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Assessment of the use of dynamic mechanical analysis to investigate initial onset of brown rot decay of Scots pine (*Pinus sylvestris L.*)

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ABSTRACT

Microbiological degradation of wood by decay fungi can cause a rapid change in the structural properties of timber which can result in both strength and mass loss. Traditional techniques for the evaluation of decay (e.g. mass loss) lack the sensitivity to evaluate the effects of the very first stages of the decay process. This paper describes the effects of initial brown rot decay, defined by the amount of *Poria placenta* genomic DNA (gDNA) present in the samples, on the dynamic mechanical properties of the timber. It was found that there is a correlation between the mean storage modulus of the timber and the amount of *P. placenta* gDNA present, and therefore the level of decay. This shows that using dynamic mechanical analysis is a viable technique that can be used to study initial decay processes.

1. Introduction

Microbiological degradation of wood by fungi can cause a rapid change in the structural properties of timber (Jellison et al., 2013), however traditional techniques for the evaluation of the decay (e.g. mass loss) lack the sensitivity to evaluate the effects of decay in the very first stages of the decay process.

The mechanism for the decay of timber via brown rot is through oxidative and enzymatic reactions (Goodell et al., 1997; Arantes et al., 2012; Alfredsen and Pilgård, 2013; Alfredsen et al., 2015; Zelinka et al., 2016). The fungus initiates the production of hydroxyl radicals by secreting hydrogen peroxide and reductants in to the cell wall. The reductants reduce the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) (Goodell et al., 1997, 2006), which then reacts with the hydrogen peroxide to form hydroxyl radicals in the Fenton reaction. The hydroxyl radicals depolymerize hemicelluloses and cellulose, modify lignin and generate sufficient rearrangements in the cell wall to allow the hydrolysing enzymes to diffuse in to the wall and degrade the polysaccharides (Goodell et al., 1997; Baldrian and Valaskova, 2008; Arantes et al., 2012). Ray et al. (2010) terms the above mechanism the Early Stage Decay Mechanism (ESDM) and suggest that it can act both rapidly and extensively. This mechanism has also been referred to as chelator-mediated Fenton (CMF) reaction (Arantes and Goodell, 2014). Green and Highley (1997) and Cowling (1961) both noted that whilst the reduction in the degree of polymerization is rapid there is low mass loss at this stage of decay. The study of early onset decay has, in recent years, been furthered by the use of molecular techniques. gDNA quantification has shown to be more sensitive than ergosterol and kition assays for estimation of fungal biomass in early stages of decay both in sterile laboratory samples (Eikenes et al. 2005) and for field samples (Pilgård et al. 2011). Hietala et al. (2014) found that the suppressive effect of suboptimal temperature on wood decay caused by *P. placenta* appeared more pronounced in Scots pine heart wood than in sapwood. At 30 °C heartwood showed no mass loss, poor substrate colonization (gDNA quantification) and marker gene transcript level profiles indicating a starvation situation.

Alfredsen et al. (2015) reviewed the role of quantitative gDNA and gene expression studies in the understanding of the mode of action of brown rot decay on modified wood, charting the method development and recent studies. Based on gene profiles the studies provide indications of a possible shift toward increased expression, or at least no down regulation, of genes related to oxidative

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metabolism and no reduction, or concomitant reduction, of genes related to the enzymatic breakdown of polysaccharides in modified wood compared to untreated control. Using gene expression Ringman et al. (2014) showed that *P. placenta* was present in acetylated, DMDHEU-treated and thermally modified wood after 2 days while at 6 days, the gene expression levels were significantly different from zero. Alfredsen et al. (2016) found *P. placenta* gDNA in acetylated samples with high treatment level (22% acetyl content) after four weeks while initial mass loss was not detected until 20 weeks. It is interesting to note that the time to mass loss of the acetylated timber was in agreement with other researchers (Hill et al., 2006). However, fungus was present in the timber much earlier in the test, which suggests a good efficiency of acetylation in the prevention of decay.

Compositional data and structural properties of wood have also been used in investigating the early stages of brown rot decay. Monrroy et al. (2011) assessed the change in the degree of polymerization of the cellulose (of *Pinus radiata*), the crystalline structure of the cellulose and the hemicellulose content of the wood over a decay period of 8 weeks (using 20 mm × 25 mm × 5 mm chips). Different species of brown rot were assessed with *Gloeophyllum trabeum* showing the highest rate of cellulose degree of polymerization reduction and *Laetiporus sulphureus* showing a comparable loss in cellulose crystallinity. Curling et al. (2002) examined the effect on the hemicellulose content and the mechanical properties of southern yellow pine exposed to brown rot fungi. Their data showed that during initial decay, where there was negligible weight loss (up to 20 days exposure), there were significant changes in hemicellulose content associated with up to a 20% loss in modulus of elasticity, 40% loss in modulus of rupture (4 point bending strength) and 80% loss in work to maximum load. This indicates that physical properties may be a more appropriate method of studying early stage decay than simple mass loss. Their method used static mechanical tests to derive the data, although there is another physical characterisation method, Dynamic Mechanical Analysis (DMA), which has become a well-established technique for dynamically measuring the viscoelastic behaviour of polymers and polymer composites. The earliest examples of DMA studies on wood were in the 1960s using torsional pendulum apparatus (Norimoto and Yamada, 1966; Becker and Noack, 1968). DMA is often used to measure the response of a material to changes at the molecular or microstructural level (often but not always brought on by a change in temperature). In their paper, Birkinshaw et al. (1986) assess the response of 10 timber species to dynamic flexing whilst being subjected to a temperature gradient raising from 10 °C to 100 °C at 10 °C h^{-1}. The species were selected for their variety in densities and in microstructure. It was noted that whilst the absolute values had a large variation the general shape of the shear storage modulus curve and the loss modulus (tan δ) were similar. Much research has been undertaken to determine the response to changes in both temperature and humidity; the effect of the humidity and moisture content of the wood on the glass transition temperature (T_g) and other relaxations of the lignin and hemicelluloses of wood; and the effect on the mechanical properties of the timber (Hillis and Rozsa, 1978; 1985; Kelley et al., 1987; Salmén and Olsson, 1998; Olsson and Salmén, 2004).

An important factor in the use of DMA is the determination of the Linear Viscoelastic Response region (LVR), and experiments should be conducted within this region. Within this region the strain is directly proportional to the stress and therefore the polymer packing is not altered by the stress applied; however, once the response becomes non-linear the polymer packing is being significantly and irreversibly altered. Sun et al. (2007) used DMA to assess the dynamic response of timber at low moisture content (<1%); they noted that the LVR was very low, between 0.03% and 0.16% strain although values were greater when bending perpendicular to the grain.

McCarthy et al. (1991) studied the fungal degradation of wood via DMA. Small samples were exposed to the brown rot fungus *Coniophora puteana* over a six week period on a weekly basis in accordance with ASTM D2017 and DIN 50 008. The response to dynamic loading was measured between −100 °C and 250 °C at a heating rate of 5 °C min^{-1}. The researchers showed that the shear modulus fell with increased amounts of decay at all temperatures. It was noticed that whilst the absolute values for the modulus changed with the increase in decay, tan δ and the shape of the shear modulus curve did not change significantly. It should be noted that this is in agreement with the earlier work of Birkinshaw et al. (1986), in that there are changes in the absolute values but not the location of the tan δ peak. Whilst we understand that the oxidative reaction depolymerizes the wood holocelluloses prior to the components being utilized by the fungi (which does not lead to a mass loss), the implication of this research is that the ultimate degradation of the polymers responsible for the stiffness of the wood is relatively uniform in rate.

The investigations undertaken to date have focused on the assessment of wood's ability to respond to a dynamic temperature range and the effects of altering the molecular and microstructure of the wood. The objective of this study was however, to determine the potential of DMA, under isothermal conditions, as a method for the assessment and quantification of initial onset brown rot decay.

2. Materials and methods

Scots pine (*Pinus sylvestris L.*) from South Norway was supplied by Norwegian Institute of Bioeconomy Research Norway. The pine was straight grained and had an average growth ring distribution of 10 rings/cm. The Scots pine was then machined to 2 mm × 2 mm strips and samples were selected to achieve 1 single latewood band through the centre of the strip. All samples were conditioned to 65% RH at 20 °C before testing.

2.1. Fungal exposure

For this study fungal cultures of *P. placenta* were used; *P. placenta* is a brown rot causing fungus that is stipulated as a test fungus in European Standard decay tests such as EN113 and cultures were obtained from the legally designated strain holding laboratory. The strain used was *Poria placenta* (Fries) Cooke sensu J. Eriksson (Synonym: *Poria monticola* Murrill) FPRL 280 (Building Research Establishment — Garston, Watford, Herts WD2 7JR — UK.

Cultures were grown on 90 mm Petri dishes containing 4% malt agar (Malt extract powder 40 g; Agar 20 g; Deionised water 1 L). *P. placenta* (FPRL 280) is a fungal isolate stipulated by a number of European testing standards for example EN113 (CEN, 1997). Once the fungal culture had grown to cover the entire surface of the plate the cultures were ready for samples. Wood samples (2 mm × 2 mm x 80 mm) were cleaned by immersing them in ethanol for 1 min. The samples were then dried and vented aseptically using a laminar flow bench. Once dry, the samples were aseptically placed onto the pre-prepared fungal cultures as follows: two samples were placed in direct contact with the agar/fungus to act as supports, with three further samples placed perpendicular and on top of the supports. This was intended to prevent water logging which may have prevented decay. The cultures were then incubated at 22 °C ± 2 at 70% RH ± 3 with replicate samples (n = 6) removed (aseptically) from incubation after 4, 8, 11 and 17 days. Upon removal from test the wood samples were dried for 12 h at 50 °C in order to stop fungal growth without degrading any DNA. Dried samples were stored in sterile tubes before subsequent DNA isolation and testing.