

Degradation of thermo-hygro-mechanically (THM)-densified wood by soft-rot fungi

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Abstract

Thermo-hygro-mechanical (THM)-densified wood is more resistant to colonisation and degradation by brown-rot fungi than untreated wood. Colonisation and degradation by soft-rot fungi was investigated in treated Norway spruce (*Picea abies*) and treated beech (*Fagus sylvatica*) to assess their suitability for utility class 4. Three different treatments were applied: thermal-hygro (TH) treatment, mechanical densification and THM-treatment including densification and post-treatment under saturated steam conditions at different temperatures. For comparison, additional wood specimens were treated with two concentrations of a chromium-copper (CC) wood preservative. After 32 weeks incubation, weight losses induced by soft-rot fungi were lowest in wood treated with CC. Highest weight losses were recorded from TH-treated wood, in which soft-rot erosion attack (type 2) was exclusively observed in spruce. In comparison to controls, significantly lower weight losses by soft-rot fungi were recorded in THM-treated spruce wood, but no such differences were found in beech wood. Microscopical examination showed that in THM-treated wood of spruce, soft-rot type 1 commenced from the outer wood surfaces and cavity formation was not found in deeper regions of the wood samples. THM-treated beech wood was more susceptible to degradation than that of spruce which can be partly explained by the higher syringyl lignin content in beech wood, which is more susceptible to all kinds of degradation. Hyphal colonisation and soft-rot was facilitated within deeper regions of beech wood mainly in the non-occluded lumina of parenchyma cells in multiseriate xylem rays. It can be concluded that TH-treated spruce wood and THM-treated beech wood is susceptible to soft-rot and therefore inappropriate for utility class 4.

Keywords: beech; Norway spruce; soft-rot fungi; thermally treated wood; thermo-hygro-mechanically (THM)-densified wood; wood modification.

Introduction

Wood is a natural material appreciated for its appearance, high ratio strength/density and suitability as a construction material. Wood is sustainable, non-toxic, recyclable and biodegradable. However, biodegradability is also an important drawback for outdoor utilisation. The low dimensional stability is another drawback. Wood adsorbs and desorbs water under different moisture conditions and swelling and shrinking is the result.

Most European wood species cannot be functional outdoors without chemical preservation. To avoid chemicals, several non-biocidal wood modification processes have been developed in recent years (Kumar 1994; Tjeerdsma et al. 1998; Goodell 2003; Mai et al. 2004; Hill 2006; Rowell 2006).

Thermo-hygro-mechanical (THM)-densified wood is a unique material in a field of engineered wood products. High temperature, moisture and compression are the parameters for its production. THM densified wood has higher strength properties than natural wood: it has decreased shape recovery, increased shear strength, better strength parallel to the grain and increased surface hardness (Navi and Heger 2004).

THM-densified wood is more resistant to colonisation and degradation by brown-rot fungi (Schwarze and Spycher 2005; Welzbacher and Rapp 2005). Microscopical examination of colonised THM-densified wood showed that the differences between treatments regarding weight loss could be partly attributed to the restriction of fungal growth by the occlusion of tracheid lumina (Schwarze and Spycher 2005). Complete occlusion of all cell lumina would be needed to prevent brown-rot activity, because degradative substances can diffuse from a single hypha in a cell lumen (Schwarze and Spycher 2005). However, if a high proportion of lumina were completely occluded, fungal ingress would be restricted and the durability would be increased.

One potential field of application for THM-densified wood is utility class 4 [wood exposed to the soil (CEN 1996)]. One requirement of class 4 is a resistance to soft-rot fungi. However, this has not been investigated to date. In contrast to brown-rot fungi, soft-rot fungi are known to degrade wood in terrestrial as well as aquatic environments (Barghoun and Linder 1944; Nilsson 1973; Eriksson et al. 1990; Eaton et al. 2004). They play a significant role in wood decay in utility class 4 (CEN 1996), particularly under conditions, such as high moisture content and preservative loading of wood, which inhibit colonisation and attack by the more aggressive basidio-

Table 1 Condition and mean density of beech and Norway spruce wood samples before incubation.

Condition of wood	Temperature (°C)		Duration (min)		Compression rate (%)		Density (g cm ⁻³)	
	Densification	Post-treatment	Densification	Post-treatment	Spruce	Beech	Spruce	Beech
Control	-	-	-	-	-	-	0.352	0.651
TH 160°C	-	160	-	75	-	-	0.373	0.648
TH 180°C	-	180	-	35	-	-	0.365	0.648
Densified	140	-	20	-	71.85	45.29	1.297	1.140
THM 140°C	140	140	20	150	72.85	46.21	1.334	1.213
THM 160°C	140	160	20	75	73.18	42.83	1.296	1.229
THM 180°C/ 80% RH	140	180	20	65	73.18	45.84	1.254	1.167
THM 180°C	140	180	20	35	73.33	44.74	1.279	1.196

SSC, saturated steam conditions. RH, relative humidity.

mycetes. The characteristic feature of soft rot is its pattern of development, which involves T-branching or L-bending and hyphal tunnelling inside lignified cell walls (Savory 1954; Hale and Eaton 1985a,b). Cavities are formed by hyphae growing within the cell wall; discrete notches of cell wall erosion are also typical due to hyphae growing within the cell lumina. The former is typical for "type 1" attack (Corbett 1965; Hale and Eaton 1985a,b; Schwarze et al. 2000). The latter, the erosion troughs, which are indistinguishable from those of white rot fungi, have been attributed to a soft rot known as "type 2".

The objective of the present study was to test the soft-rot resistance of wood modified by different thermal and densification treatments and to determine whether such resistance is related to patterns of fungal colonisation and cell wall degradation. For comparison, a chromium/copper salt solution (CC) was employed as a reference preservative. Wood specimens of Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) were investigated. The soft-rot fungi were tested according to EN 807 (CEN 2001).

Materials and methods

Thermo-hygro-mechanical densification

A total of 110 wood specimens of Norway spruce (*Picea abies* Karst) and beech (*Fagus sylvatica* L.), dimensions 150 mm × 50 mm × 50 mm (R × T × L), were subjected to the two-stage THM procedure of Navi and Girardet (2000), involving densification and post-treatment in saturated steam at 140°C, 160°C and 180°C and at 80% relative humidity (RH) and at 180°C. For a detailed scheme, see Table 1.

Inoculation of wood specimens

A slightly modified soil bed test according to EN 807 (CEN 2001) was carried out. Six wood specimens from each treatment were placed in a soil substrate. Before the test, moisture content (MC) and water holding capacity (WHC) of the substrate were determined according to EN 807. Soil substrate was taken as natural top soil from a test field in Thurgau, Switzerland. The amount of water required to bring the substrate to 95% of its WHC was calculated and added to the soil. A total of 12 containers (35 × 20 × 25 cm³) were filled with 13.5 kg of soil and were sealed with lids. To maintain enough moisture in the soil, the containers were monitored weekly by estimating weight fluctuations and MC was adjusted by adding water or removing lids of the containers. The MC of wood was monitored on wood specimens prepared from Norway spruce sapwood.

Wood specimens of three dimensions (L × R × T) were investigated: (1) beech 5 × 50 × 10 mm³, (2) spruce 5 × 40 × 10 mm³, (3) beech and spruce 30 × 10 × 5 mm³. Six virulence control wood specimens, also prepared from spruce, were positioned in each container. The wood specimens were leached according to EN 84 (CEN 1997) before determining their initial dry mass to the nearest 0.001 g. All wood test specimens and virulence specimens were placed vertically into the soil with 20 mm of their length above the surface of the substrate and with a minimum of 20 mm apart and from the sides of the container. All specimens were randomly distributed.

A CC solution was the reference preservative (CuSO₄ × 5 H₂O – 50%, K₂Cr₂O₇ – 48%, CrO₃ – 2%). Concentrations of CC solution were 0.4% (w/w) and 1.6% (w/w) for beech wood

specimens, and 0.16% (w/w) and 0.4% (w/w) for spruce wood specimens.

All test specimens (reference and virulence control) were incubated for 8, 16, 24 and 32 weeks in a conditioning room at $28 \pm 1^\circ\text{C}$ and 95–100% RH. After incubation, the specimens were removed from the soil substrate, cleaned of adhering soil particles and weighed. Determination of the initial dry mass and the final dry mass after soil exposure was carried out by oven drying the wood specimens at 103°C and weighing to the nearest 0.001 g. The mass loss of each specimen was calculated as a percentage of the initial dry mass.

One-way analysis of variance (ANOVA) of the recorded dry weight losses was performed for all wood samples in Excel with the significance level set at $P < 0.01$. A Tukey HSD post-hoc test was performed in SPSS to demonstrate differences in mean values.

Light microscopy

The incubated wood specimens were cut into sub-samples of approximately $20 \text{ mm} \times 5 \text{ mm} \times 5 \text{ mm}$, with transverse, radial and tangential surfaces exposed to examination. These were fixed in a 2% (w/w) glutaraldehyde buffered at pH 7.2–7.4, dehydrated with acetone and embedded in a methacrylate medium (Schwarze and Fink 1998). They were then sectioned at approximately 2 and $4 \mu\text{m}$ with a rotary microtome (Leica® 2040 Super-cut) fitted with a diamond knife. To compare the rate of wood colonisation and degradation by decay fungi, transversal longitudinal sections (TLS) were cut at the surface and at a depth of

10 mm of the sub-samples. For general observations of cell wall degradation and hyphal growth, sections were stained for 12 h in 1% (w/w) safranin and then counter-stained for 3 min in 0.2% (w/w) methylene blue and for 30 min in 0.7% (w/w) auramin. Micrographs were taken with a microscope fitted with a camera system (Leica® DC 50, TWAIN).

Results and discussion

Densification resulting from wood treatment

THM treatments resulted in increases of densities ranging from four-fold for Norway spruce and two-fold for beech wood (Table 1). THM wood post-treated at 140°C compressed the vessels of beech wood to a radial width from 20–24 μm to 2–7 μm in the latewood (LW) and to 5–10 μm in the earlywood (EW). After post-treatment at 180°C , the corresponding values were 0.5–5 μm in LW and 4–8 μm in EW. Occlusion of the cell lumina of tracheids in spruce wood was within the range previously described by Schwarze and Spycher (2005).

After 32 weeks, the lowest dry weight losses were recorded in wood treated with CC (Figures 1 and 2). All specimens modified by THM-treatment showed an inverse relationship between susceptibility to fungal degradation and increase in density (Figures 1 and 2). In

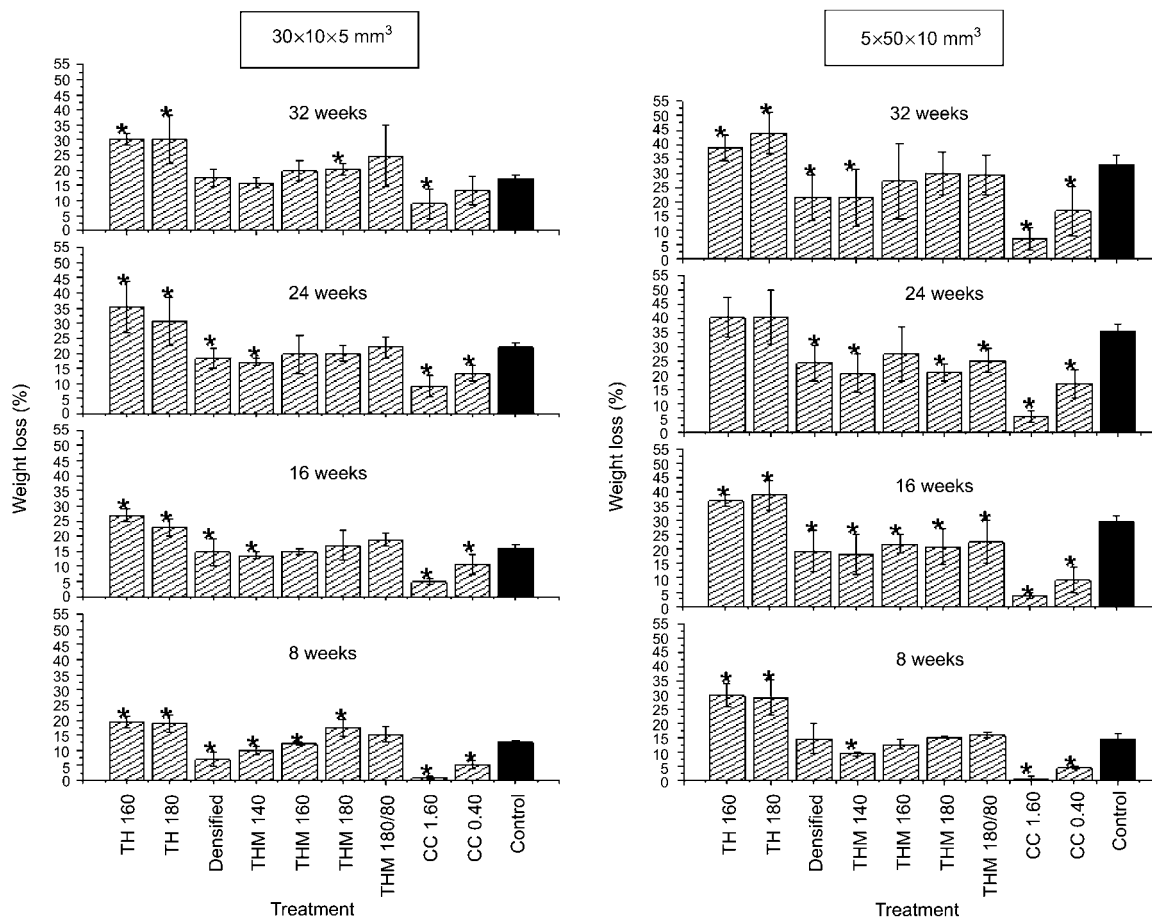


Figure 1 Dry weight losses of beech wood specimens in untreated controls, TH-treated wood (160 and 180°C), densified wood without thermal treatment, THM-densified wood (140, 160, 180°C and $180^\circ\text{C}/80\% \text{RH}$) and CC (1.6 and 0.4%) impregnated wood incubated for 8, 16, 24 and 32 weeks. Bars show standard deviation ($n=6$). Columns marked with an asterisk show a significant difference in comparison to untreated control ($P < 0.01$).

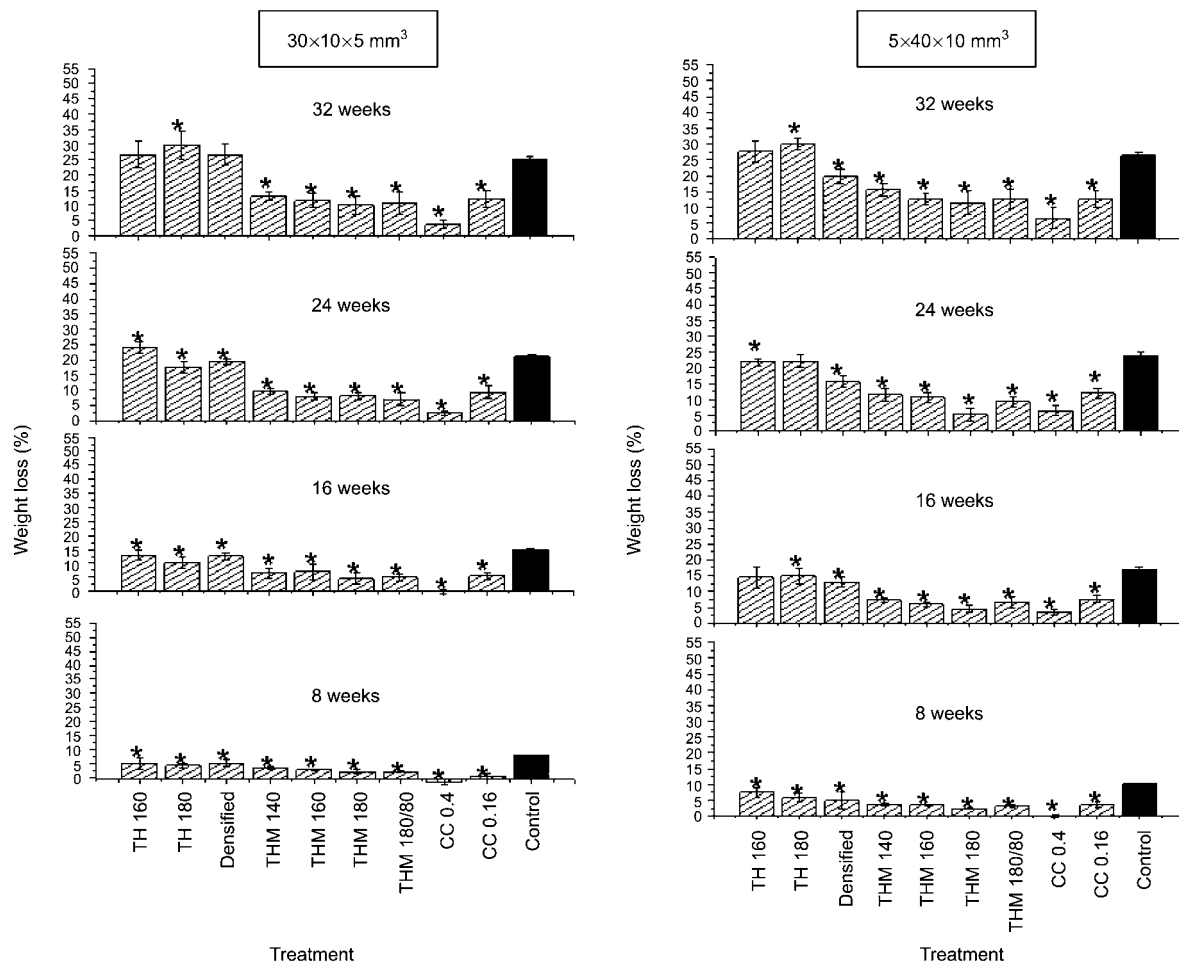


Figure 2 Dry weight losses of Norway spruce wood specimens in untreated controls, TH-treated wood (160 and 180°C), densified wood without thermal treatment, THM-densified wood (140, 160, 180°C and 180°C/80% RH) and CC (1.6 and 0.4%) impregnated wood incubated for 8, 16, 24 and 32 weeks. Bars show standard deviation ($n=6$). Columns marked with an asterisk show a significant difference in comparison to untreated control ($P<0.01$).

beech wood, most THM treatments resulted in a significant reduction in weight losses ($P<0.001$) by soft-rot fungi during initial stages of soil exposure, but after 32 weeks the treatment failed to inhibit degradation (Figures 1 and 2). In spruce wood, all THM treatments resulted in a significant reduction ($P<0.001$) in weight losses, regardless of the incubation period.

The density of wood specimens exposed to TH treatment was slightly reduced in comparison to untreated controls (Table 1) and resulted in higher weight losses by soft-rot fungi than in the untreated controls in both beech and spruce wood specimens after 32 weeks incubation. The adverse affect of TH treatment on wood resistance was previously also recorded for brown-rot fungi (Schwarze and Spycher 2005). It is well established that thermal treatment decreases the amount of accessible hydroxyl groups and results in a reduction in the hygroscopicity of treated wood (Tjeerdsma et al. 2000). TH treatment contributes to depolymerisation of polysaccharides and shifts the T_g of lignin (Heger et al. 2003). However, in the present study, the MC of incubated TH-treated wood specimens was found to be well above the fibre saturation point. During incubation, a higher MC developed in spruce than in beech wood specimens. After 8 weeks, the MC of the modified wood specimens

was in the range of $130\pm 10\%$ for TH-treated and $48\pm 10\%$ for THM-treated beech specimens, whereas TH- and THM-treated spruce wood specimens had a MC of $225\pm 40\%$ and $51\pm 15\%$, respectively.

Fungal colonisation and degradation

In THM- and TH-treated wood of spruce and beech, the impact of thermal treatment resulted in distinct alterations in the micro-morphology of cell walls in close proximity to the specimen surface. The modification of cellulose and hemicelluloses resulted in a conspicuous reddish appearance of the outer cell rows due to staining of lignin with safranin (Figure 3a). Moreover, loss of birefringence was apparent from the cell walls in this region which appeared dark when viewed between crossed Nicol prisms. In THM-treated wood of spruce, modified regions were superficial and were merely recorded in a depth of 40–45 μm , whereas in beech modifications by thermal treatment were more extensive and apparent in a depth of 170–220 μm (Figure 3a). Even in cell regions close to the surface, parenchyma cells of multiseriate xylem rays in beech wood did not appear to be affected by heat treatment and cellulose maintained a distinct birefringence.

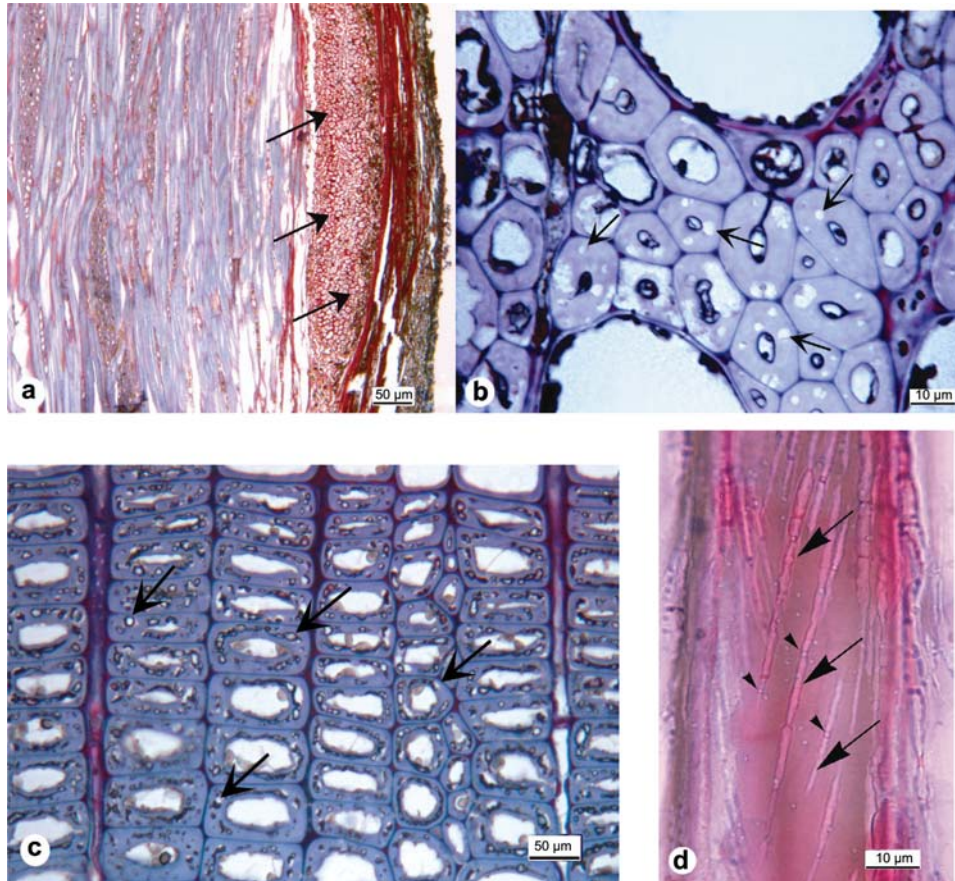


Figure 3 (a) Tangential longitudinal section (TLS) of TH-treated beech wood at 160°C. Note reddish staining of fibre tracheids and multiseriate xylem ray parenchyma (arrows) with safranin Astra blue due to combustion of polysaccharides. (b) Transverse section (TS) of TH-treated beech wood showing cavities (arrows) within secondary walls of fibre tracheids. (c) TS of untreated Norway spruce wood showing cavities (arrows) within the secondary walls of tracheids. (d) TLS of untreated Norway spruce showing bore holes (pointers) and lenticular cavities (arrows) with conical ends that follow the orientation of microfibrils in secondary walls of tracheids.

In control specimens of beech and spruce wood, hyphae had completely colonised the wood specimens. Cell wall degradation by soft-rot fungi was induced either by formation of cavities by hyphae in the S_2 layer (type 1) or erosion troughs (type 2). Soft-rot type 1 was characterised as a series of successive cavities with conical pointed ends which followed the direction of microfibrils within the S_2 layer (Figure 3b–d). Soft-rot type 1 was most prevalent in control and treated wood specimens of spruce. The latter observation is in good agreement with former studies showing that soft-rot type 1 occurs in secondary walls with high concentration of guaiacyl, whereas soft-rot type 2 is often associated with syringyl rich cell walls (Liese 1961; Nilsson et al. 1989; Schwarze et al. 2004; Singh et al. 2006). Norway spruce wood has a high concentration of guaiacyl, and thus is moderately resistant to soft-rot fungi (Liese 1961; Blanchette et al. 1988; Nilsson et al. 1989; Baum 2001; Donaldson 2001), whereas beech wood that consists predominantly of syringyl-rich fibre tracheids is more susceptible to soft-rot fungi (Schwarze et al. 2000, 2004; Baum 2001). The latter difference in the lignin composition partly explains the higher weight losses recorded by soft-rot fungi in beech wood.

Interestingly, soft-rot type 2 was exclusively observed in TH-treated wood of spruce (Figure 4a). Former studies show that many species that only cause soft-rot type 2

in hardwoods failed to exhibit any decay features or weight losses in softwoods (Anagnost 1998). One reason for this selection process appears to be related to the extremely resistant S_3 layer of tracheids that hampers degradation by hyphae from within the cell lumen outwards (Liese 1970; Schwarze et al. 2004). This does not deter brown-rot fungi, which are able to degrade the cell wall by means of diffusible secretions from hyphae within the tracheid lumen, but a resistant S_3 layer is a considerable barrier to fungi that causes only soft-rot type 2 or a simultaneous rot (Schwarze et al. 2004). In TH-treated wood, it seems that thermal treatment induces chemical alterations of the S_3 layer which strongly reduces its resistance to soft-rot type 2 attack, i.e., formation of erosion troughs by hyphae growing within the lumen (Figure 4a).

In THM-treated spruce wood, colonisation of tracheids by soft-rot fungi was evidently hampered by densification. Hyphae were not detected in deeper parts of the wood specimens and cell wall degradation was restricted to the outer surface of the wood specimens (Figure 4a). Interestingly, THM-treated beech wood post-treated at 180°C showed similar weight losses as controls. Thus, even a complete occlusion of cell lumina could not inhibit decay by soft-rot fungi, as the occlusion was simply counteracted by directional growth within the cell wall (Figure 4d).

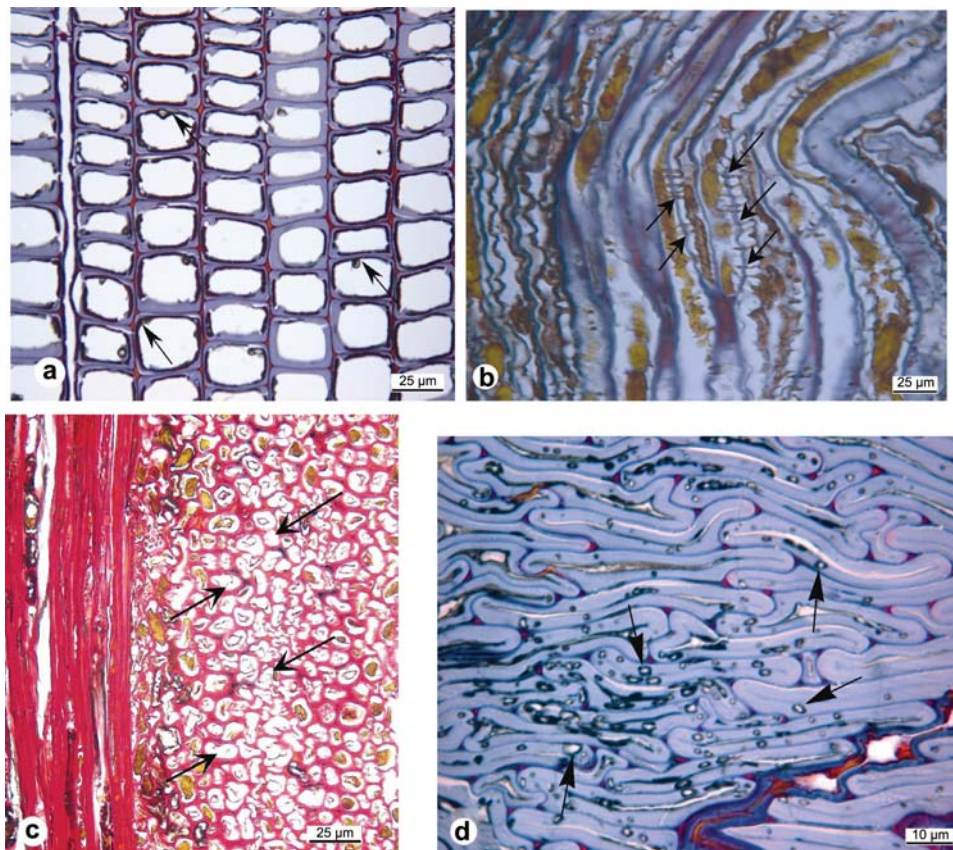


Figure 4 (a) TS of TH-treated Norway spruce wood showing soft-rot type 2 attack in secondary walls of tracheids. Note: formation of hyphal sheaths and cell wall erosion troughs (arrows). (b) TS of THM-treated beech wood post-treated at 160°C showing soft-rot attack within a multiseriate xylem ray. Note: complete degradation of the secondary walls (arrows) in xylem ray parenchyma. (c) TLS showing THM-treated spruce wood post-treated at 180°C showing non-occluded xylem ray parenchyma cells. Note: cavity formation (arrows) in the secondary walls of xylem ray parenchyma. (d) TS of THM-treated spruce wood post-treated at 180°C showing cavities within the secondary walls of tracheids (arrows).

Another important factor affecting resistance of THM-treated wood is the impact of the wood structure on compression and vice versa on hyphal colonisation and degradation. Norway spruce wood has a very homogeneous structure and consists predominantly of axially aligned tracheids (90–95%) and a low proportion of radially aligned uniseriate xylem rays. Thus, in THM-treated wood of spruce post-treated at 180°C, most cells were almost completely occluded, regardless whether wood specimens were compressed in the radial or tangential direction. In contrast, the structure of beech wood is more heterogeneous as it consists of vessels, fibre-tracheids, axial parenchyma and prominent multiseriate xylem rays comprising around 20% of all cell types (Panshin and de Zeeuw 1970). In THM-treated wood of beech post-treated at 180°C, the multiseriate xylem rays counteracted compression in the radial direction and cell lumina of xylem ray parenchyma were only slightly occluded, facilitating hyphal colonisation and access from the surface of the deeper wood regions. Thus, the strongest soft-rot attack in beech was observed in the secondary walls of xylem ray parenchyma and in fibre tracheids in close proximity to multiseriate xylem rays (Figure 4b,c).

The results show that before selecting potential wood species for THM treatment for utility class 4, it is essential

to consider whether the lignin composition and anatomical features may promote wood colonisation and degradation by soft-rot fungi despite thermal treatment and/or considerable increases in density.

Conclusions

The process of THM treatment increased the resistance of spruce but not of beech wood to degradation by soft-rot fungi. Microscopical examination showed that in THM-treated wood of Norway spruce soft rot commenced from the outer wood surfaces and cavity formation was not found in deeper regions of the wood specimens. In beech wood, hyphal colonisation and degradation was facilitated by the non-occluded lumina of parenchyma cells in multiseriate xylem rays. Moreover, the higher syringyl lignin content of beech wood renders it more susceptible to soft-rot attack than spruce wood. TH-treated wood of beech and spruce was highly susceptible to soft-rot fungi and cell wall modification induced soft-rot type 2 attack in the latter host. It is concluded that TH-treated Norway spruce wood and THM-treated beech wood are highly susceptible to soft-rot attack and therefore inappropriate for application in utility class 4.

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