. A Perkin Elmer Spectrum One spectrometer was used to collect all the infrared spectra reported in this study. The HATR accessory ZnSe crystal (Perkin Elmer) was used. Specimens were positioned on the surface of the ZnSe crystal and 64 scans were performed at 1 cm^{-1}. FTIR spectra of the specimens were collected before and after fungal exposure of oven-dried specimens. The analyses were performed at ambient temperature (23 °C). The spectra were analyzed using Perkin Elmer software. In order to elucidate decay processes, the relationship between intensities of IR absorption peaks that are assigned to C–O stretching in cellulose and hemicellulose (1030 cm^{-1}) and aromatic skeletal vibrations (stretching) at lignin (1600 cm^{-1}) were determined. Comparison of other peaks assigned to cellulose and/or lignin gave comparable results, and therefore they were not included in this paper.

2.3. Evaluation of modulus of elasticity

Nondestructive determination of modulus of elasticity (MOE) using dynamic-stress wave methods is currently an established technique in the field of wood science and technology (Stephan et al., 2000). Because of difficulties encountered in measuring the axial vibrations, it was decided to use flexural vibration modes to characterize elastic parameters. Considering the hypothesis of the homogeneity of geometrical and mechanical properties along the sample, basic dynamics theorems can be applied to obtain the motion equation of lateral vibrations. Analysis was performed on specimens with clamped–free end conditions. During the test, the lateral displacement of vibrating samples in damped vibration with known vibration mode was measured. As an inductive proximity sensor was used, a small piece of metal foil of negligible mass was glued to the surface of each sample. The damped frequency was obtained by FFT analysis of the exponentially decayed displacement signals detected in time domain. For determination of Young's modulus of samples the frequency equation deduced from Bernoulli's model was used, this was assumed to be acceptable because of the relatively high length-to-depth sample ratio (E—Young's modulus (Nm^{-2}), v—natural frequency (s^{-1}), $C = 3.51563$—constant derived from Bernoulli equation, ρ—density (kg m^{-3}), l—free sample length (m), h—sample height (m)).

$$E = \frac{48 \times \pi^2 \times l^4 \times \rho \times v^2}{C^2 \times h^2}.$$

2.4. Color of the specimens

The color of the specimens was determined from the scanned specimens and expressed in CIE $L^*a^*b^*$ system. L^*-axis represents the lightness whereas, a^* and b^* are the chromaticity coordinates. In the CIE $L^*a^*b^*$ coordinates, $+a^*$ stands for red, $-a^*$ for green, $+b^*$ for yellow, $-b^*$ for blue, and L^* varies from 100 (white) to zero (black). Any color can thus be characterized (Brock et al., 2000).

3. Results and discussion

Eight weeks of exposure to brown-rot fungi resulted in notable mass loss of control, unimpregnated specimens, indicating that both brown-rot fungi were active. The

Table 1
Mass losses and modulus of elasticity (MOE) losses of impregnated and unimpregnated-control Norway spruce wood specimens exposed to brown-rot fungi for the period between 1 and 8 weeks

Impregnation	Weeks of exposure	*G. trabeum*		*A. vaillantii*	
		Mass loss (%)	MoE loss (%)	Mass loss (%)	MoE loss (%)
CuCr	0	0.0 (0.0)	2.0 (1.1)	0.0 (0.0)	2.0 (1.1)
	1	0.1 (0.0)	4.6 (3.3)	0.0 (0.1)	3.0 (2.0)
	2	0.2 (0.0)	4.8 (1.5)	−0.4 (0.1)	3.7 (2.3)
	4	0.3 (0.1)	7.8 (3.1)	−1.0 (0.8)	5.4 (2.2)
	8	0.3 (0.1)	6.9 (4.1)	−0.1 (0.9)	5.7 (1.1)
CuE	0	0.0 (0.0)	4.4 (0.9)	0.0 (0.0)	4.4 (0.9)
	1	0.3 (0.1)	7.3 (2.3)	0.2 (0.1)	3.8 (2.1)
	2	0.4 (0.0)	6.2 (3.5)	2.8 (0.5)	19.0 (5.2)
	4	0.4 (0.1)	7.4 (1.5)	9.3 (1.4)	30.1 (7.3)
	8	0.4 (0.1)	7.3 (2.3)	10.0 (2.6)	33.0 (8.6)
Control	0	0.0 (0.0)	2.3 (1.0)	0.0 (0.0)	2.3 (1.0)
	1	−0.1 (0.2)	7.4 (1.8)	0.1 (0.0)	8.3 (3.4)
	2	2.6 (1.2)	12.0 (3.6)	4.5 (1.1)	16.4 (6.2)
	4	13.0 (3.6)	47.5 (7.4)	10.2 (1.8)	38.6 (5.9)
	8	28.0 (8.8)	76.0 (10.9)	14.3 (3.2)	40.5 (8.3)

Standard deviations of five replicates are given in the parenthesis.

decay of control spruce wood by *G. trabeum* caused higher mass loss than did exposure to *A. vaillantii*, as expected from previous studies (Humar et al., 2005). After 8 weeks of exposure to *G. trabeum,* a mass loss of 28.0% was detected, while the mass loss of spruce wood exposed to *A. vaillantii* was only half as much (14.3%). This difference was not consistent across all exposure times. For example, neither fungus caused detectible decay within the first week of exposure, but 2 weeks of decay resulted in almost two times higher mass loss by *A. vaillantii* (4.5%) in comparison to specimens decayed by *G. trabeum* (2.6%). After 4 weeks of decay, mass loss caused by *G. trabeum* (13.0%) surpassed those of *A. vaillantii* (10.2%) and then doubled again by week 8 (Table 1).

Measurements of modulus of elasticity MOE revealed similar trends as those reported for mass-loss data with one significant difference. The first sign of incipient decay was clearly seen even after 1 week of exposure of specimens to both fungal strains. This proves that a nondestructive measurement of MOE loss is a very sensitive tool. After 1 week of exposure, *G. trabeum* reduced MOE by 7.4% and *A. vaillantii* by 8.3%. Comparable findings are reported in the literature (Stephan et al., 2000; Machek and Militz, 2004). As shown with the mass-loss measurements, changes in MOE demonstrated that *A. vaillantii* was more effective during the first 2 weeks of exposure and *G. trabeum* during the remaining period. The remarkable decay capacity of *G. trabeum* can be clearly seen from MOE losses after 8 weeks of exposure of control, unimpregnated, specimens where *G. trabeum* caused MOE losses of more than 75%. On the other hand, 8 weeks of exposure of the unimpregnated specimens exposed to *A. vaillantii* resulted in only a 40.5% loss of MOE (Table 1). Thus, the data from the control specimens show that *A. vaillantii* degrades wood faster during initial stages of decay, but afterwards its ability to degrade wood decreases. On the other hand, *G. trabeum* proved again that it is one of the most capable brown-rot fungi. Curling et al. (2002) showed that hemicelluloses are decayed initially during incipient brown-rot decay, and that degradation of those components significantly influences mechanical properties. Hemicelluloses form an encrusting envelope around cellulose microfibrils, therefore degradation of cellulose depends on the prior removal of hemicelluloses. Thus, it seems that *A. vaillantii* has developed mechanisms to rapidly and effectively degrade hemicelluloses and easily accessible cellulose. On the other hand, *G. trabeum* degrades hemicelluloses, cellulose, and even lignin (Wilcox, 1993).

These data are further supported by FTIR measurements. The relationship between the intensities of IR absorption peaks that are assigned to C–O stretching in cellulose and hemicelluloses (1030 cm^{-1}) and aromatic skeletal vibrations (stretching) of lignin (1600 cm^{-1}), decreases during the first 4 weeks of exposure to *A. vaillantii* and then remains almost constant during the last 4 weeks. Data presented in Table 2 shows that the amount of cellulose and hemicellulose decreases faster than lignin content, indicating that *A. vaillantii* does not have mechanisms to degrade lignin. On the other hand, there was a different relationship between the IR absorption peaks of specimens exposed to *G. trabeum.* During the first week, the amount of cellulose and hemicellulose decreased, but afterwards the relationship between cellulose and hemicellulose peaks increased, indicating that there must be some partial degradation or change of lignin (Table 2). It is likely that these relationships are clear evidence that *G. trabeum* uses more sophisticated mechanisms to degrade wood, and that this fungus is able to utilize a higher

Table 2
Relationship between intensities of FTIR absorption peaks that are assigned to C–O stretching in cellulose and hemicellulose (1030 cm^{-1}) and aromatic skeletal vibrations (stretching) at lignin (1600 cm^{-1}), determined from the surface of wood exposed to wood-decay fungi for the period between 1 and 8 weeks

Impregnation	Weeks of exposure	*G. trabeum*	*A. vaillantii*
CuCr	0	17.3 (0.3)	17.3 (0.3)
	1	19.2 (0.3)	14.3 (0.4)
	2	17.4 (0.5)	11.9 (1.1)
	4	16.1 (1.4)	7.2 (0.9)
	8	14.2 (3.4)	6.8 (2.1)
CuE	0	19.4 (0.4)	19.4 (0.4)
	1	16.5 (0.2)	13.3 (0.3)
	2	17.6 (1.1)	10.5 (1.1)
	4	16.3 (1.1)	7.6 (1.6)
	8	16.6 (1.9)	7.5 (2.7)
Control	0	18.2 (0.2)	18.2 (0.2)
	1	16.9 (1.1)	13.3 (1.4)
	2	22.7 (1.8)	9.9 (2.1)
	4	22.1 (3.7)	8.3 (2.4)
	8	[a]	8.1 (1.9)

Standard deviations of five replicates are given in the parenthesis.
[a]The specimens were too degraded. Therefore, we were not able to determine exact spectra and calculate accurate relationships.

Table 3
Color of impregnated and unimpregnated Norway spruce wood specimens after exposure to wood-decay fungi for the period between 1 and 8 weeks

Impregnation	Weeks of exposure	*G. trabeum*			*A. vaillantii*		
		L^*	a^*	b^*	L^*	a^*	b^*
CuCr	0	57.6	1.9	7.1	57.6	1.9	7.1
	1	69.4	6.1	13.7	56.1	2.8	8.0
	2	64.7	6.6	13.7	54.9	3.8	8.0
	4	58.4	7.6	14.2	54.9	3.8	8.0
	8	49.8	7.6	13.7	52.5	4.3	8.0
CuE	0	52.2	0.9	8.5	52.2	0.9	8.5
	1	59.2	2.4	8.5	54.1	4.3	11.3
	2	56.1	2.4	8.0	51.8	4.7	10.9
	4	54.9	2.8	9.4	51.8	5.2	11.3
	8	54.9	2.8	9.0	54.9	5.2	11.8
Control	0	89.0	2.4	10.4	89.0	2.4	10.4
	1	68.6	5.2	13.2	74.9	5.7	13.2
	2	64.7	6.1	13.2	68.2	6.6	13.2
	4	58.0	6.6	13.7	64.3	7.1	13.2
	8	48.2	6.6	12.8	67.8	6.6	13.2

For each condition five parallel specimens were analyzed.

percentage of wood components than *A. vaillantii*. However, it must be considered that these spectra were determined from the surface of exposed specimens, and that the decay of surface layers is usually faster than decay of the interior portions of the specimens.

Impregnation of spruce wood with copper–chromium or copper–ethanolamine wood preservatives did not result in significant changes of MOE of wood. The average MOE of control, unimpregnated, specimens was 16,196 MPa and the average MOE's of CuCr- and CuE-impregnated wood pieces were 16,066 and 16,267 MPa, respectively. Steam sterilization (121 °C) itself affected the mechanical properties of the impregnated specimens. The greatest influence of steam sterilization was measured in CuE-impregnated specimens where MOE losses of 4.4% were determined. This is almost twice as high as the MOE losses associated with CuCr-impregnated sticks or the controls (Table 1). These data support previous studies that showed that ethanolamine in wood can cause depolymerization of wood polymers, particularly at high temperatures and in a humid environment (Claus et al., 2004; Humar et al., in press).

Neither brown-rot fungus was able to decay CuCr-impregnated specimens, regardless of copper sensitivity. In both cases, insignificant mass losses were detected (Table 1). However, despite the fact that there was almost no mass loss detected, CuCr-impregnated wood exposed to *G. trabeum* lost almost 7% of its MOE after 8 weeks, while the specimens exposed to *A. vaillantii* lost almost 6% of MOE. The retention of Cu and Cr in wood was relatively high (10 kg m^{-3}), which allows effective protection of wood against these brown-rot fungi. This result seems very promising, as we suspect that oxalic acid excreted by copper-tolerant *A. vaillantii* will depolymerize cellulose and hemicellulose, which results in notable MOE losses. Parallel experiments confirmed that this copper-tolerant fungus transforms a major portion of copper biocides in the outer layer of specimens to copper oxalate after 4 weeks of exposure (Humar et al., 2004). Therefore, it seems that oxalic acid has an affinity to form complexes with metals and does not depolymerize wood in the initial stages of decay.

Another test that showed that the specimens were overgrown and that there were significant amounts of oxalic acid excreted are the color changes and FTIR measurements. Both fungi changed the color of CuCr-impregnated specimens from a green to a more reddish color. In addition, exposure to both fungi resulted in a darkening of the wood, *A. vaillantii* from the beginning of the exposure, while *G. trabeum* initially caused a lightening of the wood within the first 4 weeks followed by a darkening of the specimens (Table 3). FTIR observation showed that similar changes after exposure to *A. vaillantii* appeared on the surface of the CuCr-impregnated specimens as on the surface of the controls. This indicates that this copper-tolerant fungus was able to overgrow, and even partially degrade the surface, but that decay could not proceed as the concentration of the copper in the wood was too high. Changes in specimens exposed to *G. trabeum* were not comparable to the controls. On control specimens, the relationship between cellulose and lignin peaks first decreased and afterward increased, while there was only a decrease associated with *G. trabeum* decay in CuCr-treated

specimens (Table 2). It is presumed that not even the surface of impregnated specimens was decayed to the same extent as the surface of control specimens, and that the decay was stopped in its initial stage.

The treatment of wood with CuE was less effective than with CuCr. The CuE-impregnated specimens exposed to copper-tolerant *A. vaillantii* on average lost 10% of their mass and 33% of MOE after 8 weeks of exposure. This is another demonstration of the lower effectiveness of copper–amine preservatives against copper-tolerant organisms, despite the fact that there was almost a two-fold higher retention of copper–ethanolamine ($19\,kg\,m^{-3}$) compared to copper chromium ($10\,kg\,m^{-3}$). FTIR analysis indicated that the mechanism of decay of CuE-impregnated wood is comparable to decay of control wood (Table 2). It seems that preservation of spruce with CuE is not sufficient to protect wood against copper-tolerant fungi. On the other hand, *G. trabeum* was not able to decay CuE-preserved wood. Mass losses lower than 0.5% were obtained, even after 8 weeks of exposure. Slightly higher MOE losses were measured; after 1 week of exposure, MOE losses of 7.3% were determined and remained almost constant throughout the remainder of the study (Table 1). It is believed that *G. trabeum* starts depolymerization of wood polymers, but that this process was halted during the first stages of decay. This presumption is further supported by FTIR measurements similar to that of CuCr-treated wood. On control specimens exposed to *G. trabeum,* the relationship between cellulose and lignin absorption peaks decreased during the first week and afterwards increased and remained almost constant, while only a decrease was observed on CuE-impregnated specimens (Table 2). FTIR measurements, mass-loss and MOE measurements indicate that the decay of CuE-treated specimens by *G. trabeum* remained at a level that was comparable to control specimens after 1 week of exposure. It is suspected that some decay process started in treated wood but was afterwards prevented by copper.

4. Conclusions

Comparison of results obtained with different experimental techniques gives very interesting details on brown-rot decay of unimpregnated and copper-preserved spruce wood. Decay caused by the copper-tolerant fungus, *A. vaillantii,* is faster during the initial stages, but then decreases compared to decay by copper-sensitive *G. trabeum.* FTIR analysis of decayed specimens resolved that *A. vaillantii* decays predominately hemicelluloses and cellulose and that lignin remains almost unaffected. On the other hand, *G. trabeum* decays hemicelluloses and cellulose, as well as lignin, which results in significantly higher mass losses compared to decay caused by *A. vaillantii.*

Impregnation of specimens with copper-based preservatives prevents decay of the specimens by *G. trabeum*. This fungus overgrows the specimens and slightly decays the wood, which can be seen from FTIR spectra, color measurements, mass losses, and MOE losses. However, decay was halted and did not proceed after initial losses. On the other hand, the copper–chromium-based preservative prevented decay by copper-tolerant *A. vaillantii,* while impregnation of spruce wood with copper–ethanolamine did not. Decay patterns of copper-impregnated wood were comparable to control specimens.

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