Holzforschung, Vol. 63, pp. 639–646, 2009 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/HF.2009.087

Resistance of thermo-hygro-mechanically (THM) densified wood to degradation by white rot fungi

Oleksandr Skyba[1,2,*], Peter Niemz[2] and Francis W.M.R. Schwarze[1]

[1] Swiss Federal Laboratories of Material Testing and Research (EMPA), Wood Laboratory, St. Gallen, Switzerland

[2] ETH Zurich, Institute for Building Materials, ETH-Hönggerberg, Zurich, Switzerland

*Corresponding author.
Swiss Federal Laboratories of Material Testing and Research (EMPA), Wood Laboratory, Lerchenfeldstr. 5, CH-9014, St. Gallen, Switzerland
Phone: +41-71-2747617
Fax: +41-71-2747694
E-mail: askyba@gmail.com

Abstract

Colonisation and degradation by the white rot fungi, *Trametes versicolor* and *T. pubescens*, were studied in wood of Norway spruce and beech subjected to three different treatments: (1) hygro-thermal treatment (160°C and 180°C), (2) mechanical densification, and (3) thermo-hygro-mechanical (THM) treatment including densification and post-treatment at different temperatures (140°C, 160°C and 180°C). The weight losses induced by the fungi were lowest in THM-densified woods. However, volume related numerical indicators for decay susceptibility did not show any significant improvements of THM-densified woods against both fungi. Analysis of the chemical composition of treated wood species revealed slight alterations in the content of polysaccharides and lignin. White rot fungi circumvented conditions restricting hyphal growth within the occluded tracheid lumina by hyphal tunnelling in the secondary walls of fibre tracheids in beech or by forming bore holes that transversally penetrated cell walls of earlywood tracheids in THM-densified spruce. The studies indicate that THM-densified beech and Norway spruce wood may have some potential in utility class 3 but are inappropriate for use in utility class 4.

Keywords: beech; decay susceptibility; lignin; Norway spruce; polysaccharides; thermo-hygro-mechanical (THM) densification; white rot fungi; wood modification.

Introduction

Owing to environmental concerns regarding certain types of wood preservatives, there has been a renewed interest in wood modification, such as thermo-hygro-mechanical (THM) densification, which combines high temperature, moisture and mechanical compression. Compressed densified wood has not become a common wood product due to its instability in the presence of moisture. An improved densification method was presented in 1998 which included steaming to give shape fixation (Ito et al. 1998). Further improvements to densification were published by Navi and Girardet (2000) that led to production of a stable compressed wood with improved set recovery. The densification consisted of two steps: at first, the samples were plasticized in saturated steam at 140°C and then densified under controlled displacement mode. After densification, the samples were post-treated with steam which was found to be effective to reduce shape recovery (Inoue et al. 1993; Ito et al. 1998). THM-densification results in a product with improved strength properties (Morsing 1998; Navi and Heger 2004) decreased shape recovery (Navi and Heger 2004) and reduced hygroscopicity (Inoue et al. 2000; Navi and Heger 2004).

Previous studies have shown that THM-densified Norway spruce wood has enhanced durability against colonisation and degradation by brown rot fungi (Schwarze and Spycher 2005), because hyphal growth is suppressed as a result of the occlusion of cell lumina. However, studies of the resistance of THM-densified beech and Norway spruce wood to soft rot fungi revealed that both types of wood are highly susceptible and therefore inappropriate for use in utility class 4, i.e., in contact with ground or fresh water (Skyba et al. 2008).

Studies on wood resistance often involve laboratory decay experiments and the results are almost always expressed in dry weight loss (WL, %). As suggested by Nilsson and Daniel (1992), WL provides a useful measure of the amount of the decay, but problems may arise when comparisons are being made between timbers of varying densities. Assessment of decay rates of density timbers provides information on *decay susceptibility* (Butcher and Nilsson 1982). As the densities for untreated wood and THM-densified wood lie in the range of 0.3–1.3 g cm^{-3}, the decay susceptibility was calculated taking into account the volume of the specimen, besides traditional weight loss measurements. The results were expressed in g cm^{-3} and corroborated the viewpoint that the amount of wood consumed per volume unit provided a better assessment for degrading activity of decay organism than WL, when comparisons are required for wood blocks of varying density.

The objectives of the present study were to assess the durability of THM-densified Norway spruce (*Picea abies* L.) and beech (*Fagus sylvatica* L.) against white rot fungi and to investigate the chemical alterations of the constituents of wood following THM treatment. The associated hyphal colonisation patterns and degradation modes should be described. For this purpose, samples of Norway spruce and beech wood were exposed to a range of thermal and densification treatments and incubated with the white rot fungi, *Trametes pubescens* and *T. versicolor*.

Materials and methods

TH treatment, mechanical densification and THM-densification

A total of 100 specimens of Norway spruce and beech wood were subjected to the two-stage THM procedure of Navi and Girardet (2000), involving densification and post-treatment in saturated steam at different conditions (SSC). The following nomenclature describes the parameters:

- Control: untreated controls;
- TH 160: heat treatment at 160°C under SSC;
- TH 180: heat treatment at 180°C under SSC;
- Densified: mechanical densification under SSC without post-treatment;
- THM 140: densification and post-treatment at 140°C under SSC;
- THM 160: densification and post-treatment at 160°C under SSC;
- THM 180: densification and post-treatment at 180°C under SSC;
- THM 180/80: densification under SSC and post-treatment at 180°C under 80% relative humidity (RH).

For precise details, see Table 1 in Skyba et al. (2008).

White rot test

The fungi investigated included *Trametes versicolor* (L.) Lloyd (EMPA isolate 159*) and *T. pubescens* (Schumach.) Pilát (EMPA isolate 229).

Pure cultures were confirmed using mycelial characteristics as observed on plates of malt extract agar (MEA). Test specimens, $50\times25\times15$ mm³, were taken from reference controls, densified, TH- and THM-treated specimens of Norway spruce and beech wood and prepared according to EN 113 (CEN 1996). The specimens were dried at 100°C for 24 h, cooled in a desiccator and weighed, sterilised with ethylene oxide for approximately 5 h, dried at 65°C for 48 h and cooled, and then two wood specimens were inoculated and incubated in Kolle flasks with fungi according to EN 113 (CEN 1996).

For each treatment and period of fungus combination and incubation, six specimens and six controls were investigated. The specimens were incubated in a random array at 22 ± 1°C and 70 ± 5% RH for 16 weeks. After incubation, the wood specimens were removed from the Kolle flasks, cleaned from the adjacent mycelium and weighed. Chips of negligible weight were removed from randomly selected sites, and placed on MEA to check whether the decay fungi were the only micro-organisms present. This was confirmed in all cases. Then, the test samples were dried for WL measurements.

Decay susceptibility (DS) according to Nilsson and Daniel (1992) was used for assessing the decay rate of wood of different densities:

$$DS = \frac{W_0\text{-}W_1}{V} \quad (1)$$

where DS is decay susceptibility, W_0 is original dry weight of the wood specimen, W_1 is dry weight after decay, and V is volume of the specimen.

The result is the amount of wood consumed per volume and expressed as g cm^{-3}.

Microscopy

The incubated test specimens were subdivided into samples of approximately $20\times5\times5$ mm³, with the transverse, radial and tangential surfaces exposed to examination, fixed in 2% glutaraldehyde buffered at pH 7.2–7.4, dehydrated with acetone and embedded in a methacrylate medium (Schwarze and Fink 1998), then sectioned at approximately 2 or 4 μm using a rotary microtome (Leica® 2040 Supercut) fitted with a diamond knife. For general observation of cell wall degradation and hyphal growth, sections were stained for 12 h in safranin and then counterstained for 3 min in methylene blue and 30 min in auramine. Micrographs were taken with a microscope fitted with a camera system (Leica® DC 50, TWAIN). Longitudinal sections were cut at a depth of 10 mm and at the surface.

Carbohydrate analysis

Due to technical restrictions, one wood sample (prior to white rot decay test) per treatment was subjected to analysis.

Table 1 Relative composition of monomeric sugars in hydrolysates (rel. %), contents of lignin and extractives in untreated controls, densified, TH- and THM-treated Norway spruce and beech.

	Composition of sugars found in hydrolysates (relative %)							Klason lignin	Extractives
Treatment	Glu	Xyl	Man	Gal	Ara	Ram	4-O-Me-GluA	(%)[a]	(%)[b]
Spruce									
Control	71	6.9	17.7	1.8	1.5	0.2	0.9	26.7	1.61
TH 160	71.9	6.9	18.2	1.7	0.5	0.2	0.6	29.7	4.72
TH 180	81.5	4.6	13.4	0.5	–	–	–	32.2	10.9
Densified	69.8	7.4	18.7	1.9	1.2	0.3	0.8	26.1	2.17
THM 140	72.2	7.2	17.2	1.8	0.8	0.2	0.7	26.9	2.11
THM 160	73.1	6.6	17.8	1.5	0.5	0.1	0.4	30.9	2.76
THM 180	77.5	5.7	15.9	0.8	–	–	0.2	32.3	5.56
THM 180/80	73.8	6	19	1	–	–	0.2	31.2	2.9
Beech									
Control	63.4	28.8	4.1	0.8	0.5	0.5	1.9	24.1	0.73
TH 160	67.2	27.1	2.9	0.7	0.3	0.5	1.4	24.4	7.27
TH 180	67.4	27.3	2.3	0.7	0.3	0.4	1.6	23.9	4.85
Densified	64.9	28.9	2.4	0.8	0.7	0.5	1.9	24.8	0.96
THM 140	63.7	30.8	2.1	0.8	0.4	0.6	1.6	25.1	2.89
THM 160	67.2	27.5	2.4	0.6	0.2	0.4	1.6	25.1	3.73
THM 180	77.3	18.2	0.5	2.4	0.2	0.6	0.8	24.8	4.51
THM 180/80	68	27.7	1.9	0.7	–	0.4	1.4	27	6.41

[a]Based on oven-dry and extracted wood; [b]related to dry wood.

Two-step hydrolysis

Prior to hydrolysis, all wood samples were ground in a vibration mill (Herzog, Germany) and the cellulose samples were conditioned and fluffed (Ika Labormühle, Germany). All samples were conditioned at 20°C and 65% RH. The moisture content was measured and recorded. The following hydrolysis is a modified process of Effland (1977).

1. Pre-hydrolysis (depolymerisation of carbohydrates) 2 ml of 72% H_2SO_4 was added to 200 μg (±10 μg) of wood sample, which was pre-swelled for 1 h at 30°C (incubated in a water bath) in a short test tube while being constantly stirred. A glass rod was used to press the sample against the side of the test tube; thus, the particle size of the softened wood was broken down and facilitated the wood/acid interaction. After 1 h, the reaction was terminated by adding 6 ml of distilled H_2O. Each test sample was transferred into a 100-ml volumetric flask with 50 ml of distilled water and the vessel was sealed with a small condenser (i.e., glass ball).

2. Post-hydrolysis (further hydrolysis of reversion products) Samples were hydrolysed under pressure (0.12 MPa) in an autoclave for 40 min at 120°C. After cooling to room temperature, the flask was filled to a volume of 100 ml, shaken and the condensed lignin residue removed by filtration through a G4 sinter glass crucible. Next, 1 ml of the solution was transferred to a sample vial for analysis in the Borate system: 1.5 ml was preserved in a plastic tube for a second analysis and then the sample was frozen. The solid residue was washed thoroughly with distilled water, the crucible was dried at 105°C and the amount of non-hydrolysable residue (i.e., Klason lignin) was determined gravimetrically after hydrolysis of holocellulose.

Borate anion exchange chromatography

Stationary phase Anion exchange MCI Gel CA08F (Mitsubishi) packed in Omnifit empty columns 7×11.5 mm (60°C).

Mobile phase 0.7 ml min^{-1} gradient elution, first with 0.3 M potassium tetraborate and then 0.9 M potassium tetraborate.

Post-column derivatisation was obtained by the addition of cubicinchoniate (0.35 ml min^{-1}) and subsequent heating to 105°C in 0.3 mm Teflon coil. Detection at 560 nm was performed with a UV-detector.

Determination of extraneous compounds

Shavings were taken from all differently treated samples of Norway spruce and beech wood, immediately freeze-dried, and then ground in a mill with a rotating knife (Retsch, Germany, equipped with 3-mm screen).

Extraction was performed by the accelerated solvent method (ASE 200, Dionex). The best method of extracting phenolic compounds from beech and spruce wood (Mayer et al. 2006) was acetone/water (9:1) at 70°C and at a constant pressure of 1.01 MPa; static equilibration treatment of 7 min. For each treatment, 2 g of freeze-dried wood powder was submitted to extraction and the extract was made up to 25 ml with acetone/water (9:1). The extractive content was determined gravimetrically after removing the solvent in a rotary evaporator under vacuum and 40°C.

Statistical analysis

One-way analysis of variance (ANOVA; Excel) of the recorded dry weight losses was performed for wood specimens and the significance level was set at $P<0.01$. The Tukey HSD post-hoc test was performed to demonstrate the differences in mean values (SPSS, Chicago, IL, USA).

Results

White rot test

After the 16-week incubation period, most of the wood specimens were completely colonised by surface mycelium as assessed by EN 113 (CEN 1996). Beech wood showed a lower resistance to white rot fungi than Norway spruce (Figure 1). *T. pubescens* induced higher weight losses than *T. versicolor* in most test specimens (Figure 1). The dry WLs in the beech samples were significantly higher than in the Norway spruce specimens (Figure 1), and the highest WLs for beech were recorded in untreated controls, TH-treated (160°C and 180°C) and densified specimens without post-treatment incubated with *T. ver-*

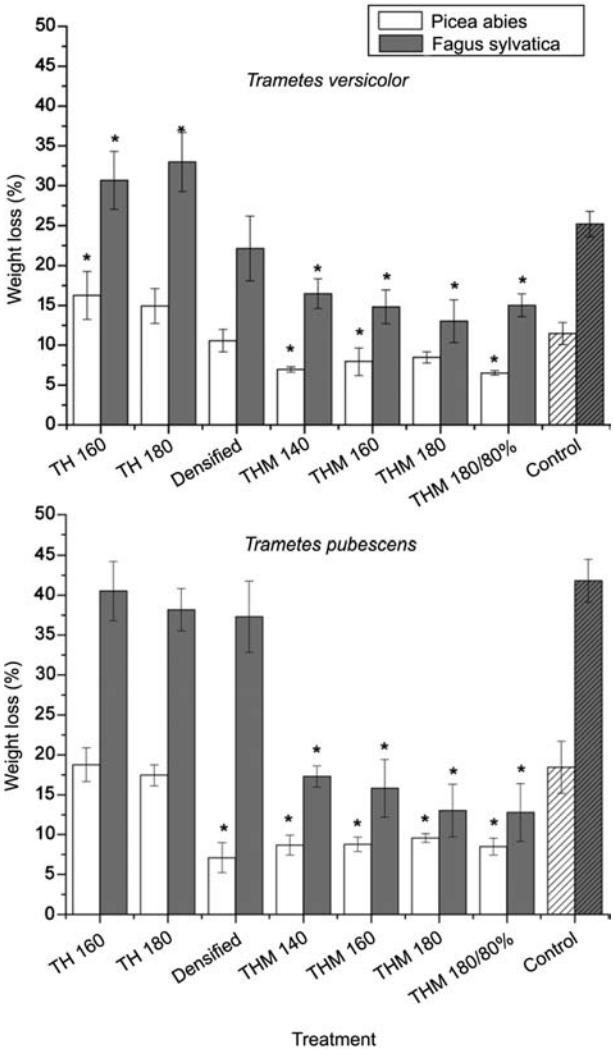

Figure 1 Dry weight losses of beech and Norway spruce samples. Control: untreated; TH-treated (160°C and 180°C); densified: without thermal post-treatment; THM-densified: (at 140°C, 160°C, 180°C and 180°C/80% RH) and incubated for 16 weeks with *T. versicolor* or *T. pubescens* ($n=6$ each treatment/fungus combination). Bars: standard deviations. Columns marked with an asterisk denote significant differences compared to untreated controls ($P<0.01$).

sicolor (Figure 1). All the THM treatments (140°C, 160°C, 180/80°C, 180°C) resulted in significantly lower WL of beech, compared to untreated controls, with either of the test fungi.

The lowest WLs in Norway spruce wood occurred with all the THM treatments and densified Norway spruce wood incubated with *T. pubescens* (Figure 1). The moisture content of the beech specimens after 16 weeks of incubation with the white rot fungi was in the range of 70±10% for TH treatment and 35±10% for THM treatment of beech, compared to 58±8% and 30±5%, respectively, for Norway spruce.

For DS calculated according to Eq. (1) (Figure 2), all the treated specimens of Norway spruce showed higher DS both against *T. versicolor* and *T. pubescens*. In the case of beech wood, higher DS (Figure 2) was observed exclusively in THM-densified (180°C and 180/80°C) specimens incubated with *T. pubescens*. TH 180, densified and THM 140 exposed to *T. versicolor* were the treatments that induced a significant increase of DS in beech wood; in fact, densified beech incubated with *T. pubescens* showed a two-fold increase in DS (Figure 2).

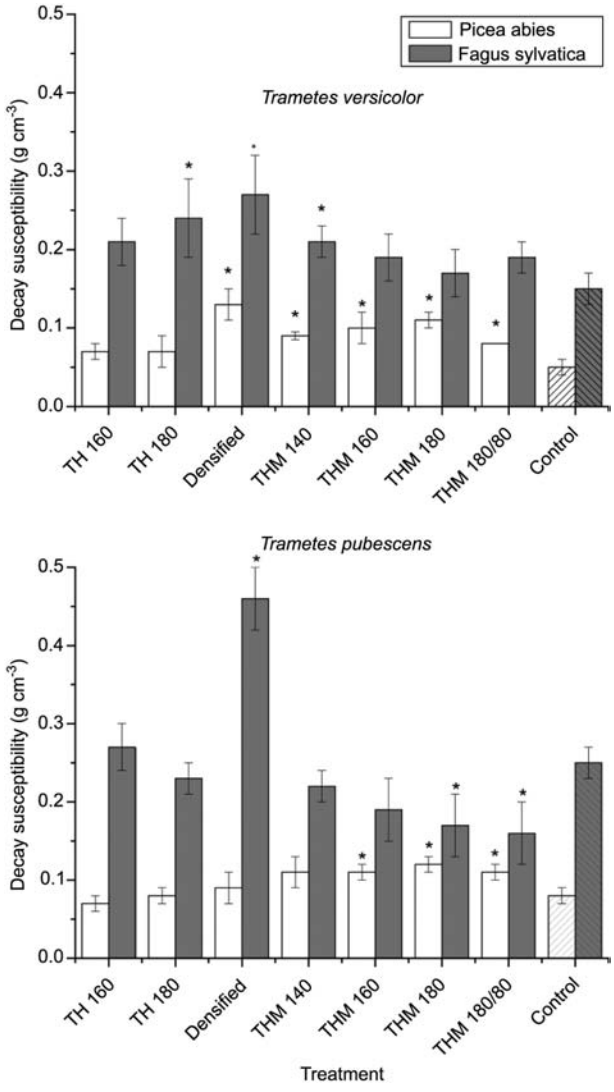

Figure 2 Decay susceptibility of beech and Norway spruce samples. The notations are explained in Figure 1.

Light microscopy

Control specimens of beech and Norway spruce wood were completely colonised by surface mycelium after 16 weeks of incubation. Microscopic studies revealed the presence of both types of white rot. In TH-treated specimens of Norway spruce and beech selective delignification predominated, whereas in THM-densified specimens of both wood species revealed the presence of selective delignification and simultaneous rot. Selective delignification was predominantly associated with *T. pubescens* (Figure 3a, d), whereas *T. versicolor* induced a simultaneous rot; the latter is well characterised by cell wall erosion and formation of bore holes in the secondary wall of tracheids (Figure 3b, c).

Delignification of the secondary walls commenced from the lumen towards the middle lamella and occurred in the immediate vicinity of hyphae growing in the cell lumen. In stained wood cell, wall delignification resulted in a distinct colour change of the inner secondary wall from light blue to dark blue. At a more advanced stage of degradation, selective delignification resulted in separation of the outer secondary wall (S1 layer) from the compound middle lamella, resulting in the separation of cells from another (Figure 3b). In THM-densified Norway spruce, post-treated at 180°C and 160°C, bore holes and hyphal tunnelling was observed within the secondary walls of latewood tracheids (Figure 4a, b). THM 160°C treatment of beech wood, incubated with *T. pubescens*, resulted in formation of minute cavities (Figure 4c) within secondary walls of fibre tracheids.

Chemical alterations

The results of hydrolysis, presented in Table 1, reveal the presence of glucose, xylose, mannose, galactose, arabinose, ramnose and 4-O-Me glucuronic acid in both species. In control samples of spruce, glucose (70%), mannose (18%) and xylose (7%) were typical. In hydrolysates of beech control, these sugars occurred in 65%, 28% and 3% yield.

TH and THM treatments reduced the amount of carbohydrates in Norway spruce wood, though to a small extent, with the exception of the absolute amount of sugars in densified samples. TH180 treatment resulted in increased glucose (from 70% to 81%) and decomposition of both mannose (from 18% to 13%) and xylose (from 7% to 5%). THM-densified wood post-treated at 180°C had an increased glucose content (77%) and this treatment slightly reduced the content of mannose (16%) and xylose (5.7%) (Table 1).

TH treatments did not alter the chemical composition of sugars in beech wood (glucose 67%, xylose 27%, mannose 3%), although THM 180 resulted in an increased glucose content of 77% and a reduction in the content of xylose (17%) and mannose (0.5%).

Klason lignin of beech wood did not show significant differences after TH and THM treatment. However, the effect of the treatments was particularly pronounced in spruce wood specimens, especially those that had been post-treated at elevated temperatures (Table 1). TH 180 increased the Klason lignin content of Norway spruce

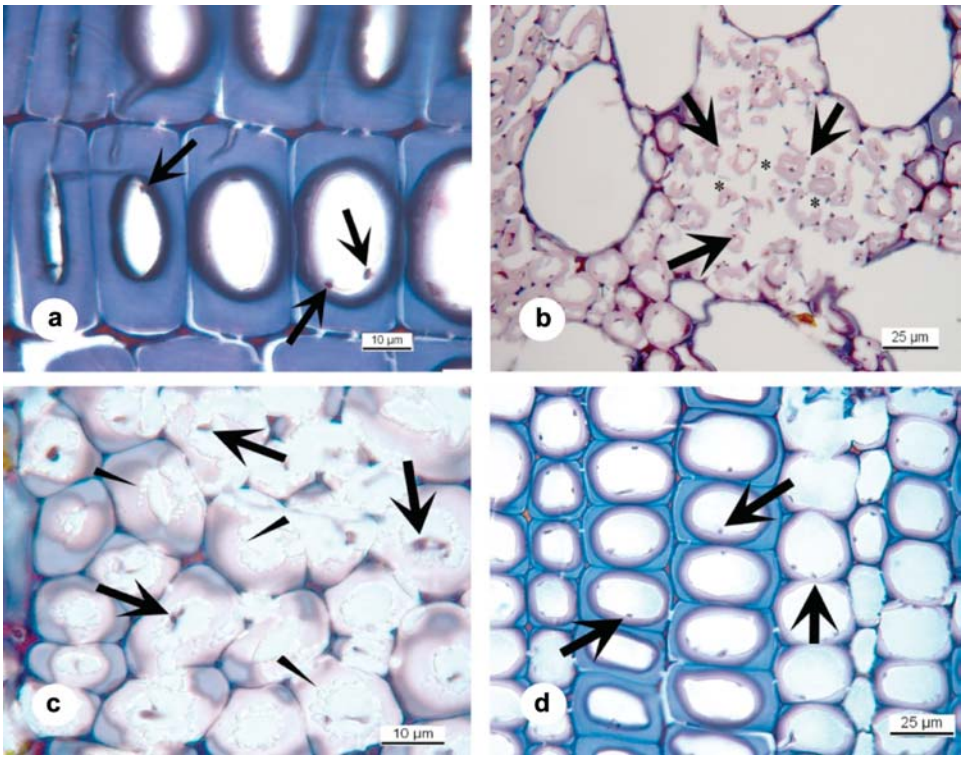

Figure 3 (a) Transverse section (TS) of untreated Norway spruce wood incubated with *T. pubescens*. Note that the early stage of selective delignification in the secondary walls by hyphae (arrows) growing in the cell lumina of tracheids results in a distinctive colour change. (b) TS of untreated beech wood incubated with *T. versicolor* showing selective delignification by hyphae (arrows) growing within the cell lumen. *Separation of fibre tracheids at advanced stages of degradation. (c) TS of TH 180 beech wood incubated with *T. versicolor* showing selective delignification in the secondary walls of fibre tracheids caused by hyphae (arrows) growing in the cell lumen. Note small cavities in the secondary walls, which are separated from one another by radial structures (arrowheads). (d) TS of Norway spruce wood after TH 180 treatment and incubation with *T. pubescens*. Note selective delignification commenced by hyphae growing on the S3 layer within the cell lumen (arrows).

from 27% to 32%, similar to the effect of THM 180 (32% of Klason lignin).

For both species, the acetone/water (9:1) extract from the TH- and THM-treated samples appeared considerably darker to the naked eye when compared to the extract from untreated controls or densified wood samples. The data presented in Table 1, together with the visual observations, indicate that the amount and com-

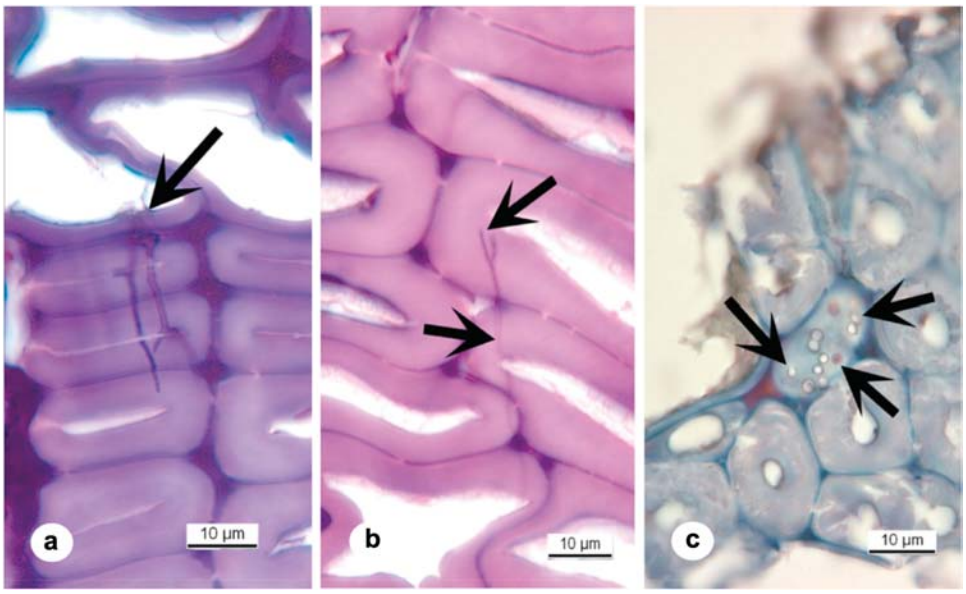

Figure 4 (a) Transverse section (TS) of THM-densified Norway spruce post-treated at 180°C and incubated with *T. versicolor*. Note hyphal (arrow) tunnelling in the secondary walls of latewood tracheids. (b) TS of THM-densified Norway spruce post-treated at 160°C and incubated with *T. versicolor*. Note hyphal tunnelling (arrow) within the secondary wall. (c) TS of THM-densified beech wood post-treated at 160°C and incubated with *T. pubescens*. Note cavities (arrows) within the secondary walls of fibre tracheids.

position of the extractives were significantly influenced by the conditions (i.e., temperature and degree of steam saturation) of the wood modification process and that this influence is species-dependent.

In the Norway spruce specimens, the total amount of extractives increased for all treatments. The highest extractive content was recorded for non-densified TH 180-treated wood. Densified spruce, which was post-treated at 180°C under saturated steam conditions, showed a significant increase in extractive content. All TH- and THM-treated beech wood samples showed increased extractive contents, but no significant changes were recorded for densified and non-post-treated specimens.

Discussion

Microscopic examination of THM-treated wood showed that the differences between treatments regarding weight loss could be partly attributed to restriction of fungal growth by the occlusion of the tracheid lumens. In wood subjected to THM 180 treatment, fungal hyphae were absent from latewood tracheids that had completely occluded lumina and were only observed in the partially occluded lumina of the earlywood tracheids.

Formation of cavities in the cell walls of wood artificially incubated with white rot basidiomycetes is reminiscent of a soft rot (Schwarze and Engels 1998). In the past, cavity formation in the secondary wall of lignified cell walls was regarded as a distinct, relatively reliable character of a soft rot that could be used to readily differentiate it from other modes of degradation (Hale and Eaton 1985a,b; Eriksson et al. 1990; Zabel and Morrell 1992). The distinguishing feature of soft rot is T-branching and L-bending (Nilsson et al. 1989), but in the present study we observed different phenomena. Hyphal growth was not detected within the cell walls and – atypically for a soft rot, or any other commonly described degradation pattern – substantial selective delignification of the cell wall always preceded cavity formation.

The most decisive factor governing delignification of wood by white rot fungi is the quantity and type of lignin in a tree (Agosin et al. 1990; Schwarze et al. 2004). Conifer wood has a high concentration of guaiacyl lignin in the S2 and the S3 layer of tracheids and it is very resistant to white rot fungi that cause a simultaneous rot (Liese 1961; Blanchette et al. 1988; Nilsson et al. 1989; Obst et al. 1994). Beech wood, which consists predominantly of syringyl-rich fibre tracheids, is more susceptible to this type of decay (Baum et al. 2000; Schwarze et al. 2000). Differences in the lignin composition may partly explain the higher WL recorded in the beech wood specimens incubated with *T. versicolor* and *T. pubescens*. However, the interpretation of the decay behaviour of various woods as a function of the syringyl content of their lignin would be too simple (Obst et al. 1994). There are more significant variations in wood, e.g., the amount and distribution of wood constituents, relative proportion of fibres, vessels and parenchyma cells, cell wall thickness, vessel element size, the presence of tyloses, and the ratio of earlywood to latewood.

Apart from the effects of diminution of the size of cell lumina, TH treatment also appeared to enhance the susceptibility to degradation. Thus, *T. pubescens* circumvented occlusion of cell lumina by switching its mode of action to hyphal tunnelling within the fibre tracheids of beech wood (Figure 4c) and *T. versicolor* formed branching hyphae that transversely penetrated the cell walls of latewood tracheids in Norway spruce (Figure 4a, b). This degradation pattern was exclusively observed in THM-densified wood and to a lesser extent in TH-treated wood, but not in untreated control specimens. Such behaviour of decay fungi in adverse conditions is known as "plasticity of metabolism" and has been previously described (Rayner and Boddy 1988; Schwarze et al. 1995, 2000, 2004). This mode of action was also observed by Schwarze and Spycher (2005) for *Gloeophyllum trabeum* in TH 160°C treated Norway spruce. It seems likely that in addition to cell lumina occlusion and increase in gross wood density, THM-densification increases cell wall porosity due to the depolymerisation of polysaccharides. This may also explain the significantly higher decay susceptibility values recorded from TH and THM-densified Norway spruce.

In a previous study on the soft rot resistance of THM-densified wood, Skyba et al. (2008) recorded a pronounced effect of THM treatment in the outer wood surface that suppressed colonisation and degradation of inner regions of the wood samples during incubation. The same phenomenon was observed in the present study in which hyphae were not detected in deeper regions of the wood and cell wall degradation was restricted to the outer surfaces of THM-densified wood.

Many of the fungal species that cause simultaneous rot in hardwoods rarely do so in conifers (Schwarze et al. 2004). This appears to be related to the extremely resilient S3 layer of the tracheids, which hampers degradation by hyphae from within the cell lumen outwards. In contrast, the low-molecular weight substances causing brown rot and selective delignification simply diffuse through the S3 layer of the secondary wall (Koenigs 1974; Murmanis and Highley 1987; Kerem et al. 1999; Schwarze and Spycher 2005). THM treatment may modify the chemical composition of the S3 layer and render it more susceptible of tracheids to penetration of fungal enzymes and hence increase the susceptibility of softwood to selective delignification.

In the present study, decay susceptibility calculations based on WL and density measurements of specimens did not reveal any improvement in the decay resistance of Norway spruce and only a slight reduction in the susceptibility of beech wood against *T. pubescens* (Figure 2). This result questions the value of wood modification by compression in general and THM-densification in particular concerning the resistance against fungi.

Chemical alterations

Chemical analyses revealed that wood treated in a THM reactor undergoes slight alterations in terms of the composition of its chemical constituents. Degradation of hemicelluloses by THM treatment resulted in the production of simple sugars, which might undergo further dehy-

dration reactions to form highly reactive furfural, hydroxymethylfurfural and other substances.

It is generally accepted that cellulose degradation occurs at higher temperatures than that of hemicelluloses, although there are some contradictory results (Hill 2006). The rate of cellulose degradation seems to be reduced if water (saturated steam in the case of THM treatment) is present. This observation is assumed to be related to the enhanced ability of the paracrystalline regions of cellulose to change its structure towards more thermally stable crystalline regions (Fengel and Wegener 1984). Steam accelerates the formation of organic acids (e.g., acetic acid) that catalyse the hydrolysis of hemicelluloses and, to a lesser extent, the paracrystalline cellulose (Mitchell 1988).

Hydrothermal processing leads to a partial hydrolysis of polysaccharides, owing to the action of oxonium ions that are generated by the auto-ionisation of water, although the formation of oxonium ions from acetic acid is generally believed to be more important (Garrote et al. 1999). Temperatures between 150°C and 230°C are used because hydrolysis is very slow in this temperature range and cellulose degradation commences at 210–220°C, peaking at 270°C (Hillis 1984). Hardwoods are less thermally stable than softwoods, which is attributable to their hemicellulose content and composition. Pentosans, which are found in greater proportion in hardwood hemicelluloses, are more susceptible to thermal degradation than hexans (Fengel and Wegener 1984). In addition, hardwoods, in general, have a higher proportion of hemicelluloses that have a higher acetyl content compared to those of softwoods.

It is generally accepted and has also been demonstrated in this study that lignin is the most stable component of the woody cell wall. However, some thermal degradation of lignin may occur at relatively low temperatures accompanied by the production of various phenolic breakdown products (Sandermann and Augustin 1964). Presumably, the degradation of polysaccharides and the denaturation of lignin (i.e., its elevated cross-linking density) leads to an apparently increased lignin content.

Hardwood lignin is more easily softened than softwood lignin owing to its lower mole mass and lower degree of condensation, i.e., the additional methoxy group occupies the reactive position no. 5 on the aromatic ring. Furthermore, beech has more xylem ray cells and its tissue is more heterogeneous. This restricts uniform penetration of saturated steam during post-treatment and results show that chemical alterations at the surface are not as uniform as in deeper regions. Sudo et al. (1985) demonstrated that lignin from beech wood steamed at 215°C was richer in syringyl units and was only slightly modified compared to treatments at higher temperatures in which significant losses in methoxy groups occurred. It is widely accepted that TH treatment initially produces soluble lignin fragments, but re-polymerisation occurs as reaction time progresses. Furfural and other polysaccharide degradation products also participate in the cross-linking reactions (Garrote et al. 1999). The guaiacyl/syringyl ratio is also affected by THM treatment and contributes to the resistance against white and soft rot decay fungi.

Several researchers have shown that the yield of free sugars formed from hemicelluloses during steaming decreases (Lawther et al. 1996; Rowell et al. 2002). Obviously, monomeric sugars or oligomeric fragments of hemicelluloses released from the thermal degradation undergo further chemical reactions and/or are chemically changed, so that they are no more detectable in the hydrolysate as sugars. TH treatment under saturated steam conditions intensifies the brown colour, presumably because of polymerisation of lignin and hydrolysed carbohydrate derivatives (McDonald et al. 1997). But TH treatment also dissolves some extractives and their degradation products.

Nuopponen et al. (2004) observed migration of fats and waxes along axial parenchyma cells to the surface of steam heat-treated Scots pine wood at temperatures between 100°C and 160°C, whereas at temperatures above 180°C the fats and waxes disappeared from the surface. Mobilisation of extractives during THM-densification is responsible for darkening of the wood substrate; however, partial removal of extractives facilitates the access of water molecules to the cell wall.

Conclusions

THM treatment did not improve the durability of Norway spruce or beech wood against *T. versicolor* and *T. pubescense*. The fungi circumvented conditions restricting hyphal growth. In the case of beech the secondary walls of the fibre tracheids were tunnelled, and in the case of spruce bore holes were formed that transversely penetrated the cell walls of earlywood tracheids in THM-densified samples. Chemical analyses revealed slight changes in the composition of its chemical constituents in THM-treated wood. THM-treated beech and Norway spruce wood are still susceptible to decomposition by white rot fungi and therefore inappropriate for long-term application in utility class 4 but may have some potential in utility class 3.

Acknowledgements

This work was funded by the Staatssekretariat für Bildung und Forschung, SBF-Cost Project Nr. C04.0083, Cost Action E 37 “Sustainability for Enhanced Wood Durability”. The authors thank Mr. Philippe Vuilliemin from École Polytechnique Fédérale de Lausanne for preparing THM-densified wood samples. We also acknowledge technical assistance of Susanne Röske and Kari Waldmann of Freiburg University in the preparation of sections for light microscopy.

References

Agosin, E., Blanchette, R.A., Silva, H., Lapierre, C., Cease, K.R. (1990) Characterization of Palo Podrido, a natural process of delignification in wood. Appl. Environ. Microbiol. 56:65–74.

Baum, S., Schwarze, F.W.M.R., Fink, S. (2000) Persistence of the gelatinous layer within altered tension-wood fibres of beech degraded by *Ustulina deusta*. New Phytol. 147:347–355.

Blanchette, R.A., Obst, J., Hedges, J.I., Weliky, K. (1988) Resistance of hardwood vessels to degradation by white rot basidiomycetes. Can. J. Bot. 66:1841–1847.

Butcher, J., Nilsson, T. (1982) Influence of variable lignin content amongst hardwoods on soft-rot susceptibility and performance of CCA preservatives. The International Research Group on Wood Preservation. Document No. IRG/WP/1151-82.

CEN (1996) EN 113. Wood preservatives: Test method for determining the protective effectiveness against wood destroying basidiomycetes – Determination of toxic values. European Committee for Standardization (CEN), Brussels, Belgium.

Effland, M.J. (1977) Modified procedure to determine acid-insoluble lignin in wood and pulp. Tappi J. 60:143–144.

Eriksson, K.-E., Blanchette, A., Ander, P. Microbial and Enzymatic Degradation of Wood and Wood Components. Springer, Berlin, 1990.

Fengel, D., Wegener, G. Wood – Chemistry, Ultrastructure, Reactions. De Gruyter, New York, 1984.

Garrote, G., Domínguez, J., Parajó, J.C. (1999) Hydrothermal processing of lignocellulosic materials. Holz Roh Werkst. 57:191–202.

Hale, M., Eaton, R.A. (1985a) Oscillatory growth of fungal hyphae in wood cell walls. Trans. Br. Mycol. Soc. 84:277–288.

Hale, M., Eaton, R.A. (1985b) The ultrastructure of soft rot fungi. II: cavity forming hyphae in wood cell walls. Mycologia 77:594–605.

Hill, C. Wood Modification: Chemical, Thermal and Other Processes. John Wiley & Sons, London, 2006.

Hillis, W.E. (1984) High temperature and chemical effects on wood stability. Part 1: General considerations. Wood Sci. Technol. 18:281–293.

Inoue, M., Norimoto, M., Tahanashi, M., Rowell, R.M. (1993) Steam or heat fixation of compressed wood. Wood Fibre Sci. 25:224–235.

Inoue, M., Hamaguchi, T., Morooka, T., Higashihara, T., Norimoto, M., Tsunoda, T. (2000) Fixation of compressive deformation of wood by wet heating under atmospheric pressure. Mokuzai Gakkaishi 46:298–304.

Ito, Y., Tanahashi, M., Shigematsu, M., Shinoda, Y. (1998) Compressive-molding of wood by high-pressure steam-treatment: Part 2. Mechanism of permanent fixation. Holzforschung 52:217–221.

Kerem, Z., Jensen, K., Hammel, K. (1999) Biodegradative mechanism of the brown-rot basidiomycete *Gloeophyllum trabeum*: evidence for an extracellular hydroquinone-driven Fenton reaction. FEBS Lett. 446:49–54.

Koenigs, J.W. (1974) Production of hydrogen peroxide by wood-rotting fungi in wood and its correlation with weight loss, depolymerization and pH changes. Arch. Microbiol. 99: 129–145.

Lawther, M., Sun, R., Banks, W.B. (1996) Effect of steam treatment on the chemical composition of wheat straw. Holzforschung 50:365–371.

Liese, W. (1961) Über die natürliche Dauerhaftigkeit einheimischer und tropischer Holzarten gegenüber Moderfäulepilzen. Mitt. Dtsch. Ges. Holzforschung 48:18–28.

Mayer, I., Koch, G., Puls, J. (2006) Topochemical investigations of wood extractives and their influence on colour changes in American black cherry (*Prunus serotina* Borkh.). Holzforschung 60:589–594.

McDonald, A.G., Fernandez, M., Kreber, B. (1997) Chemical and UVI-VIS spectroscopic studies on kiln brown stain formation in Radiata pine. In: 9th International Symposium of Wood and Pulping Chemistry, Montreal, Canada.

Mitchell, P. (1988) Irreversible property changes of small lobolly pine specimens heated in air, nitrogen or oxygen. Wood Fibre Sci. 20:320–335.

Morsing, N. (1998) Densification of wood. The influence of hygrothermal treatment on compression of beech perpendicular to the grain. PhD thesis. Institute for Baerende Konstruktioner og Materialer, Danmarks Tekniske Universitet.

Murmanis, L., Highley, T. (1987) Cytochemical localisation of cellulases in decayed and nondecayed wood. Wood Sci. Technol. 21:101–109.

Navi, P., Girardet, F. (2000) Effects of thermo-hydro-mechanical treatment on the structure and properties of wood. Holzforschung 54:287–293.

Navi, P., Heger, F. (2004) Combined densification and thermo-hydro-mechanical processing of wood. MRS Bulletin 29: 332–336.

Nilsson, T., Daniel, G. (1992) On the issue of % weight loss as a measure for expressing results of laboratory decay experiments. The International Research Group on Wood Preservation. Document No. IRG/WP/2394-92.

Nilsson, T., Daniel, G., Kirk, K., Obst, J. (1989) Chemistry and microscopy of wood decay by some higher ascomycetes. Holzforschung 43:11–18.

Nuopponen, M., Vuorinen, T., Jaämsä, S., Viitaniemi, P. (2004) Thermal modifications in softwood studied by FT-IR and UV resonance raman spectroscopies. J. Wood Chem. Technol. 24:13–26.

Obst, J., Highley, T., Miller, R.B. (1994) Influence of lignin type on the decay of woody angiosperms by *Trametes versicolor*. In: Biodeterioration Research 4. Eds. Llewellyn, G.C., Dashek, W.V., O'Rear, C.E. Plenum Press, New York. pp. 357–374.

Rayner, A.D.M., Boddy, L. Fungal Decomposition of Wood: Its Biology and Ecology. Wiley, Chichester, 1988.

Rowell, R.M., Lange, S., McSweeny, J., Davis, M. (2002) Modification of wood fibre using steam. In: The 6th Pacific Rim Bio-Based Symposium. Workshop on the chemical modification of cellulosics. Ed. Humphrey, P.E. Wood Science and Engineering Department, Oregon State University, Portland, OR, USA. pp. 606–615.

Sandermann, W., Augustin, H. (1964) Chemical investigations on the thermal decomposition of wood. Part III: Chemical investigation on the course of decomposition. Holz Roh Werkst. 22:377–386.

Schwarze, F.W.M.R., Engels, J. (1998) Cavity formation and the exposure of peculiar structures in the secondary wall (S_2) of tracheids and fibres by wood degrading basidiomycetes. Holzforschung 52:117–123.

Schwarze, F.W.M.R., Fink, S. (1998) Host and cell type affect the mode of degradation by *Meripilus giganteus*. New Phytol. 139:721–731.

Schwarze, F.W.M.R., Spycher, M. (2005) Resistance of thermo-hygro-mechanically densified wood to colonisation and degradation by brown-rot fungi. Holzforschung 59:358–363.

Schwarze, F.W.M.R., Lonsdale, D., Fink, S. (1995) Soft-rot and multiple T-branching by the basidiomycete *Inonotus hispidus* in ash and London plane. Mycol. Res. 99:813–820.

Schwarze, F.W.M.R., Baum, S., Fink, S. (2000) Dual modes of degradation by *Fistulina hepatica* in xylem cell walls of *Quercus robur*. Mycol. Res. 104:846–852.

Schwarze, F.W.M.R., Engels, J., Mattheck, C. Fungal Strategies of Wood Decay in Trees. Springer, Heidelberg, 2004.

Skyba, O., Niemz, P., Schwarze, F.W.M.R. (2008) Degradation of thermo-hygro-mechanically (THM)-densified wood by soft rot fungi. Holzforschung 62:277–283.

Sudo, K., Shimizu, K., Sakurai, K. (1985) Characterisation of steamed wood lignin from beech wood. Holzforschung 39: 281–288.

Zabel, R.A., Morrell, J.J. Wood Microbiology: decay and its prevention. Academic Press, San Diego, CA, USA, 1992.

Received September 24, 2008. Accepted March 6, 2009.
Previously published online June 3, 2009.