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Effect of thermal modification on the durability and decay patterns of hardwoods and softwoods exposed to soft rot fungi

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ABSTRACT

The durability and decay patterns of thermo-vacuum (Termovuoto process for 3–4 h at 160–220 °C) treated hardwoods (ash, beech) and softwoods (spruce, fir) TMWs exposed to three soft rot fungi (*Chaetomium globosum*, *Phialophora malorum*, *P. mutabilis*) were investigated using the soil-block test, light- and electron microscopy. Monitoring of mass loss over 1 year indicated that soft rot fungi do not attack softwood TMWs as rapidly or as extensively as hardwood TMWs. Decay resistance progressively increased in hardwood TMWs with increase in temperature but was unclear/or varied in softwood TMWs depending on fungal/wood species, particularly at lower temperatures (160–180 °C). Soft- and hardwood TMWs showed a major increase in decay resistance at 200–220 °C and 210–220 °C, respectively. Light microscopy of decayed hardwood TMWs showed formation of typical soft rot Type-I cavities in fibres at lower temperatures (190–200 °C). However, cavities were significantly inhibited or delayed at higher temperatures (210–220 °C). Cavity formation in vessels and parenchyma cells were only observed in beech TMW treated at 190 °C or references, indicating higher resistance than fibres. Transmission electron microscopy of decayed ash TMW treated at 200 °C showed a radial-like distribution of electron dense materials in cavities and lack of fibrillar-like materials within degraded fibre walls, which differed from reference.

1. Introduction

Thermal modification (TM) of wood shows great promise in terms of an economically viable way for production of non-toxic wood materials with improved dimensional stability and biological durability (Esteves and Pereira, 2009; Welzbacher and Rapp, 2007). In this regard, thermally modified woods (TMWs) have gained a rapid market share in recent years (Boonstra, 2008). Over the last few decades, several industrial scale TM processes have been developed with extensive research carried out on changes in physical properties, chemical composition and decay resistance (e.g. Boonstra, 2008; Cademartori et al., 2013; Windeisen and Wegener, 2008, 2009). The Termovuoto (thermo-vacuum) process used in this study represents a more recent industrial scale TM technology aimed at modifying wood by combining efficient vacuum drying with thermal treatment. These conditions ensure high energy efficiency, less corrosion and rust problems and reduced mass loss of wood during treatment (Allegretti et al., 2012; Ferrari et al., 2013).

Resistance against fungal decay is of key interest to evaluate before using materials like TMWs *in-service*. The most popular approach for studying wood durability against fungi involves soil-block (AWPA standard E10, 2008) or agar-block (European standard EN 113, 2004) tests, where mass loss serves as an indicator of decay. The fungal durability of TMWs has been intensively studied using these two tests (e.g (Candelier et al., 2012; Chaouch et al., 2010; Hakkou et al., 2006; Kamdem et al., 2002; Šušteršic et al., 2010), including Termovuoto TMWs (Gao et al., 2016). However, most previous studies concerning fungal resistance of TMW have focused on brown- and white rot fungi (basidiomycete) and few studies have been conducted on soft rot fungi (Brischke and Hanske, 2016; Sivonen et al., 2003). Decay resistance of Termovuoto TMWs against soft rot fungi has also not been studied. In contrast to brown- and white rot fungi, soft rot commonly occurs in wood exposed to high moisture situations and wood treated with preservatives that can hinder both colonization and attack by more aggressive basidiomycetes (Daniel, 2003; Daniel and Nilsson, 1998). This emphasizes the importance of decay tests against soft rot fungi to evaluate the possibility of use of TMWs in outdoor out of ground situations where high moisture conditions can occur, even if only intermittent.

Micromorphologically, soft rot decay differs from both brown- and

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white rot decay by involving a process of T-branching and/or L-bending and hyphal development inside lignified cell walls by producing characteristic cavities (Daniel and Nilsson, 1998). Chemically wood degraded by soft rot is more similar to brown rot fungi, with greatest degradation of cellulose and hemicelluloses. With respect to lignin, although several earlier studies report the capability of soft rot fungi to degrade lignin (Eslyn et al., 1975; Haider and Trojanowski, 1975), degradation is generally poor and more limited compared to white rot fungi. It is also commonly considered that the nature of lignin including concentration, condensation and composition (i.e. guaiacyl and syringyl) significantly affect soft rot degradation of wood cell walls, especially formation of soft rot cavities (Daniel, 2016). These characteristics of soft rot decay suggest that decay patterns of soft rot fungi in TMWs can differ from general decay patterns of soft rot fungi since TM of wood commonly induces significant changes in lignin chemistry as well as changes in the chemistry of polysaccharides and wood cell wall structure (Esteves and Pereira, 2009). However, the effect of TM on morphological decay patterns of soft rot fungi is almost unknown.

The aims of this study were to 1) examine the decay resistance of Termovuoto process treated hard- and softwood TMWs against soft rot fungi to evaluate the process for improvement aginst soft rot decay; 2) determine the effect of the Termovuoto TM on changes in micromorphology of soft rot decay and their relationship with improved decay resistance in TMWs.

2. Materials and methods

2.1. Wood materials

Thermo-vacuum (Termovuoto process for 3–4 h under 240–260 mbar at 160–220 °C) treated sapwoods of two hardwoods [European ash (*Fraxinus excelsior* L.), European beech (*Fagus sylvatica* L.)] and two softwoods [Norway spruce (*Picea abies* Karst.), silver fir (*Abies alba* Mill.)] were used in the study. Detailed procedures for sample preparation and TM process were described previously (Allegretti et al., 2012; Ferrari et al., 2013). Unmodified equivalent wood samples served as reference. For the decay test, small wood blocks (5 × 10 × 30 mm) were cut randomly from modified boards (30 × 100 × 1000 mm).

2.2. Fungal species and strains

Three soft rot fungi [*Chaetomium globosum* (Kunze: Fries, Telemorph) (strain F-171-1, ATCC 34152) (syn = *Chaetomidium japonicum*), *Phialophora malorum* (M. N. Kidd & A. Beaumont) McColloch and *P. mutabilis* (J. F. H. Beyma) Schol-Schwarz (1970) (syn = *Lecythophora mutabilis*)] obtained from the culture collection maintained at the Department of Forest Products, Swedish University of Agricultural Sciences were used for the decay test. The fungi were recultured on 2.5% w/v malt extract agar (MEA) plates for two weeks. One plate of each fungus was homogenized with 100 mL deionized water for further inoculation.

2.3. Decay test

Resistance to soft rot decay was determined according to the AWPA standard E10-08 soil-block test (2008) with modification. In brief, Erlenmeyer glass flasks (100 mL) were used and half-filled with moist commercial planting soil. Test wood blocks were vertically placed in the soil with 5 mm of their length protruding above the soil surface. Subsequently, the flasks were sterilized at 103 kPa and 121 °C for 30 min. After cooling, the flasks were inoculated with 4 mL homogenized fungal solution and placed in a dark culture room at 25 °C and 70% relative humidity, to promote fungal growth. Flasks were weighed monthly to control the moisture content (~70–75%) of the soil by adding autoclaved deionized water. The moisture content of the tested wood blocks was also monitored during the whole decay process. Mass loss (%) of decayed wood blocks was determined at regular time intervals over 1 year after incubation. Mass loss data after 1 year's decay were subjected to one-way ANOVA with a post-hoc Tukey's test to examine the influence of treatment temperature, wood type and fungal species tested.

2.4. Light- and transmission electron microscopy (TEM)

Small pieces (~1 × 1 × 3 mm) taken from ash and beech blocks incubated with *P. mutabilis* for 18 weeks were fixed in 2.5% v/v glutaraldehyde + 2% v/v paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 3 h at room temperature. After washing in 3 × buffer (20 min each), post-fixation was carried out in 2% w/v osmium tetroxide in 0.1 M sodium cacodylate buffer for 3 h at room temperature. After subsequent washing in 3 × buffer (20 min each), samples were dehydrated in a graded ethanol series (20–100%) and embedded in London Resin White (Basingstoke, UK).

For light microscopy, semi-thin resin sections (~2 μm) cut on a Leica RM 2265 rotary microtome (Wetzlar, Germany) were mounted on glass slides and stained with 1% w/v toluidine blue or 1% w/v safranin O. Sections were observed using a DMBL Leica light microscope (Wetzlar, Germany) equipped with an Infinity X-32 digital camera (DeltaPix, Samourn, Denmark). For TEM, ultrathin sections (~90 nm) cut on a Reichert ultra-microtome (Wien, Austria) were examined using a Philips CM12 TEM (Eindhoven, Netherlands). Negative TEM films were prepared by a film scanner (Epson Perfection Pro 750, USA). To obtain information on the colonization process of fungi from the outside to inside of wood blocks, sections prepared from entire wood blocks (i.e. non-resin embedded blocks) using a Leitz 1300 sledge microtome (Wetzlar, Germany) were also observed.

3. Results and discussion

3.1. Fungal resistance of hardwood and softwood TMWs against soft rot fungi

Fig. 1 and Table S1 show mass loss of ash and beech TMWs (hardwoods) and the corresponding reference samples after 1 year's decay by the three soft rot fungi. The mass loss showed the order *P. mutabilis* > *C. globosum* ≫ *P. malorum* in TMWs treated at relatively low temperatures (190–200 °C) and reference wood, emphasizing a variation in decay ability between fungal species. In contrast, no notable difference in mass loss was detected in TMWs treated at high temperatures (210–220 °C) between fungal species (Table S3). In all fungi/wood species tested, mass loss in TMWs was always less than that for the reference during the same exposure period. After 1 year decay, the higher temperature TMWs always showed greatest decay resistance, with ash greater than beech (Fig. 1). In particular, TMWs treated at 210–220 °C ($TMW_{210–220°C}$, for 4 h) showed a major increase in the durability class, i.e. ~71–93% mass loss reduction compared to reference after 1 year's decay (Fig. 1). A similar trend in relation to TM temperature of the Termovuoto process was also shown in brown- and white rot decay in our previous study (Gao et al., 2016). Durability class 1–3 (i.e. very durable − moderately durable) was only achieved in ash and beech $TMW_{220°C}$ (i.e. high TM temperature), with greater decay resistance in ash TMWs than beech TMWs shown.

Interestingly mass loss patterns showed differences between reference and TMWs in the two hardwoods. References always showed lower mass loss in beech than ash wood for all fungal species (only exception was *P. malorum* after 1 year's decay) during the entire decay period (Fig. 1, Table S1). In contrast, TMWs showed opposite patterns and particularly ash $TMW_{200°C}$ degraded by *P. malorum* and *C. globosum* always showed more than twice as high mass loss than beech $TMW_{200°C}$ during the same exposure period (e.g. 10.1% in ash $TMW_{200°C}$ and 21.4% in beech $TMW_{200°C}$ after 1 year decay by *P. mutabilis*) (Fig. 1,