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LACCASE TREATMENT OF NORWAY SPRUCE WOOD SURFACE IMPROVES RESISTANCE AND COPPER FIXATION OF TREATED WOOD

The effects of laccase from the white-rot basidiomycete fungus Trametes versicolor on Norway spruce wood (Pices abies) surface were studied. Experiments were performed at room temperature and at pH 4.6, without the addition of mediators. Biological, chemical and physical properties of the treated wood surface were examined by a wood decay test, a scanning electron microscopy (SEM), a Fourier transform infrared (FTIR) spectroscopy and a copper leaching test. Laccase pre-treatment of Norway spruce wood surface was shown to reduce wood decay by brown-rot fungus, Gloeophyllum trabeum and white-rot fungus, Trametes versicolor. SEM images showed expanded wood cell walls, closed pits and a more even surface after laccase treatment. FTIR analysis indicated that laccase not only catalyse depolymerisation of lignin, but also affect other wood cell wall components, such as hemicellulose and cellulose. We showed that laccase treatment altered wood properties in a way that improved wood resistance to decay and prevented leaching of copper from impregnated wood.

Keywords: laccase, *Trametes versicolor*, Norway spruce, wood surface, wood decay, copper leaching

Introduction

Laccase (EC 1.10.3.2., p-diphenol:dioxygen oxidoreductase) are common enzymes in nature belonging to the multicopper oxidases family and are called blue enzymes. The first laccase was reported as a component of the resin ducts of the Japanese lacquer tree *Rhus vernicifera* [Yoshida 1883]. Laccases have since been discovered in numerous plants and bacteria [Martins et al. 2002]. The majority of laccases characterized have been detected in wood decay fungi, especially white-rot basidiomycetes, which are efficient lignin degraders.

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Well-known laccase producers also include fungi belonging to the ascomycetes, deuteromycetes and basidiomycetes [Sharma et al. 2007]. White-rot fungi laccase are among the main enzymes involved in delignification [Kirk and Cullen 1998]. Recent studies on the immunocytochemical characterization of wood decayed by white rot, give not only information about component removal, but also direct visualization of the cell wall components in decayed wood [Kim et al. 2015a, b]. A good understanding of lignin structure and chemistry is helpful in the development of laccase based treatment technology for wood [Widsten and Kandelbauer 2008]. The enzyme may catalyse the polymerization of lignin fragments to lignin or de-polymerization of lignin. It has been proposed that laccases are involved in cell wall formation in plants and together with peroxidases, in the lignification process [Mayer and Staples 2002]. The balance between these opposing mechanisms depends on the nature of redox mediators [Felby et al. 2002].

Lignin is an amorphous polymer; it comprises approximately 20% to 32% of dry wood mass and functions as a cementing material in wood cells. There is a 5% to10% greater concentration of lignin found in softwood than hardwood, less hemicelluloses and about the same amount of cellulose 40% to50%. The chemical composition of softwood is also different with different types of lignin primarily of guaiacyl propane units and mannose being the most prominent constituent in softwood hemicelluloses [Blanchette 2000]. Lignin consists of p-hydroxyphenyl, guaiacyl and syringyl-type phenylpropane units in which the aromatic units bear 1, 2 or 3 free or etherified hydroxyl groups. The phenylpropane units are linked together by alkyl – aryl ether bonds, aromatic ether bonds and carbon-carbon bonds. The oxidation of lignin components creates reactive radicals that can undergo non-enzymatic reactions such as cross--linking of monomers, degradation of polymers and ring cleavage of aromatic compounds [Claus 2003].

Enzyme technology offers an environmentally friendly method for modifying solid wood, pulp or other lignocellulosics by biografting of phenols and other molecules onto their surfaces. An important topic related to wood products is the chemical modification of their surface properties to improve their resistance, such as wood-polymer composites prepared by swell-bonding and in-situ polymerization of monomers into the wood cell wall [Li et al. 2011, 2013]. Properties such as antimicrobial, antifungal, UV and weathering stability and fire retardancy have been or can potentially be imparted to lignocellulosic substrates [Chandra and Ragauskas 2002; Chandra et al. 2004; Fackler et al. 2008; Widsten and Kandelbauer 2008].

Investigations into Norway spruce wood (*Pices abies*) surfaces treated with laccases from basidomycetes *Trametes versicolor* at room temperature were undertaken. No additional redox mediators or other chemical compounds were used. The biological, chemical and physical properties of the treated wood surface were determined with a wood decay test, Scanning electron microscopy

(SEM), Fourier transform infra-red spectroscopy (FTIR), as well as copper penetration and leaching of copper based wood preservatives from impregnated wood.

Materials and methods

Penetration of copper into wood

Three specimens of Norway spruce (*Picea abies*) sapwood (dimensions 1.5 × 2.5 × 5.0 cm) were submerged in 10-3 mg/ml laccase from *Trametes versicolor* (SIGMA 51639-5G, ≥10 U mg^{-1}) and phosphate-citrate buffer (pH 4.6) solution for 30 min at room temperature and then conditioned for one week at room temperature [Humar and Lesar 2009]. Temperature, pH and dipping time were determined with previous experiments to optimize parameters of laccase activity. UV/VIS spectroscopy was used to measure the laccase catalysed conversion of ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) and DMP (2,6-dimethoxyphenol) at different pHs, temperatures and time of exposure (data not shown). The laccase treated specimens were then impregnated with a 0.43 (w/w) copper solution of copper(II)sulphate pentahydrate (Kemika, Croatia) for 30 min at 10 kPa pressure, together with three additional control samples. Copper ions were determined using an aqueous solution of 2% (w/w) potassium hexacyanoferrate(II). Following the chemical reaction: $2\ Cu^{2+} + K_4[Fe(CN)_6] \rightarrow Cu_2[Fe(CN)_6] + 4\ K^+$. The penetration of copper was detected visually with brown complex $Cu_2[Fe(CN)_6]$.

Copper leaching test

For leaching tests, specimens of Norway spruce (*Picea abies*) sapwood (1.5 × 2.5 × 5.0 cm) were impregnated with the 0.43% (w/w) copper solution of copper(II)sulfate pentahydrate (Kemika, Croatia) for 30 min at 100 mbar pressure. Average solution uptake was 428 $kg \cdot m^{-3}$, which indicates that the wood samples were fully treated. After impregnation, nine specimens were submerged in 10^{-3} mg/ml laccase from *Trametes versicolor* (SIGMA 51639-5G, ≥10 $U \cdot mg^{-1}$) and phosphate-citrate buffer (pH 4.6) solution for 30 min at room temperature and nine were used as control. Wood specimens were conditioned for one week at room temperature and the specimen's axial surfaces were then end sealed with paraffin wax. Leaching was performed according to the modified EN 1250-2 [1994] method with two modifications: instead of five, three specimens were positioned in the same vessels and instead of a magnetic stirrer, a shaking device was used in order to achieve water mixing. In order to have three parallel leaching procedures, nine specimens per solution/treatment were put into three vessels (three specimens per vessel). The samples in the vessel were afterwards positioned with a weight, 300 g of distilled water was

added and the vessel with its content was shaken with a frequency of 60 min^{-1} [Humar and Lesar 2008]. Leachates from the same vessel were collected and compiled. The concentration of copper in leachates was determined after the first, second and fourth day of leaching. The copper concentration was measured with XRF Twin-X (Oxford instruments).

Determination of contact angles

The sessile drop method was applied to determine the contact angles of distilled water on the surfaces of specimens using a Theta (Optical Tensiometer) from Biolin Scientific Oy, Espoo – Finland. After calibration, the goniometer microscope was focused and adjusted to the image of the drop. The contact angles were measured by means of computer-aided analysis (OneAttension, Version 2.4 (r4931), Biolin Scientific, Young-Laplace contact angle analysis mode) of the shapes of the liquid drops, as observed in an optical goniometer and recorded by a digital camera installed in the axial extension of the lens. Droplets of 4 µl were applied by means of a dispenser at 3 different places, 10 mm apart from each other, on the radial surface (regardless of whether these places were earlywood or latewood). Image recording was set for 62 s (15 FPS), and the time when contact angles started to be calculated (0 s) was after detachment of the dispenser tip from the drop, which happened approximately 2 s after the first contact of the drop with a substrate. The measurements were taken at a constant temperature of 23°C.

Wood decay test

The resistance of wood treated with 10^{-3} mg/ml laccase from *Trametes versicolor* (SIGMA 51639-5G, $\geq 10\ U \cdot mg^{-1}$) against wood decay fungi was determined according to the EN 113 procedure [EN 113:1989]. Specimens of Norway spruce (*Picea abies*) sapwood (1.5 × 2.5 × 5.0 cm) were submerged in laccase solution for 30 min, as described above, and air dried for two weeks. Before exposure to white-rot fungi *Trametes versicolor* (*T. versicolor*, ZIM L057) and brown-rot fungi *Gloeophyllum trabeum* (*G. trabeum*, ZIM L018) wood samples were steam sterilized [Raspor et al. 1995]. Jars with PDA (potato dextrose agar) medium were inoculated with small pieces of mycelium after which the wood samples (one each of treated and untreated) were placed on a plastic net in each inoculated jar. In parallel jars, only control specimens were exposed in order to completely avoid the influence of treatment on the vitality of the test fungi. The samples were incubated in a growth chamber at 25°C, relative humidity (RH) 75%. After 16 weeks, specimens were isolated and mass losses were gravimetrically determined and expressed as a percentage. The experiment was replicated five times.

Scanning Electron Microscopy analysis

Scanning Electron Microscopy (SEM) was carried out in a FEITM Quanta 200 3D. The samples were cut from the specimens of Norway spruce (*Picea abies*) wood, used in the wood decay test. With each sample, two cross-sections were examined: the first was parallel to the treated surface and the second was transverse to the surface. With the latter sample, the changes in structure from the surface towards the interior (max. 250 µm deep) were investigated. All samples were coated with a thin layer of gold. The program, Soft analysis was used for image analysis.

Fourier transform infra red spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) spectra of laccase treated specimens were measured. For this part of the investigation, Norway spruce wood specimens were first extracted, in order to avoid the signals of resins. The continuous extraction in 50% (v/v) methanol/water for 10 hours was performed with the automatic extraction system Soxlet Büchi B-811. Wood specimens were then oven-dried at 60°C and submerged in10^{-3} mg/ml laccase from *Trametes versicolor* (SIGMA 51639-5G, ≥10 U·mg^{-1}) in phosphate-citrate buffer (pH 4.6) for 30 min at room temperature. FTIR analysis of the wood was performed with a Perkin Elmer FTIR Spectrum One Spectrometer, using Abrasive Pad 600 Grit-Coated, PK/100 (Perkin Elmer) paper. DRIFT spectra were collected between 3000 cm^{-1} and 600 cm^{-1}, 64 scans were performed at 1 cm^{-1}.

Results and discussion

Penetration of copper

In figure 1 (right column), it can be clearly seen that copper did not penetrate into the laccase treated wood specimens during impregnation with respect to the control specimens (left column), which had not undergone laccase treatment. It is evident that enzyme catalysis reactions occur, that change either the permeability or the surface energy of the wood samples. The results were surprising, we did not expect laccase treatment would completely prevent penetration of copper into wood. The results of this simple experiment led us to investigate further the effect laccases have on the Norway spruce wood surface, in terms of a fixative preventing biocides from leaching.

Copper leaching test

In contrast to the previous experiment, where wood specimens were treated with laccase prior to impregnation with copper(II)sulphate pentahydrate, the copper leaching test wood was first impregnated and then exposed to the enzyme.

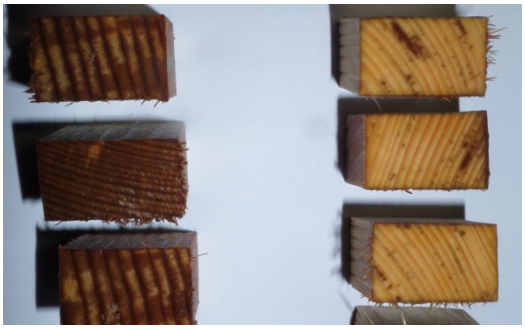


Fig. 1. The penetration of copper is detected visually with brown complex $Cu_2[Fe(CN)_6]$. In the left column are three control Norway spruce wood specimens impregnated with a solution of copper(II) sulphate pentahydrate and on the right are three specimens which were first treated with laccase and then impregnated with a copper solution

As can be seen in table 1, laccase treated specimens lost 12 percentage points (pp) less copper, after four days of leaching than untreated specimens. The results confirmed the change in permeability or surface energy of the wood specimens. Laccase treatment could be used as an agent to reduce copper leaching. Laccase application might be potentially useful to slow down leaching from micronized copper treated wood that does not have any compounds added for leaching reduction, but the fixation phenomenon is based on physical mechanisms only.

Table 1. Percentages (w/v) of copper determined in leachates from copper impregnated wood determined according to the modified EN 1250-2 [1994] procedure, in relation to the laccases treated wood surface. Standard deviations are given in parenthesis

	Retention of Cu (kg/m^{-3})	Leached Cu % (w/v)	Leached Cu % (w/v)	Leached Cu % (w/v)	Leached Cu % (w/v)
		1.day	2.day	4.day	SUM
Laccase	0.18 (0.1)	10.4 (1.3)	5.6 (0.6)	1.9 (1.2)	17.9 (1.0)
Control	0.18 (0.1)	16.7 (3.3)	8.2 (0.6)	4.7 (0.5)	29.6 (1.5)

Lower surface energy of laccase treated Norway spruce wood

To determine a possible hydrophobic effect after laccase treatment of Norway spruce wood surface, the water contact angels were determined. Control spruce samples have higher contact angels after 0, 10 and 60 s in comparison to wood samples treated with laccase and buffer indicating that laccase treated samples have lower surface energy and are less hydrophobic (table 2). These results are in agreement with another experiment. Long term water uptake, which was measured in order to explain further the results of the copper leaching test, confirmed that laccase treatment does not affect the water uptake of wood samples (data not shown). In view of this, further methods were used to investigate the low permeability of wood after laccase treatment.

Table 2. Contact angles of distilled water on the surfaces of Norway spruce wood specimens treated with laccase and phosphate-citrate buffer (pH 4,6), determined by the sessile drop method

	Time		
	0 s	10 s	60 s
Laccase	61°	43°	35°
Control	70°	51°	39°
Buffer	55°	39°	27°

Wood decay test

The contribution of laccase treatment to Norway spruce wood against fungi biodegradation was also examined. Brown rot fungus *G. trabeum* and white rot fungus *T. versicolor* were applied for testing, since these two fungal species are the most important degrading organisms of wood in outdoor applications in central Europe. No biocides were used in the experiment. In figure 2 we can observe 10 percentage points (pp) lower mass loss after *G. trabeum* exposure in favour of laccase usage and in the case of *T. versicolor* biodegradation, 12 pp less mass loss than in the control. Although protection provided for Norway spruce wood by laccase is not deemed to be adequate, the results are surprising,

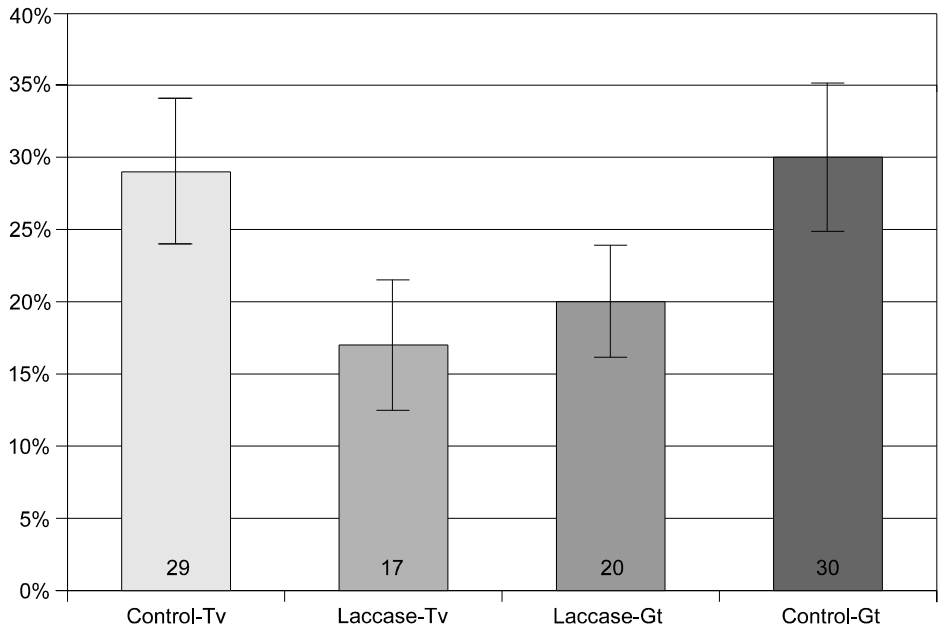
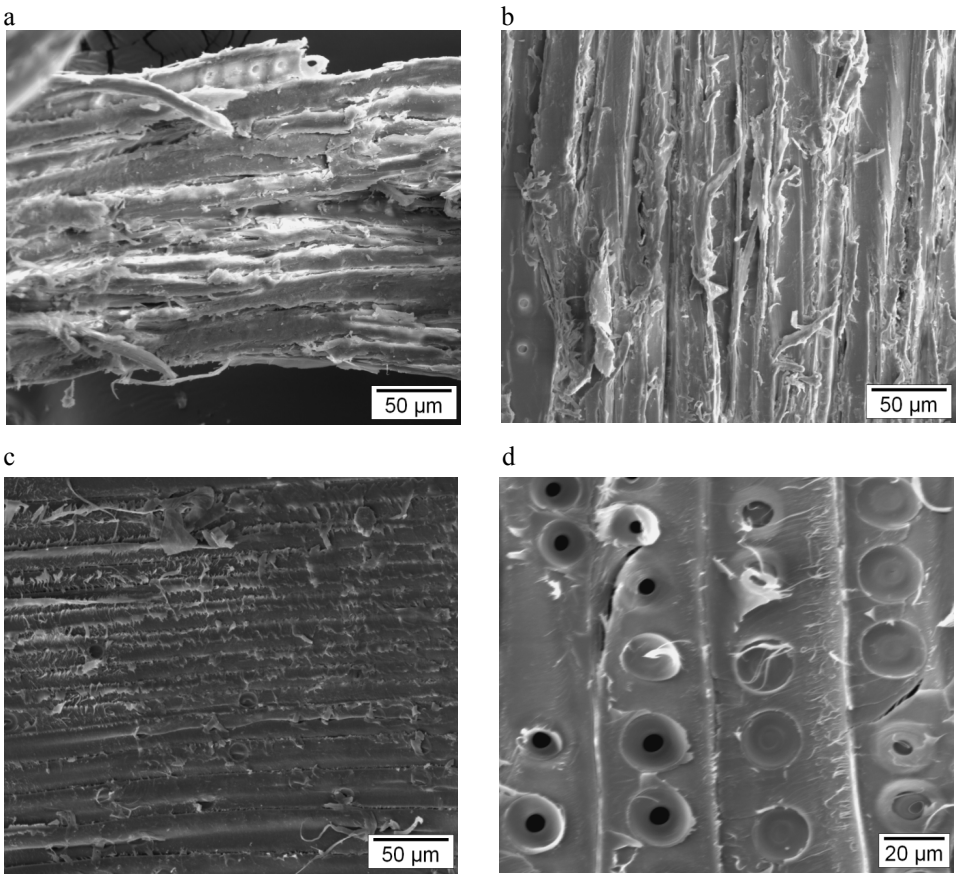


Fig. 2. Mass loss (%) of control and laccase treated Norway spruce (*Picea abies*) specimens after sixteen weeks of exposure to wood decay fungi *T. Versicolor* and *G. trabeum*

as laccases are native in *T. versicolor* and one could expect basidomyces to identify and overcome the laccase contribution to a changed wood surface. Furthermore, it should be noted that only the surface of the wood was treated with laccase. If the whole cross-section of the specimens had been impregnated with this pre-treatment, an even better result might have been achieved.

SEM images of laccase treated Norway spruce wood surface after fungal degradation

We used SEM to take a closer look at the changed Norway spruce wood surface and biodegradation by *T. versicolor* and *G. trabeum*. The first four images in figure 3 are the Norway spruce wood surface as control (a), the second control is Norway spruce wood treated with just buffer (b), treated with laccase (c) and a closer look at pits on the surface of the laccase treated wood surface. It can be clearly seen in (fig. 3c) that the surface is not as rough or fibreless and many pits are fully closed (fig. 3d). Closed pits could explain the results of the wood decay test, since the hyphae were commonly seen moving cell-to-cell by penetrating bordered pits [Schilling et al. 2013]. The same observations apply to figure 3f and figure 3h after the wood surface had been exposed to *T. versicolor* and



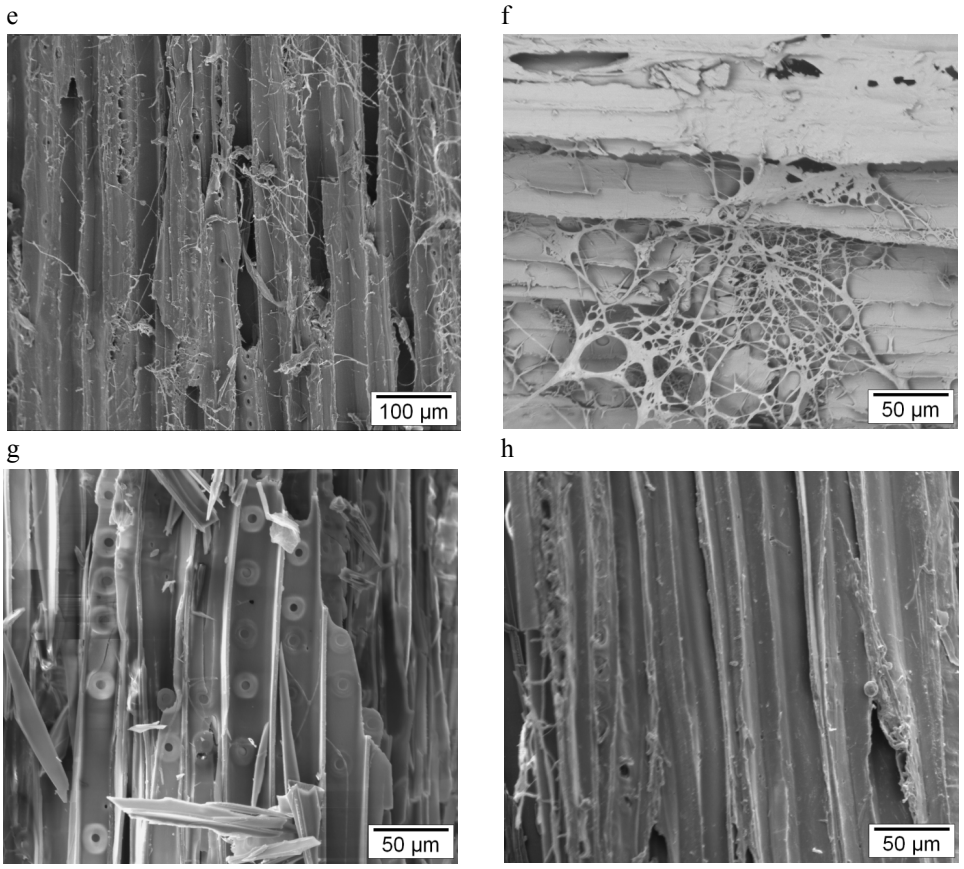


Fig. 3. Scanning electron micrograph of the surface of Norway spruce wood (*Picea abies*): a – as control, b – treated with buffer, c and d – treated with laccase, e – after 16 weeks of exposure to *T. versicolor*, f – treated with laccase and exposed to *T. versicolor*, g – after 16 weeks of exposure to *G. trabeum*, h – treated with laccase and exposed to *G. trabeum*

G. trabeum. In the case of brown rot degradation (fig. 3g), cellulose has been removed leaving lignin as the cell wall component, the structure left behind looks clean and the pits well exposed. *T. versicolor*, on other hand, also degrades lignin so the structure after white rot degradation (fig. 3e) looks rough. On figure 3 (e) and (f), hyphae from *T. versicolor* can be seen, in comparison the surface of the laccase treated specimen (fig. 3f) again looks more even, with no pits.

What is surprising is that after *G. trabeum* biodegradation of laccase treated samples (fig. 4d), the wood cell wall (WCW) is three times thicker (c. 3 µm) than in samples that were not treated (c. 1 µm) and also thicker than control samples treated with laccase that did not undergo biodegradation (fig. 4b) and

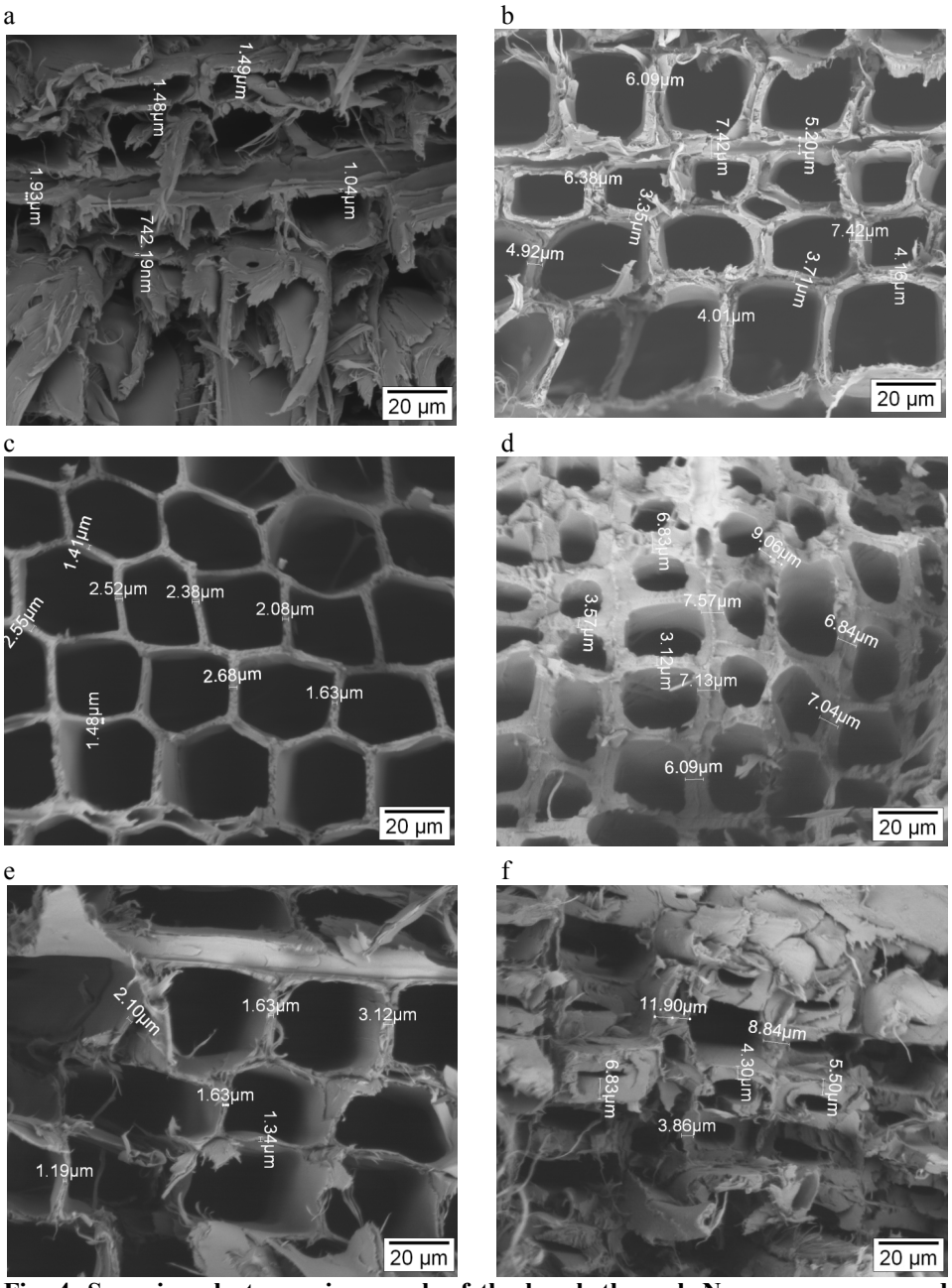


Fig. 4. Scanning electron micrograph of the break through Norway spruce wood (*Picea abies*) circa 250 µm from the surface: a – as control, b – treated with laccase, c – after 16 weeks of exposure to *G. trabeum*, d – treated with laccase and exposed to *G. trabeum*, e – after 16 weeks of exposure to *T. versicolor*, f – treated with laccase and exposed to *T. versicolor*

are approximately 2.5 µm. This could be due to acidification correlating to a drop in pH behind the hyphal front, leading to carbohydrate depolymerization [Schilling et al. 2013]. Figure 4c shows the very even structure left after biodegradation of *G. trabeum.* Since *G. trabeum* is a brown rot fungus it can be assumed that the substance left behind does not contain cellulose and is mainly from lignin [Green and Highley 1997]. The same can be said for the sample in figure 4d. After biodegradation of the treated samples with white rot fungus *T. versicolor* (fig. 4f), the WCW also expanded (c. 2.5 µm) in relation to untreated samples (c. 1 µm). In figure 4e it can also be seen that the structure left behind after *T. versicolor* is not as clean as with *G. trabeum*; it is more fibrous but far less so, than the Norway spruce wood control in figure 4a. As mentioned above, the samples were not cut, but broken through and a clean break was difficult to achieve with untreated Norway spruce wood. It can be said that laccase catalyses depolymerization of the polymer molecules that make up these fibrous structures in spruce wood. It looks as if the primary structures of Norway spruce wood components are transformed by laccase activity into a new secondary structure, which makes WCW thicker. Since no additional substances have been added, only compounds of primary material and water are used in biochemical reactions. Why the WCW is even thicker after biodegradation can only be speculated upon. There is the factor of other fungi enzymes that need to be taken into consideration. The main extracellular enzymes that can act on lignin directly or indirectly are laccase, manganese peroxidase and lignin peroxidase [Kirk and Cullen 1998].

Chemical changes of laccase treated Norway spruce wood surface

Fourier transform infrared (FTIR) analysis is a suitable tool that enables an understanding of the wood surface structure and possible laccase catalysed reactions in wood. It can be clearly seen from the spectra (fig. 5) that samples treated with laccase have a higher transmission (R %) than untreated samples, correlating to the SEM results indicating that the surface is more even. As expected, there is no band at 1128 cm^{-1} because softwood contains only guayacil units and no syrinmannosegil [Faix 1992]. The best band for lignin observation is 1510 cm^{-1} assigned to C=C stretching of the aromatic ring [Colom et.al. 2003]. The band decreased after laccase treatment, indicating that some aromatic rings open. Rico et al. [2015] suggested that guayacil units degraded by laccase undergo either condensation reactions forming 4-O-5' linkage or reaction leading to the aromatic ring opening. The latter seems to be the case; according to our results, there is a decrease of C-O-C vibrations at 1035 cm^{-1}. The band at 1740 cm^{-1} also mentioned in correlation with lignin degradation did not increase but decrease; fewer C=O stretching vibrations are an indicator that lignin units are not in an oxidized state.

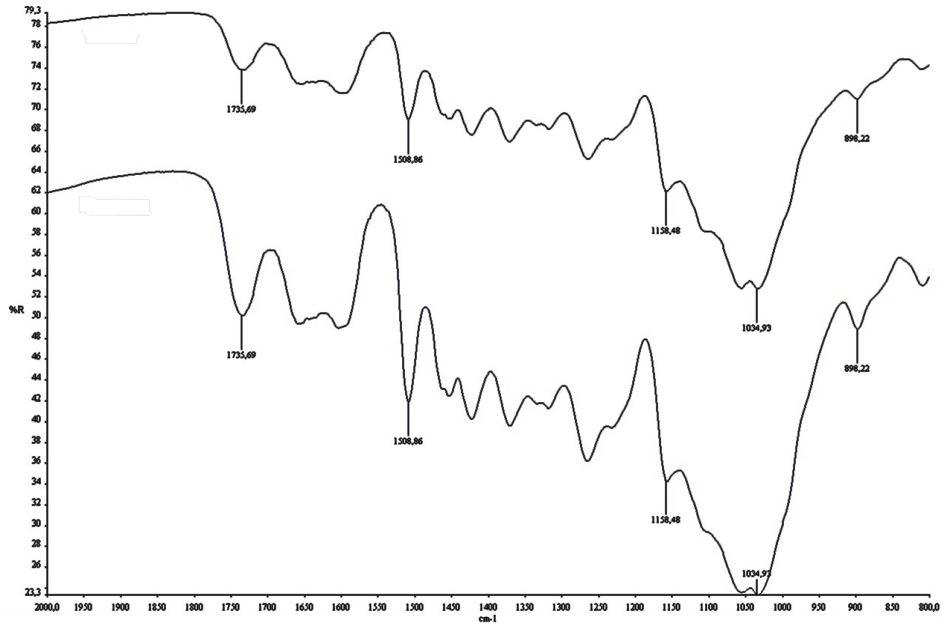


Fig. 5. DRIFT spectra of Norway spruce wood surface collected between 1800 cm^{-1} and 800 cm^{-1}. The upper curve shows spectra of the laccase pretreated wood surface (lakaza) in comparison to the lower curve – the control (kontrola)

Laccase treatment also decreases unconjugated C=O in xylans (hemicelluloses), seen at 1734 cm^{-1}. Colom et al. [2003] investigated the correlation between crystallized and amorphous cellulose, based on the doublet at 1335 cm^{-1} and 1316 cm^{-1}. According to their research, a decrease in the ratio 1335/1316 indicates that the content of crystallized cellulose I and/or II is increasing. Taking this into consideration, it can be argued that the crystalline cellulose content in our study increased after laccase treatment. It is surprising that laccase has a simultaneous effect on lignin and carbohydrate modifications. Schilling et al. [2013] reported it was the other way around, where unexpected lignin modifications were mentioned as a side effect of carbohydrate depolymerization induced by brown rot fungi *Postia placenta*.

Furthermore, there is also an increase of asymmetric bridge C-O-C at 1158 cm^{-1} associated with cellulose. The decrease of asymmetric out-of-phase ring stretching in the pyranose ring at 898 cm^{-1} confirms the transformation of amorphous into crystalline cellulose. The SEM images show that the expanded WCW after laccase treatment may be due to cellulose changes. Native cellulose consists of amorphous and crystalline regions, and the amorphous regions have a lower density than the crystalline ones, so when cellulose fibres are subjected to harsh acid treatment, the amorphous regions break up, releasing individual crystallites called Nano-Cristalline-Cellulose (NCC) [Peng et al. 2011]. Pääkkö et al. [2007] introduced mild enzymatic hydrolysis combined with mechanical

shearing and high-pressure homogenization, leading to controlled fibrillation down to nanoscale and a network of long and highly entangled cellulose I elements. The resulting strong aqueous gels exhibit more than 5 orders of magnitude tunable storage modulus G' on changing the concentration. The resulting material was referred to as MFC (micro fibrillated cellulose). Dynamical rheology has shown that the aqueous suspensions behaved as gels. This could explain the thicker WCW after laccase treatment in acid conditions at pH 4.6.

Further experiments must be done to determine the exact chemical structure of the thickened WCW, such as Two-Dimensional Nuclear Magnetic Resonance (2D-NMR) and Matrix-Assisted Laser Desorption/ Ionization MALDI.

Conclusions

This study has shown that laccase influences the Norway spruce wood surface without the addition of mediators. It altered the properties of Norway spruce wood, improving wood resistance to wood decay and reducing copper leaching.

FTIR analysis indicated that laccase catalysed the depolymerization of wood cell wall components, not only lignin but also cellulose. The wood cell wall thickened, the pits were closed, suggesting that reduced leaching of copper was due to a mechanical barrier as a consequence of cell wall swelling.

To determine the exact components of the thickened WCW, further experiments should be performed using other available analytical methods. The mechanical properties of laccase treated Norway spruce wood should also be tested and further application of the material considered, since there is a strong future interest in using natural materials in biotechnology applications.

References

Blanchette R.A. [2000]: A review of microbial deterioration found in archaeological wood from different environments. International Biodeterioration and Biodegradation 46: 189--204

Chandra R.P., Ragauskas A.J. [2002]: Evaluating laccase – facilitated coupling of phenolic acids to high-yield kraft pulps. Enzyme and microbial tehnology 30: 855-861

Chandra R., Felby C., Ragauskas A.J. [2004]: Improving laccase-facilitated grafting of 4-hydroxybenzoic acid to highkappa kraft pulps. Journal of Wood Chemistry and Technology 24: 69-81

Claus H. [2003]: Laccase: structure, reactions, distribution. Micron 35: 93-96

Colom X., Carillo F., Nogues F., Gariga P. [2003]: Structural analysis of photodegraded wood by means of FTIR spectroscopy. Polymer Degradation and Stability 80: 543-549

Fackler K., Kuncinger T., Ters T., Srebotnik E. [2008]: Laccase-catalyzed functionalization with 4-hydroxy-3-methoxybenzylurea significantly improves internal bond of particle boards. Holzforschung 62: 223-229

Faix O. [1992]: Furier transform infrared spectroscopy. In: S.Z. Lin, C.W. Dnce (ed.), Methods in lignin chemistry. Springer, Berlin: 83-109

Felby C., Hassingboe J., Lund M. [2002]: Pilot scale production of fiberboards made by laccase oxidized wood fibers: board properties and evidence for cross-linking of lignin. Enzyme and Microbial Technology 31: 731-741

Green F., Highley T.L. [1997]: Mechanism of brown-rot decay: paradigm or paradox. International Biodeterioration and Biodegradation 39: 113-124

Humar M., Lesar B. [2008]: Fungicidal properties of individual components of copper--ethanolamine based wood preservatives. International Biodeterioration and Biodegradation 62 (1): 46-50

Humar M., Lesar B. [2009]: Influence of dipping time on uptake of preservative solution, adsorption, penetration and fixation of copper-ethanolamine based wood preservatives. European Journal of Wood and Wood Products 67: 265-270

Kim J.S., Gao J., Daniel G. [2015a]: Cytochemical and immunocytochemical characterization of wood decayed by the white rot fungus Pycnoporus sanguineus. I. preferential lignin degradation prior to hemicelluloses in Norway spruce wood. International Biodeterioration and Biodegradation 105: 30-40

Kim J.S., Gao J., Daniel G. [2015b]: Cytochemical and immunocytochemical characterization of wood decayed by the white rot fungus Pycnoporus sanguineus. II. Degradation of lignin and non-cellulosic polysaccharides in European ash wood. International Biodeterioration and Biodegradation 105: 41-50

Kirk T.K., Cullen D. [1998]: Enzymology and molecular genetics of wood degradation by white-rot fungi. In: R.A. Young, M. Akhtar (ed.), Environmentally friendly technologies for the pulp and paper industry. John Willey and Sons, Inc., New York: 273-307

Li Y., Dong X., Liu Y., Li J., Wang F. [2011]: Improvement of decay resistance of wood via combination treatment on wood cell wall: Swell-bonding with maleic anhydride and graft coploymerization with glycidyl methacrylate and methyl methacrylate. International Biodeterioration and Biodegradation 65: 1087-1094

Li Y., Liu Z., Dong X., Fu Y., Liu Y. [2013]: Comparison of decay resistance of wood and wood-polymer composite prepared by in-situ polymerization of monomers. International Biodeterioration and Biodegradation 84: 401-406

Martins L.O., Soares C.M., Pereira M.M., Teixeira M., Costa T., Jones G.H., Henriques A.O. [2002]: Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the Bacillus subtilis endospore coat. The Journal of Biolgical Chemistry 277: 18849-18859

Mayer A.M., Staples R.C. [2002]: Laccases: new functions for an old enzyme. Phytochemistry 60: 551-565

Pääkkö M., Ankerfors M., Kosonen H., Nykänen A., Ahola S., Österberg M., Ruokolainen J., Laine J., Larsson P.T., Ikkala O., Lindström T. [2007]: Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels. Biomacromolecules 8 (6): 1934-1941, doi:10.1021/bm061215p. PMID 17474776

Peng B.L., Dhar N., Liu H.L., Tam K.C. [2011]: Chemistry and applications of nanocrystalline cellulose and its derivatives: A nanotechnology perspective. The Canadian Journal of Chemical Engineering 89 (5): 1191-1206, doi:10.1002/cjce.20554

Raspor P., Smole-Možina S., Podjavoršek J., Pohleven F., Gogala N., Nekrep F.V., Rogelj I., Hacin J. [1995] ZIM: Collection of industrial microorganisms. Ljubljana, University of Ljubljana, Biotechnical Faculty 98

Rico A., Rencoret J., del Rio J.C., Martinez A.T., Gutierez A. [2015]:. In-Depth 2D NMR study of lignin modification during treatment of Eucalyptus wood with laccase and mediators. Bioenergy Resources 8: 211-230

Schilling J.S., Duncan S.M., Presley G.N., Filley T.R., Jurgens J.A., Blanchette R.A., [2013]: Colocalizing incipient reactions in wood degraded by the brown rot fungus *Postia placenta*. International Biodeterioration and Biodegradation 83: 56-62

Sharma P., Goel R., Capalash N. [2007]: Bacterial laccases. World Journal of Microbiology and Biotechnology 23: 823-832

Widsten P., Kandelbauer A. [2008]: Laccase application in the forest products industry: A review. Enzyme and Microbial Technology 42: 293-307

Yoshida H. [1883]: Chemistry of lacquer (urushi). Journal of Chemical Society 43: 472-486

List of standards

EN 1250-2:1994. Wood preservatives – Methods for measuring losses of active ingredients and other preservative ingredients from treated timber – Part 2: Laboratory method for obtaining samples for analysis to measure losses by leaching into water or synthetic sea water

EN 113:1989. Wood preservatives, Determination of the toxic values against wood destroying basidiomycetes cultured an agar medium

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