International Biodeterioration & Biodegradation 133 (2018) 230–237

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Acetylation increases relative humidity threshold for ion transport in wood cell walls – A means to understanding decay resistance

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ARTICLE INFO

Keywords:
Decay resistance
Diffusion
X-ray fluorescence
Humidity
Acetylated wood
Reduced equilibrium moisture content
Wood modification

ABSTRACT

The improved fungal decay resistance exhibited by modified wood has been attributed to inhibited diffusion of decay precursors and subsequent degradation products through the wood cell wall. However, data relating the effect of modification to diffusion through wood cell walls is lacking. Synchrotron X-ray fluorescence microscopy paired with an *in situ* humidity chamber was used to observe the transport of an implanted model metabolite, potassium (K^+) ions, in wood cell walls as a function of relative humidity (RH) and extent of the wood modification acetylation. The RH threshold for K^+ transport in wood cell walls increased with increasing levels of acetylation between 0 and 20 wt percentage gain (WPG), which clearly indicates that acetylation inhibits ion transport in the modified wood cell walls. The reduced equilibrium moisture content (EMC_R or moisture based on wood polymer mass) thresholds were also calculated, but the trend of EMC_R thresholds with WPG was inconclusive. Although the results provided support to the proposed mechanism that diffusion inhibition in acetylated wood caused decay resistance, the results could not confirm that diffusion inhibition was the most important mechanism. The observed inhibition of K^+ transport in acetylated wood should motivate additional work to understand how chemical modifications affect cell wall diffusion and the implications for producing decay-resistant wood.

1. Introduction

Wood is a desirable building material because it is economical, it is renewable, it typically requires relatively little energy to produce, and it sequesters carbon (Bergman et al., 2014; Jakes et al., 2016). One of the limitations of wood is that it is subject to decay when exposed to high moisture conditions. Wood decay is primarily caused by filamentous fungi, which are characterized as either brown, white, or soft rot fungi. Brown rot fungi pose an increased threat to wood and wood-based materials due to their prevalence on coniferous species, which predominate the construction market, and because they rapidly depolymerize all wood polymers, which can lead to rapid structural failure (Curling et al., 2002; Martinez et al., 2005; Arantes and Goodell, 2014) These fungi have 1–3 μm diameter hyphae that can grow through the open micron-scale spaces in the wood cellular structure and adhere to interior surfaces. In the initial stages of brown rot, these fungi secrete low-molecular-weight reactants or oxidant precursors that diffuse into the wood cell wall to oxidize and cleave cell wall polymers (Hammel et al., 2002; Arantes and Goodell, 2014). In brown rot, the oxidative mechanism is thought to be dominated by the fenton reaction of Fe^{2+} with hydrogen peroxide (H_2O_2) to create the highly reactive hydroxyl radical (Suzuki et al., 2006; Arantes and Goodell, 2014). Therefore H_2O_2 and Fe^{2+} inside the cell wall must be continually replenished by enzymatic action in the lumen. Fungal enzymes make H_2O_2 directly but are thought to reduce Fe via small diffusible reducing agents (Kerem et al., 1998; Tanaka et al., 1999; Cohen et al., 2002; Arantes and Goodell, 2014). White rot fungi are thought to produce a variety of diffusible oxidants including, but not limited to, veratryl alcohol cation radical, fatty acid peroxyl radicals, and manganese III chelates (Harvey et al., 1986; Popp et al., 1990; Tanaka et al., 1999). All these oxidants,

Abbreviations: WPG, Weight Percentage Gain; RH, Relative Humidity; EMC, Equilibrium Moisture Content; EMC_R, Reduced Equilibrium Moisture Content; XFM, X-ray Fluorescence Microscopy; ROI, Region of Interest; AMU, Atomic Mass Unit

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https://doi.org/10.1016/j.ibiod.2018.06.014
Received 22 February 2018; Received in revised form 31 May 2018; Accepted 16 June 2018
0964-8305/ Published by Elsevier Ltd.

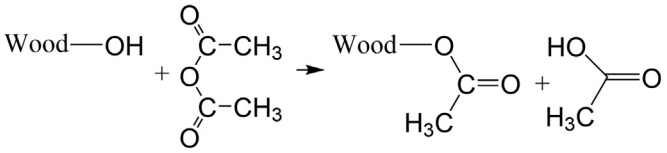

Fig. 1. Acetylation reaction.

after diffusing into and attacking the cell wall, release soluble sugars that then diffuse out of the cell wall and can be taken up as an energy source by the fungus (ten Have and Teunissen, 2001; Martinez et al., 2005; Arantes and Goodell, 2014). To protect wood from decay, copper-based preservative treatments are often prescribed. However, these wood preservatives are registered pesticides and their availability depends on future regulations and maintaining their registration with environmental regulators (Lebow, 2004). Nontoxic alternatives to wood preservatives are being sought as manufacturers and consumers are increasingly demanding decay-resistant wood products with non-biocidal treatments (Hill, 2011; Mantanis, 2017; Sandberg et al., 2017).

An alternative to preservative treatments is to make wood resistant to decay by modifying wood chemistry. Although numerous wood modification methods have been developed and found effective, acetylation is currently the most widely studied and is finding commercial success (Hill, 2006; Mantanis, 2017). During acetylation, wood is treated with acetic anhydride and the hydroxyl groups on wood polymers are replaced with acetate esters (Fig. 1). The effectiveness of the treatment depends on the extent of modification, which can be quantified using weight percentage gain (WPG), defined as the change in mass resulting from the modification divided by the original wood mass. Mass increases because the acetate ester (59 AMU) is heavier than the hydroxyl group (17 AMU) it replaces. Volumetric swelling of the wood cell wall is approximately the same as WPG (for example, 12% dry volume swelling at 12 WPG) (Hill and Graham, 2004). This irreversible swelling from acetylation decreases pore volume and the amount of water vapor that is sorbed at a given relative humidity (RH) (Stamm and Tarkow, 1947; Hill, 2006; Engelund et al., 2013; Popescu et al., 2014). With regards to decay resistance, acetylation to 20 WPG is very effective at inhibiting decay, whereas 10 WPG is only mildly inhibitory to decay (Stamm and Baechler, 1960; Ibach and Rowell, 2000; Larsson Brelid et al., 2000; Hill 2006, 2009).

Despite the empirically known effectiveness of acetylation, the material property changes caused by acetylation that lead to the improved decay resistance are not thoroughly understood and continue to be an active topic of research (Rowell et al., 2009; Ringman et al., 2014; Alfredsen et al., 2015; Hosseinpourpia and Mai, 2016b; Thybring, 2017). Proposed mechanisms for how wood modifications, including acetylation, inhibit decay, along with supporting references and analyses of which mechanisms are most likely to contribute, are available elsewhere (Ringman et al., 2014; Zelinka et al., 2016). The proposed mechanisms for decay resistance of modified wood include (1) acetylated hemicelluloses do not serve as a nutrient source for fungi (Rowell et al., 2009; Rowell, 2015); (2) fungal degradative enzymes that break down modified wood polymers are inhibited (Rowell, 2005; Rowell et al., 2009); (3) fungal degradative enzymes are unable to enter cell wall because micropores are blocked by the modification (Hill et al., 2005); and (4) diffusion within the cell wall is inhibited because the modification decreases the wood equilibrium moisture content (EMC) (Papadopoulos and Hill, 2002; Boonstra et al., 2007; Jakes et al., 2013; Xie et al., 2015; Hosseinpourpia and Mai 2016a, 2016b). Of the proposed mechanisms, Ringman and coworkers concluded that (4) is likely to be the most important (Ringman et al., 2014; Zelinka et al., 2016). Lowered diffusion rates would inhibit fungi by slowing the transport of degrading agents into the wood and also by slowing the transport of degradation products out of the wood to feed the fungus (Goodell et al., 2017).

Inhibiting cell wall diffusion could be an important mechanism by which acetylation imparts decay resistance to wood. However, there are no studies to our knowledge in which diffusion in untreated and acetylated wood has been compared. One study observed that veneers acetylated to 18.1 WPG immersed 48 h in a dilute solution of Fe^{++} absorbed approximately 20 times less Fe than control wood (Hosseinpourpia and Mai, 2016b). However, because no time course data were acquired, we suspect that this study reflects a higher Fe ion binding capacity of unmodified wood compared with acetylated wood, rather than a higher diffusion rate (Hunt et al., 2017).

In this study, experiments were performed to test the hypothesis that the mechanism by which chemical modifications, such as acetylation, impart decay resistance to wood could be the inhibition of diffusion through wood cell walls. We directly observed the moisture-dependence of potassium ion diffusion in different wood cell walls with varying levels of acetylation. .

2. Materials and methods

2.1. Wood

A kiln-dried loblolly pine (*Pinus taeda*) board (2 by 12 in., 5 by 30 cm) was obtained from Shuqualak Lumber in Shuqualak, Mississippi, USA. A single latewood band without compression wood, approximately 3 mm thick and located about 30 cm from the pith, was chosen and used throughout. Specimens prepared for acetylation treatment were typically 3 mm in the radial direction, 5–20 mm in the tangential direction, and 25 mm in the longitudinal direction. Samples were randomly assigned to a level of acetylation.

2.2. Acetylation

The samples used in this study were from the same batch of acetylation treatments used for a previous study, and full treatment details can be found there (Passarini et al., 2017). After immersion in acetic anhydride and reaction for various times at 140 °C, samples were removed, washed in water, and dried. Five individual specimens were used in this study, including the control and acetylation WPGs of 8.1 and 8.2 (pooled to get enough mass for sorption isotherm), 10.9, 13.2, and 20.3, which will be referred to as 8, 11, 13, and 20 WPG, respectively.

2.3. X-ray fluorescence microscopy (XFM)

X-ray fluorescence microscopy (XFM) and an *in situ* RH chamber were used to observe RH-dependent ion diffusion in wood cell walls. XFM is a synchrotron-based technique capable of mapping ions in wood cell walls with high sensitivity and submicron spatial resolution. In previous work on unmodified wood, this technique showed that implanted ions (K^+, Cl^-, Cu^{2+}, Zn^{2+}) in unmodified wood cell walls only diffuse above a threshold RH (Zelinka et al., 2014). Because acetylation is proposed to inhibit diffusion of all chemical species produced by any wood decay organism, any diffusible species would be sufficient to test this hypothesis. The K^+ ion in K_2SO_4 is a good model for decay agents or released degradation products because smaller species such as K^+ generally diffuse faster and K^+ ions tend not to form complexes (Dean, 1992). In addition, K_2SO_4 has a high deliquescence point (96% RH); therefore, the solid salt will not liquefy during the experiments. Finally, the K signal was strong enough to obtain high quality data.

Two-μm-thick sections measuring approximately 2 mm in length and 0.5 mm wide were cut using a diamond knife fit into a Leica EM UC7 ultramicrotome (Wetzlar, Germany). Sections were prepared with both transverse and radial–longitudinal orientations. Sample holders were made from a piece of 0.13-mm-thick Kapton™ (DuPont, Wilmington, Delaware, USA) film with a 1-mm-wide by 5-mm-long slot cut in the center. The wood section ends were secured to the Kapton™ film using small pieces of Kapton™ tape such that the section freely