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Assessing the destructive behaviors of two white-rot fungi on beech wood

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ABSTRACT

This research assessed the destructive behaviors of two white-rot fungi, *Pleurotus ostreatus* and *Trametes versicolor* and compared their degradation capabilities on solid oriental beech wood (*Fagus orientalis* Lipsky). Beech wood specimens were exposed to both fungi for a period of 120 days based on the specifications in the EN-113 standard. Mechanical properties and chemical composition of the specimens were measured every 15 days. Mass loss (ML) caused by the two fungi were not significantly different at the last stage of exposure, although *T. versicolor* showed higher ML at the middle exposure intervals. Compression strength parallel to grain, hardness, and impact bending values were higher in specimens exposed to *P. ostreatus*, although differences were not statistically significant. Microscopic images showed nearly the same decay patterns for both fungi. The results of the chemical assessment indicated that degradation of cell wall components was approximately the same for both fungi, but in some case *T. versicolor* showed a slightly higher potential to decay compared to *P. ostreatus*, especially in the early stages of exposure. The two fungi differed in their ability to reduce degree of polymerization (DP) holocellulose of beech wood. It was concluded that the destructive behaviors ability of both fungi was considerable.

1. Introduction

In nature, wood undergoes physical degradation (Maresi et al., 2013; Ozdemir and Tutus, 2013) and biological decay by white-rot, brown-rot, and soft-rot fungi. Bacteria attack wood after long exposure beneath soil and water in applications such as foundation piles, sleepers, cooling tower wood, harbor construction and boats (Eriksson et al., 1990; Zabel and Morrell, 1992; Mohebby, 2003; Schmidt, 2006). Saprotrophic wood decaying fungi, particularly white-rot fungi, carry out an essential role in the carbon cycle of terrestrial biomass, especially in a forest ecosystem, (Hatakka and Hammel, 2010). These processes are necessary for natural recycling of organic substances in different ecosystems (Blanchette et al., 1990; Stokland et al., 2012). They are also one of the equilizers in environmental cycling (Schmidt, 2006; Kubicek, 2013), because they are able to disintegrate the lignin cell wall complex (Kirk and Farrell, 1987; Eriksson et al., 1990; Hatakka, 2001; Koshijima and Watanabe, 2003).

The fungi causing rot are multi-celled fibrous organisms which use wood as food. Their hyphae spread throughout the wood and secrete enzymes which dissolve woody cells and lead to decay (Eaton and Hale, 1993). Wood undergoes a number of changes such as mass loss (ML) and decrease of the mechanical properties. These changes have a severe effect on the characteristics of wood such as

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moisture variability, electrical conduction, acoustics, convection, elasticity and plasticity. It is worth noting that changes in one characteristic are always accompanied with changes in other characteristics. For example, ML decreased the level of thermogenesis and strength (Winandy and Rowell, 1984; Zabel and Morrell, 1992). Various studies have so far been carried out on the influence of white-rot fungi on wood properties (Cowling, 1961; Wilcox, 1978; Blanchette, 1984a,b; Camarero et al., 1994; Adaskaveg et al., 1995; Martínez et al., 2005; Taghiyari et al., 2014); some studies were also specifically done on the *Pleurotus* species (Martínez et al., 2001, 2005; Bari et al., 2015a,b,c,d; Karim et al., 2016). The results of the previous studies demonstrated the selective and simultaneous decay patterns of white-rot fungi at the same time. The aim of this study was to determine and compare the biological degradation capabilities of two predator and terminator the white-rot fungi, the oyster fungus (*Pleurotus ostreatus*) and the EN-standard fungus (rainbow fungus, *Trametes versicolor*), to assess whether *P. ostreatus* would be an equivalent substitute for *T. versicolor* in EN-113. In addition to biological degradation capabilities at different stages of the decay process, changes to chemical and physical properties were compared to evaluate differences and similarities between the two test fungi. Changes in biological, chemical and physical properties were evaluated in 15-day intervals up to 120 days.

2. Material and methods

2.1. Source of fungi

The two white-rot fungi *Pleurotus ostreatus* (Jacq.: Fr.) Kummer isolate 11 and the standard fungus *Trametes versicolor* (L.: Fr.) Pilát isolate 325 were purified by Bari (Bari et al., 2015e) in accordance with Schmidt (Schmidt et al., 2012). *Pleurotus ostreatus* and *T. versicolor* were collected and purified at University of Sari; they were re-identified by rDNA-ITS sequencing (Bari, 2014). Fungal pure cultures were held on malt extract agar in Petri dishes.

2.2. Specimen preparation

Disks were cut from five 200-year old beech trees (*Fagus orientalis* Lipsky) at breast height. The thickness of the disks was 10–12 cm. To avoid any micro cracks, they were first air-dried to 23 ± 2% moisture content and then oven-dried at 103 ± 3 °C. Beech wood specimens 8 × 8 × 8 mm were prepared for light microscopy. Specimens 25 × 20 × 15 mm prepared according to EN 113 (1997) were used for determination of ML, 60 × 20 × 20 mm were prepared according to ASTM-D143-94 for hardness (ASTM, 2000), 60 × 20 × 6 mm were prepared according to ASTM-D256-04 (ASTM, 2004) for impact strength, and 50 × 20 × 20 mm were prepared according to ASTM-D143-94 for compression strength parallel to grain. Ten replicate specimens were prepared from different disks for each test. All specimens were kept in a conditioning chamber (25 °C, and 40 ± 3% RH) for 4 weeks prior to testing because wood has a thermo-hygromechanical behavior and its properties depend on the combined action of temperature, relative humidity, and mechanical load variations (Figueroa et al., 2012).

2.3. Biological resistance

Beech wood samples were oven dried at 103 ± 3 °C, then weighed, and after that sterilized at 121 °C for 20 min. After complete growing the fungal mycelia of both fungi in Kolle-flasks, the wood blocks were exposed to grown mycelia on 4.8% malt extract agar (Merck, Germany) in Kolle-flasks. Conditioned specimens were incubated for 120 days at 22 ± 2 °C and 65 ± 5% RH. At the end of the incubation time, mycelia were removed from the block surfaces. The blocks were oven dried and weighed again to calculate ML according to Equation (1) (EN 113, 1997).

$$ML(\%) = \frac{M_1 - M_2}{M_1} \times 100 \quad (1)$$

Where ML is the mass loss (%), M_1 is the dry matter before incubation (g), and M_2 is the dry matter after incubation (g).

2.4. Mechanical experiments

2.4.1. Compression parallel to grain

The samples for measuring compression parallel to grain were evaluated on the basis of ASTM-D143-94 with a loading rate of 5 mm/min in order to calculate their resistance according to Equation (2).

$$P_{II} = \frac{F_{max}}{A_{\circ}} \quad (2)$$

Where P is the resistance to compression parallel to grain (kN/mm^2), F = force (kN), A = the cross section area (mm^2).

2.4.2. Hardness

In order to measure hardness before and after fungal attack, the samples were evaluated according to ASTM-D143-94 (Equation (3)).

$$H = \frac{F}{\pi \times r^2} \times 1000 \quad (3)$$

Where H is resistance to hardness (N/mm^2), F = force (J), r = radius of the sphere.

2.4.3. Impact bending

Impact bending strength was determined according to ASTM-D256-04 using Equation (4).

$$I = \frac{F_{max}}{A_{\circ}} \quad (4)$$

Where I is resistance to impact (J/m^2), F = force (J), A = cross section area (m^2).

2.5. Chemical analysis

Changes in the chemical composition of the wood cell wall in healthy and decayed wood were evaluated according to TAPPI standards as well as a modified method for carbohydrate analysis (Davis, 1998). Decayed specimens were oven-dried, milled, and then passed through a sieve with mesh size of 40 to determine the content of lignin according to T 222 om-98 (TAPPI, 1998), the content of cellulose according to T 17 wd-70 (TAPPI, 1997), and the content of hemicelluloses according to T249 cm-85 (TAPPI, 1992).

2.6. Light and SEM microscopy

2.6.1. Light microscopy

Blocks of 8 × 8 × 8 mm were prepared from the degraded wood specimens and sectioned (10–15 μm) using a sliding microtome (GSL-1 microtome, WSL). The sections were stained with a 1:1:1 mixture of safranin 0.5% and astrablue 0.3% as well as picrin-anilinblue 1% (Gärtner and Schweingruber, 2013; Bari et al., 2015c) and dried sections were studied under a light microscope and photographed with the Olympus microscope and an Olympus