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Effect of *Physisporinus vitreus* on wood properties of Norway spruce. Part 2: Aspects of microtensile strength and chemical changes

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Abstract

The biotechnological application of the white rot fungus *Physisporinus vitreus* named ''bioincising'' is currently being investigated for permeability improvement of Norway spruce (*Picea abies* (L.) Karst.) wood. During short-term (<9 weeks) incubation, fungal activity induces degradation of pit membranes and a simultaneous alteration of the tracheid cell wall structure. In Part 1 of this article series, the occurrence of selective delignification and simultaneous degradation was shown by UV-microspectrophotometry (UMSP). Moreover, significant reduction of Brinell hardness was recorded after 7 and 9 weeks incubation. For a better understanding of the chemical alterations in the wood constituents and the corresponding changes of mechanical properties due to fungal activity, we applied microtensile tests on thin strips that were prepared from the surface of incubated Norway spruce wood. Indications for the occurrence of selective delignification and simultaneous degradation were evident. Determination of lignin content and carbohydrate analysis by borate anion exchange chromatography confirmed the results. The present study verifies the findings from Part 1 of this article series and from previously conducted microscopic investigations. Now, the degradation characteristics of *P. vitreus* are established and the bioincising process can be further optimized with higher reliability.

Keywords: chemical analysis; finite span; selective delignification; simultaneous degradation; zero span.

Introduction

Permeability improvement of Norway spruce (*Picea abies* (L.) Karst.) wood by means of a biotechnological method called ''bioincising'' has been investigated intensively during the past years (Schwarze and Landmesser 2000; Schwarze et al. 2006; Schubert et al. 2009; Schwarze and Schubert 2009; Lehringer et al. 2010; Lehringer et al. 2011). Hereby, the white rot fungus *Physisporinus vitreus* is supposed to selectively degrade the membranes of the bordered and half-bordered pits during short-term incubation times. The bioincised material shows significant improvement of liquid uptakes (Schwarze et al. 2006) but also anatomical changes of the cell wall structure due to fungal activity are reported by Lehringer et al. (2010). Not only pit membranes are degraded but also cell wall deterioration occurs, which is induced by a selective delignification and/or a simultaneous degradation of lignin and polysaccharides. The term selective delignification is applied to a successive decomposition of the cell wall components by highly diffusible ecto-enzymes. Preferably, the degradation of lignin and hemicelluloses occurs at the beginning of fungal colonization, while the cellulose fraction is degraded at rather later stages (Liese 1970; Schwarze et al. 1997; Martínez et al. 2005; Ray et al. 2005). Simultaneous rot is characterized by fungal ecto-enzymes, which have the capacity to degrade cellulose, hemicelluloses, and lignin at the same time (Schmidt 2006).

Analysis of Brinell hardness revealed a significant hardness reduction in the subsurface area after 7 and 9 weeks incubation (Lehringer et al. 2011). In the same work, the application of cellular UV-microspectrophotometry (UMSP) indicated chemical alterations of the main wood components in close vicinity of fungal hyphae.

For a successful upscaling of the bioincising process, the incubation period must be short (<4 weeks) and wood colonization homogeneous, in order to minimize adverse side effects such as structural alterations. To this purpose, a detailed knowledge of the degradation pattern of *P. vitreus* and of the resulting chemical alterations is crucial. Factors during incubation such as temperature, water activity, nutrient and oxygen supply have an influence on the homogeneity, the speed of substrate colonization and finally on the selectivity of fungal activity towards different cell wall components (Schubert et al. 2009, 2010). Especially, the carbon/nitrogen-ratio (C/N-ratio) has recently been discussed to affect the degradation characteristics of *P. vitreus* during incubation (Lehringer et al. 2010). The specimens in the present study were incubated on malt extract agar (MEA) that has a narrow C/N-ratio (50/1). It is well established that

a narrow C/N-ratio usually facilitates the degradation of polysaccharides by wood decay fungi (Levi and Cowling 1969; Kirk et al. 1978; Reid 1983; Dill and Kraepelin 1986; Rios and Eyzaguirre 1992). For investigating these aspects of chemical changes and the resulting micromechanic effects, the microtensile testing method appears to be a suitable approach. In the present study, the mechanical changes of the tracheid cell (wall) matrix during incubation times between 0 and 9 weeks were observed by this method.

Microtensile tests of microtomed (approx. 80 μm thick) wood strips were conducted in the past by various authors (Kennedy and Ifju 1962; Grozdits and Ifju 1969; Derbyshire et al. 1995; Turkulin and Sell 2002). Usually two measurement setups are applied. With the zero span method, the jaws holding the thin strip are initially in contact. All cellulose microfibrils in the cross section are bridging the infinitesimal gap between the jaws and the test is principally a measure of microfibril strength. As the cellulose microfibrils are the cell wall elements responsible for the longitudinal tensile strength of the wood structure, the zero span tensile strength is generally greater than any value of tensile strength determined in a finite span test. For the latter, the thin strips are mounted between the jaws with a free distance of 10 mm. This approach provides further information about the bonding properties between the fibers, because the middle lamella (where the highest lignin content is found) will additionally react sensitively on the tensile stresses.

In the present study, the selective delignification and simultaneous degradation were in focus. The hypothesis was that finite span strength (f-strength) and zero span strength (z-strength) will be indicative with this regard. Materials were characterized by microtensile tests in various contexts (Derbyshire et al. 1995; Jirous-Rajkovic et al. 2004; Gierlinger and Burgert 2006; Keunecke and Niemz 2008; Eder et al. 2009; Xiao et al. 2010; Xie et al. 2010). However, to our knowledge, the application of this approach to decayed wood is limited to the study of Wilcox and Garcia (1968). The method of microtensile testing is advantageous if mechanical properties in the border zone are in focus, where fungal activity is the highest and where the permeability improvement occurs. In contrast to impact bending-, tension-, compression- or shear-tests aiming at the properties of the entire specimen, this method affords detailed information exclusively from the surface of the specimen, as was demonstrated by Derbyshire et al. (1995), Jirous-Rajkovic et al. (2004) and Turkulin et al. (2006).

Subsequently, wet chemical analysis was performed in the present study to provide further information about the degradative activity of *P. vitreus*. Wet chemical analysis is useful for investigation of wood decaying fungi, as reported by Crawford et al. (1982), Rabinovich et al. (2004), Istek et al. (2005) and Schmutzer et al. (2008).

The main objective of the present study is to elucidate the mechanical and chemical changes that are induced by *P. vitreus* at the subsurface area during short-term incubation of spruce wood. The enzymatic activity of *P. vitreus* was in focus in terms of a discrete selective delignification and a simultaneous degradation under certain incubation conditions. The expectation was that the results will contribute to the further improvement of the bioincising incubation process.

Material and methods

Specimen material

One board of defect-free and kiln-dried wood from a Norway spruce tree [*Picea abies* (L.) Karst.] was investigated. For sapwood (SW) and heartwood (HW), three specimen collectives were prepared. In each collective, five specimens were always taken in one longitudinal sequence in order to minimize the influence of natural property variation within the single tree. As the samples were subjected to four different incubation periods and one control, this axial pairing provided a good comparability of the results. Each specimen measured $200\times30\times30$ mm^3 (L×R×T). After sterilization, the specimens were incubated with the white rot fungus *P. vitreus* for 3, 5, 7, and 9 weeks at 22°C and 70% RH. For a detailed description of material selection and specimen preparation, see Lehringer et al. (2011). Density and mass loss were calculated as described in Lehringer et al. (2010).

Chemical analysis was conducted on smaller wood specimens of Norway spruce SW and HW ($L\times R\times T=100\times15\times10$ mm^3) that were incubated separately, but exactly following the same incubation routine as used for the larger specimens in Lehringer et al. (2011). Three replicates for each incubation time were prepared. In contrast to the larger wood specimens, mass losses were determined after drying to a moisture content of 0% to provide a definite basis for further calculations.

Microtensile testing

SW and HW specimens from all three collectives were taken for thin strip preparation (Table 1). Blocks of $L\times R\times T=80\times10\times30$ mm^3 were prepared from the bottom surface of the incubated specimens, where fungal activity was most pronounced (see also Lehringer et al., 2011).

The blocks were vacuum impregnated with distilled water at ambient temperature until fully saturated. Sequential microtoming of 80 μm thin strips ($n=20$ per block) in the longitudinal-radial plane was conducted with slight inclination of the specimens at an angle of approximately 5° to the radial plane in order to avoid disturbing influences by the wood rays.

Instrument for thickness control of each single strip (on an average of 5 points on each strip): electronic thickness gauge (Mitutoyo, Kawasaki, Japan, accuracy ±0.1 μm). All strips missing the target thickness by ±5% were rejected, resulting in 12–19 strips per block,

Table 1 Experimental plan for microtensile strip preparation for sapwood (SW) and heartwood (HW) from Norway spruce treated with *Physisporinus vitreus*.

Description	Variable	Factor
Wood type	SW, HW	2
Collective	A, B, C	3
Incubation time	0*, 3, 5, 7, 9 weeks	5
Side on specimen	Bottom	1
Replicates	No. of prepared strips	20
Result from factor multiplication:		n=600

*Control.

thus the total number of tested strips was $n = 528$. Each strip was then cut transversally into two parts, which then were measured separately at finite span and zero span.

Instrument for microtensile testing: paper tester (Pulmac International Inc., Montpelier, VT, USA) under standard laboratory conditions of 20°C, 65% RH. The ultimate breaking load was recorded in pounds per square inch (Psi) and recalculated to Newton (N). The clamping pressure was set to 0.55 MPa for finite span and 0.62 MPa for zero span and the loading rate was set to 70 kPa s^{-1} for all measurements.

Chemical analysis

For two step hydrolysis and lignin determination all incubated specimens were ground in a vibration mill (Herzog, Osnabrück, Germany). The milled powder was then conditioned at 20°C and 65% RH and moisture content was determined for further calculations. A two step-hydrolysis with 72% H_2SO_4 for 1 h at 30°C and 2.6% H_2SO_4 for 40 min at 120°C was conducted. After filtration, 1 ml of the hydrolyzate was removed for analysis in the Borate anion exchange chromatography. Lignin content was determined as hydrolysis residue (Willför et al. 2009) and acid-soluble lignin was measured by UV-spectroscopy ($\lambda = 205$ nm) according to Tappi 250 (2005).

Borate anion exchange chromatography for carbohydrate analysis Columns (Omnifit®, Bio-Chem Valve, Boonton, NJ, USA; 7×11.5 mm) were filled with anion exchange MCI Gel CA08F (Mitsubishi Chemical Corporation, Tokyo, Japan) resin. The mobile phase: (A) 0.3 M potassium tetraborate and (B) 0.9 M potassium tetraborate at 0.7 ml min^{-1}; conditions of gradient elution: 0 min: 90% A, 10% B; 35 min: 10% A, 90% B; 47 min: end. Post-column derivatization: by addition of cubicinchoniate (0.35 ml min^{-1}) and subsequent heating to 105°C in a 0.3 mm Teflon coil. Detection at 560 nm: UV-VIS-detector (Sinner et al. 1975; Sinner and Puls 1978; Willför et al. 2009).

The amounts of detected monosaccharides were corrected for water uptake during hydrolysis. The % losses for cellulose, xylan, and mannan (Table 2, columns 12–15) were determined corresponding to the constitution of their monosaccharides, respectively, the arabinoglucuronoxylans and galactoglucomannas of softwoods. According to (Timell 1967) and Janzon et al. (2008) the carbohydrates were calculated with Eq. (1), (2), and (3) where the mannose/glucose-ratio is defined with 3:1.

$$\text{Xylan} = \text{Xyl} + \text{4-O-MeGlcA} + \text{Ara} \quad (1)$$

$$\text{Mannan} = \text{Man} + \text{Gal} + (\text{Man}/3) \quad (2)$$

$$\text{Cellulose} = \text{Glc} - (\text{Man}/3) \quad (3)$$

Data analysis

For all collected data, a one way ANOVA and a Tukey honesty test were conducted with the statistic software Systat12® (Systat Software Inc., Chicago, IL, USA). A probability value of $P < 0.05$ was considered to print to significant differences.

Results and discussion

Mictrotensile

Expectedly, the f-strength was always at least one-third lower than the z-strength (Table 3). In the former, the bigger influ-

Table 2 Contents of lignin, composition of monomeric sugars in hydrolyzates and percent losses of wood constituents after incubation of Norway spruce with *Physisporinus vitreus*.

	Incub. time (weeks)	Mass loss (%)	Results of total hydrolysis (abs. %)							Resulting losses of polymers (%)				
			Lignin*	Glc	Xyl	Man	Ara	Gal	Ram	4-O-Me-GluA	Lignin	Cellulose	Xylan	Mannan	Selectivity**
SW	Control	–	26.5	50.1	5.6	12.8	1.1	1.1	0.1	0.6	–	–	–	–	–
	3	0.1±0.1	26.5	49.0	5.4	12.6	1.1	1.1	0.1	0.6	-0.01	-0.9	-0.2	-0.4	0.01
	5	1.8±0.2	25.9	48.3	5.2	12.0	1.2	1.0	0.1	0.7	-0.6	-1.4	-0.3	-1.1	0.46
	7	5.5±0.3	25.0	46.3	4.9	11.4	1.1	0.9	0.1	0.6	-1.5	-2.9	-0.6	-1.8	0.5
	9	8.2±0.6	24.7	45.2	4.9	11.7	1.1	0.9	0.1	0.6	-1.8	-4.0	-0.6	-1.6	0.45
HW	Control	–	27.7	50.1	4.6	12.6	1.1	0.9	0.1	0.6	–	–	–	–	–
	3	0.1±0.05	27.4	49.7	4.5	12.5	1.1	0.8	0.1	0.5	-0.3	-0.3	-0.1	-0.3	0.8
	5	2.4±0.7	27.0	47.7	4.3	12.0	1.1	0.8	0.1	0.5	-0.7	-1.9	-0.2	-1.0	0.35
	7	3.9±0.8	26.1	47.3	4.3	11.7	1.0	0.8	0.1	0.5	-1.6	-2.2	-0.3	-1.2	0.72
	9	6±1.6	25.7	47.3	4.3	11.7	0.9	0.9	0.1	0.4	-2.0	-2.2	-0.4	-1.2	0.91

*Hydrolyses residue and acid soluble lignin, **selectivity: lignin loss/cellulose loss.

Table 3 Absolute values of microtensile strength of Norway spruce sapwood (SW) and heartwood (HW) incubated with *Physisporinus vitreus*.

	Incubation time (weeks)	Mass loss (%)	Zero span (N)	Finite span (N)	Ratio finite span/zero span
SW	Control	–	96.8±4.4	66.6±6.2	0.7
	3	0.1±0.2	96.8±6.8	55.1±7.1	0.6
	5	1.2±0.5	79.9±8	50.2±7.9	0.6
	7	2.4±0.7	69.4±9.6	30.9±4.5	0.4
	9	2.9±0.8	49.5±5	23±6.2	0.5
HW	Control	–	91±9.6	60.4±10.5	0.7
	3	1.1±0.5	89.3±9.5	64.8±10.2	0.7
	5	1.3±0.6	98.9±8.1	68±9.3	0.7
	7	2.1±0.8	69.6±8.7	44.7±8.2	0.6
	9	2.4±1.4	60.2±14.7	41.5±5.3	0.7

ence of plastic deformations and inter-tracheid bonding in the compound middle lamella (CML) generally causes lower strength values (Derbyshire et al. 1995; Turkulin and Sell 2002).

The SW specimens showed a significant loss of z-strength after 5 weeks incubation (Figure 1). After 9 weeks, z-strength retention was 51.1 ± 10%. In comparison, f-strength of the SW specimens decreased significantly after 3 weeks incubation and resulted in a minimum strength retention of 34.6 ± 26.9% after 9 weeks. The strength decrease follows a linear function.

The effect of *P. vitreus* on the HW specimens was less pronounced. A significant drop of both z- and f-strength was recorded after 7 weeks and the strength retention after 9 weeks was 66.1 ± 24.4% for z-strength and 68.7 ± 12.9% for f-strength. Obviously and expectedly, the degradation rate in SW is higher than in HW, as the degradative activity of wood decay fungi is higher in SW than in HW due to a lower level of extractives and a better accessibility of fungi to nutrients and carbohydrates (Rypacek 1966; Schmidt 2006).

The consequence is that the results for HW specimens are not as clear as for SW specimens. For the former, partly increasing values of f-strength (3 and 5 weeks) and of z-strength (5 weeks) were recorded (Figure 1). To our knowledge, it has not yet been reported that increasing tensile

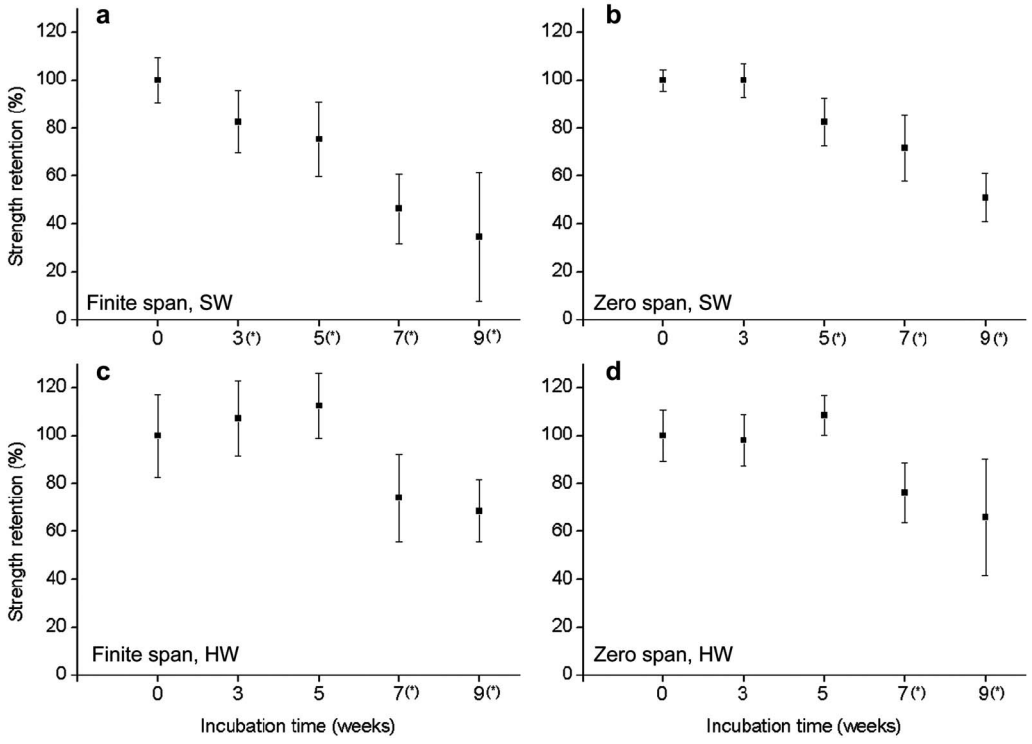

Figure 1 Effect of incubation with *Physisporinus vitreus* on microtensile strength of Norway spruce sapwood (SW) and heartwood (HW). (*) = significant changes compared to control (zero weeks).

strength may not occur during white rot, brown rot, and soft rot degradation. Enzymatic depolymerization, hydrolysis and oxidative reactions occur that commonly reduce the mechanical integrity of the wood. Turkulin and Sell (2002) discussed the initial increase of z-strength that they observed during photodegradation of softwoods by ultraviolet radiation. They proposed an initial 'radiation induced cross-linking' between cellulose microfibrils that was later followed by high strength losses. However, this hypothesis is not convincing for fungal degradation, unless one accepts the dominance of radical degradation mechanisms by means of mediators. Thus, the minor increases of microtensile strength must rather be explained with statistical effects, such as a deviation of the measured data from the normal distribution. The lower mass losses in HW may further support this hypothesis.

In HW, the ratio f/z-strength ranged for all incubation times between 0.6 and 0.7, while for SW a larger span was recorded with ratios down to 0.4 and 0.5 after 7 and 9 weeks incubation, respectively (Table 3). Turkulin and Sell (2002) conducted microtensile tests on photodegraded thin strips and reported similar ratios of f- and z-strength. The reduced values for SW samples with longer incubation time are due to a relatively stronger decrease of f-strength than z-strength, as can also be seen in Figure 1. It thus can be hypothesized that in SW the ratio between delignification and degradation of polysaccharides is changing while in HW all components are degraded with a more or less constant ratio.

The higher losses in SW f-strength can be explained by a combination of the delignification processes and the degradation of cellulose microfibrils. The microscopic and topochemical investigations on bioincised wood by Lehringer et al. (2010, 2011) revealed local regions of a selective delignification of the tracheid cell walls by *P. vitreus* that is commencing from the lumen through the secondary cell wall towards the CML. Even though the cell wall structure remains mostly intact during the first 9 weeks of incubation, it is probable that the progressing delignification also weakens the bonding properties of the CML, resulting in interfiber slippage effects.

Additionally, the degradation of SW cellulose, as will be shown by chemical analysis below, contributes to a loss of f-strength. The progressing weakening of the tracheid cell wall due to hyphal tunneling, cavities, and notches (as reported by Lehringer et al. 2010, 2011), chain length reduction of the microfibrils (indicated by decreasing degrees of polymerization, DP), and lignin depolymerization leads to a pronounced reduction of tensile strength.

The microtensile strengths showed a stepwise decrease after 7 and 9 weeks (Figure 1a,c,d). Correspondingly, studies on morphological changes, Brinell hardness and delignification determined by UMSP also displayed stronger effects of *P. vitreus* after 7–9 weeks incubation (Lehringer et al. 2010, 2011). Hence, incubation times should be kept significantly below 7 weeks to avoid major adverse effects on the mechanical wood properties.

Chemical analysis

Weight losses of the specimens from chemical analysis ranged between $0.1 \pm 0.1\%$ and $8.2 \pm 0.6\%$ in SW and $0.1 \pm 0.05\%$ and $6 \pm 1.6\%$ for HW (Table 2). These values are higher than the recorded mass losses of the microtensile tesed specimens (Table 3). This is due to the fact that the specimens incubated for chemical analysis provided a smaller volume and thus the absolute losses by fungal degradation resulted in virtually higher mass losses. Still, *P. vitreus* is shown to be a wood decay fungus with comparable low degradation rates, as discussed previously by Lehringer et al. (2011).

In SW and HW, the lignin degradation commences at almost equal rates and shows absolute losses of -1.8% and -2%, respectively, after 9 weeks incubation (Figure 2). The xylan reduction after 9 weeks was slightly lower in HW specimens (-0.6% SW > -0.4% HW) as well as the mannan loss (-1.6% SW > -1.2% HW).

A difference was recorded for cellulose degradation; in SW, the cellulose degradation proceeded rapidly after 3 weeks incubation and showed a continuously strong reduc-

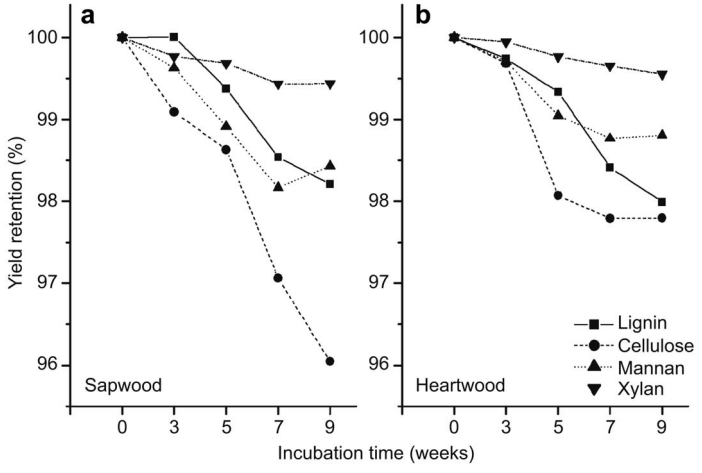

Figure 2 Effect of incubation with *Physisporinus vitreus* on chemical composition of Norway spruce sapwood and heartwood.

tion rate of -4% after 9 weeks incubation. This relatively strong linear decrease could be an explanation for the reduction of f- and z-strengths (Figure 1a,b). The relatively high diminishing content of cellulose in SW appears to suggest the activity of a soft rot type I and II in the latewood tracheids cell walls, as shown by Lehringer et al. (2010, 2011). In contrast, the HW specimens showed a strong cellulose decrease after 3 and 5 weeks but then the degradation level remained stable with -2.2% after 7 and 9 weeks incubation.

The selectivity for lignin degradation can be expressed by the ratio of lignin loss/cellulose loss (Table 2, column 16). The ratio was approximately 0.5 for SW and between 0.3 and 0.9 for HW, which indicates a rather low selectivity for lignin degradation. When Hakala et al. (2004) conducted a study with 86 isolates of white rot fungi on Norway spruce at 10 weeks incubation, they found 17 strains that showed a selectivity ratio >1.0; among other fungi, *Physisporinus rivulosus T241i* also showed a high selectivity for lignin degradation (ratio 3.3). In the latter study, wood specimens were incubated on vermiculite that is known to possess a wide C/N-ratio and stimulates a range of white rot fungi (probably also *P. vitreus*) and causes a selective delignification (Lehringer et al. (2010). The present study provides additional evidence that incubation of wood specimens on malt extract agar (MEA) (i.e., with a narrow C/N-ratio), as was the case in this work, results in sub-optimal incubation conditions for a selective delignification or a selective pit membrane degradation (see also Dill and Kraepelin 1988; Rios and Eyzaguirre 1992).

Conclusions

The method of microtensile testing was successfully applied to bioincised specimens in order to investigate the mechanical and chemical changes in the border zone occurring during incubation. *P. vitreus* is a variable decay fungus that leads to different degradation patterns in the same wood sample with moderate degradation rates. The high data variability complicates an unambiguous interpretation of the results (see also Lehringer et al. 2010, 2011). Nevertheless, results from microtensile testing confirm the findings of our previous studies. Many effects caused by the fungus are close to the detection limit of some wet chemical analytical methods. However, chemical analysis in the present paper shows the degradation of lignin, hemicelluloses and, even to a higher extent, of cellulose.

Incubation on MEA obviously triggered *P. vitreus* to induce both a selective delignification and a simultaneous degradation, reflecting the degradation patterns of a white rot fungus and soft rot type I and II. Thus, incubation with a wide C/N-ratio nutrient medium might be favorable to increase selective delignification and pit membrane degradation. These findings will be implemented in the future optimization of the incubation process for the bioincising technology.

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References

Crawford, D.L., Barder, M.J., Pometto Iii, A.L., Crawford, R.L. (1982) Chemistry of softwood lignin degradation by *Streptomyces viridosporus*. Arch. Microbiol. 131:140–145.

Derbyshire, H., Miller, E.R., Turkulin, H. (1995) Investigations into the photodegradation of wood using microtensile testing – Part 1: the application of microtensile testing to measurement of photodegradation rates. Holz Roh-Werkst. 53:339–345.

Dill, I., Kraepelin, G. (1986) Palo podrido: model for extensive delignification of wood by *Ganoderma applanatum*. Appl. Environ. Microbiol. 52:1305–1312.

Dill, I., Kraepelin, G. (1988) Degradation of Lignin/Cellulose by white-rot fungi: influence of Specific Ecological Factors. Forum Mikrobiol. 11:484–489.

Eder, M., Jungnikl, K., Burgert, I. (2009) A close-up view of wood structure and properties across a growth ring of Norway spruce (*Picea abies* [L] Karst.). Trees-Struct. Funct. 23:79–84.

Gierlinger, N., Burgert, I. (2006) Secondary cell wall polymers studied by confocal raman microscopy: spatial distribution, orientation, and molecular deformation. NZ J. Forest Sci. 36:60–71.

Grozdits, G.A., Ifju, G. (1969) Development of tensile strength and related properties in differentiating coniferous xylem. Wood Sci. 1:137–147.

Hakala, T.K., Maijala, P., Konn, J., Hatakka, A. (2004) Evaluation of novel wood-rotting polypores and corticioid fungi for the decay and biopulping of Norway spruce (*Picea abies*) wood. Enzyme Microb. Technol. 34:255–263.

Istek, A., Sivrikaya, H., Eroglu, H., Gulsoy, S.K. (2005) Biodegradation of *Abies bornmülleriana* (Mattf.) and *Fagus orientalis* (L.) by the white rot fungus *Phanerochaete chrysosporium*. Int. Biodeter. Biodegr. 55:63–67.

Janzon, R., Saake, B., Puls, J. (2008) Upgrading of paper-grade pulps to dissolving pulps by nitren extraction: properties of nitren extracted xylans in comparison to NaOH and KOH extracted xylans. Cellulose 15:161–175.

Jirous-Rajkovic, V., Turkulin, H., Miller, E.R. (2004) Depth profile of UV-induced wood surface degradation. Surf. Coating Int. B Coating. Trans. 87:241–247.

Kennedy, R.W., Ifju, G. (1962) Applications of microtensile testing to thin wood sections. Tappi 45:725–733.

Keunecke, D., Niemz, P. (2008) Axial stiffness and selected structural properties of yew and spruce microtensile specimens. Wood Res-Slovakia 53:1–14.

Kirk, T.K., Schultz, E., Connors, W.J. (1978) Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol. 117:277–285.

Lehringer, C., Hillebrand, K., Richter, K., Arnold, M., Schwarze, F.W.M.R., Militz, H. (2010) Anatomy of bioincised Norway spruce wood. Int. Biodeter. Biodegr. 64:346–355.

Lehringer, C., Koch, G., Adusumalli, R.B., Mook, W.M., Richter, K., Militz, H. (2011) Effect of *Physisporinus vitreus* on wood properties of Norway spruce. Part 1: Aspects of delignification and surface hardness. Holzforschung 65:711–719.

Levi, M.P., Cowling, E.B. (1969) Role of nitrogen in wood deterioration. VII. Physiological adaptation of wood-destroying and other fungi to substrates deficient in nitrogen. Phytopathology 59:460–468.

Liese, W. (1970) Ultrastructural aspects of woody tissue disintegration. Annu. Rev. Phytopathol. 8:231–257.

Martínez, Á.T., Speranza, M., Ruiz-Dueñas, F.J., Ferreira, P., Camarero, S., Guillén, F., Martínez, M.J., Gutiérrez, A., Del Río, J.C. (2005) Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol. 8:195–204.

Rabinovich, M.L., Bolobova, A.V., Vasil'chenko, L.G. (2004) Fungal decomposition of natural aromatic structures and xenobiotics: a review. Appl. Biochem. Microbiol. 40:1–17.

Ray, M., Kleist, G., Murphy, R. (2005) Decay assessment in a hardwood handrail at the South Bank, London. J. Inst. Wood Science. 17:51–58.

Reid, I.D. (1983) Effects of nitrogen supplements on degradation of aspen wood lignin and carbohydrate components by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 45:830–837.

Rios, S., Eyzaguirre, J. (1992) Conditions for selective degradation of lignin by the fungus *Ganoderma australis*. Appl. Microbiol. Biotechnol. 37:667–669.

Rypacek, V. Biologie holzzerstörender Pilze. Gustav Fischer Verlag, Jena, 1966.

Schmidt, O. Wood and tree fungi. Springer Verlag, Berlin, Heidelberg, 2006.

Schmutzer, M., Schwanninger, M., Fackler, K., Messner, K., Gradinger, C. (2008) Comparison of methods to evaluate the potential of fungal growth on decay of spruce wood after short-time treatment. Int. Biodeter. Biodegr. 61:319–324.

Schubert, M., Dengler, V., Mourad, S., Schwarze, F.W.M.R. (2009) Determination of optimal growth parameters for the bioincising fungus *Physisporinus vitreus* by means of response surface methodology. J. Appl. Microbiol. 106:1734–1742.

Schubert, M., Mourad, S., Schwarze, F. (2010) Radial basis function neural networks for modeling growth rates of the basidiomycetes *Physisporinus vitreus* and *Neolentinus lepideus*. Appl. Microbiol. Biotechnol. 85:703–712.

Schwarze, F.W.M.R., Lonsdale, D., Fink, S. (1997) An overview of wood degradation patterns and their implications for tree hazard assessment. Arboric. J. 21:1–32.

Schwarze, F.W.M.R., Landmesser, H. (2000) Preferential degradation of pit membranes within tracheids by the basidiomycete *Physisporinus vitreus*. Holzforschung 54:461–462.

Schwarze, F.W.M.R., Landmesser, H., Zgraggen, B., Heeb, M. (2006) Permeability changes in heartwood of *Picea abies* and *Abies alba* induced by incubation with *Physisporinus vitreus*. Holzforschung 60:450–454.

Schwarze, F.W.M.R., Schubert, M. (2009) Enhanced uptake of wood modification agents in ''bioincised'' wood. International Research Group on Wood Protection Paper prepared for the 40th Annual Meeting Beijing, China 24–28 May 2009:1–8.

Sinner, M., Puls, J. (1978) Non-Corrosive dye reagent for detection of reducing sugars in borate complex ion-exchange chromatography. J. Chromatogr., A 156:197–204.

Sinner, M., Simatupang, M.H., Dietrichs, H.H. (1975) Automated quantitative analysis of wood carbohydrates by borate complex ion exchange chromatography. Wood Sci. Technol. 9:307–322.

Tappi 250 (2005) Acid-soluble lignin in wood and pulp. Tappi Usefull Methods 250.

Timell, T.E. (1967) Recent progress in the chemistry of wood hemicelluloses. Wood Sci. Technol. 1:45–70.

Turkulin, H., Sell, J. (2002) Investigations into the photodegradation of wood using microtensile testing. Part 4: tensile properties and fractography of weathered wood. Holz Roh-Werkst. 60:96–105.

Turkulin, H., Arnold, M., Richter, K., Strub, E., Jirouš Rajković, V., Mihulja, G. (2006) Improvements in photoprotection of wood. Final report of a research project BÖHME AG Lack- und Farbenfabrik:67.

Wilcox, W.W., Garcia, B.J. (1968) Changes in wood properties at the boundary of polyporus amarus decay pockets. Wood Sci. Technol. 2:115–127.

Willför, S., Pranovich, A., Tamminen, T., Puls, J., Laine, C., Suurnäkki, A., Saake, B., Uotila, K., Simolin, H., Hemming, J., Holmbom, B. (2009) Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides – A comparison between different hydrolysis and subsequent chromatographic analytical techniques. Ind. Crops Prod. 29:571–580.

Xiao, Z., Xie, Y., Militz, H., Mai, C. (2010) Effects of modification with glutaraldehyde on the mechanical properties of wood. Holzforschung 64:475–482.

Xie, Y., Xiao, Z., Goodell, B., Jellison, J., Militz, H., Mai, C. (2010) Degradation of wood veneers by Fenton's reagents: effects of wood constituents and low molecular weight phenolic compounds on hydrogen peroxide decomposition and wood tensile strength loss. Holzforschung 64:375–383.

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