BIODEGRADATION OF WOOD

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Introduction

This presentation will overview the degradation of wood by white rot fungi, brown rot fungi and also soft rot decay; however, brown rot decay will be then be the primary focus because this is the primary type of decay found in Canada and is responsible for significant damage to softwood lumber, poles and ties.

White rot fungi have been extensively studied over the last 25 years because of their potential in biorefinery applications, but until more recently, brown rot fungi and soft-rot fungi had not been the focus of most decay studies. More information is needed not only about how brown rot fungi are able to attack solid wood and also about how soft rot fungi invade wood and, for example, how some soft-rot fungi produce strings of unique diamond-shaped cavities within the wood cell wall.

Increasing interest particularly in the brown rot fungi, has allowed new information to be uncovered over the past 10-15 years with regard to the unique decay mechanisms employed by these fungi. These mechanisms are unique in all of biological systems and the ways in which brown rot fungi have developed these mechanisms will be a primary focus of the talk, not only because of their importance as destructive agents in Canada, but also because the unique brown rot fungal chemistries have potential application in the bioprocessing of wood (biorefinery applications). These oxygen radical chemistries also present both a challenge and an opportunity for the development of protection systems that can be used for wood protection/preservation.

Overview of Soft Rot and White Rot Fungal Degradation of Wood

Soft rot fungi typically attack the outer surface of wood in relatively wet environments (Zabel, Lombard, Wang, & Terracina, 1985). However they are known to cause extensive and deep degradation (extending several centimeters deep into the wood) of utility poles in Scandanavia and northern Europe with other reports of deep penetrating soft rot from other areas of the world appearing periodically (Greaves, 1977). Soft rot decay that penetrates deeply into the wood occurs only when the wood is at a high moisture content, but not saturated. It is unknown whether this type of deep-penetrating degradation occurs more extensively in other parts of the world other than Scandanavia, or if it simply has been less frequently reported because of the limited number of studies on this type of fungal attack.

As soft rotted wood dries it develops surface checks across the grain as the wood shrinks. The wood becomes brown in color. In most areas of the world the decay occurs primarily only at the wood surface and the surface appearance may be similar to brown rot decay. In advanced stages of soft rot decay the wood can be brash or brittle, similar to that of brown rotted wood. Typically the wood is described as having a weathered appearance like unpainted "barn board".

White rot fungi are known to produce a complete enzymatic system for either directly or indirectly oxidizing and deconstructing lignin (Hatakka, 1994; Hatakka, Lundell, & Jeffries, 2002). Enzymes causing the degradation of lignin include: lignin peroxidase, manganese peroxidase, versatile peroxidase and laccase (Goodell, 2003). However, specific white rot fungi typically secrete only one or a select suite of these enzymes.

Cellulose and hemicellulose degradation in the white rot fungi is also known to occur via enzymatic processes and the white rot fungi produce both endo-glucanases and exo-glucanases which can act synergistically on the crystalline cellulose. The non-enzymatic processes which are known to be involved (Daniel, 2003; Messner et al., 2003) particularly for hemicellulose depolymerization and selective white rot attack are not well understood. Enzymatic systems for breakdown of holocellulose include endoglucanases, ß-glucosidases and cellobiohydrolase enzymes (T.K. Kirk & Cowling, 1984; Uzcategui, Ruiz, Montesino, Johansson, & Pettersson, 1991) as well as xylosidase, xylanase, acetyl xylan esterase, glucuronidase and arabinofuranosidase; these later enzymes being necessary for complete depolymerization and oxidation of hemicellulose (Blanchette, Abad, Farrell, & Leathers, 1989; T.K. Kirk & Cowling, 1984).

Beyond the physical appearance of the wood undergoing decay, two types of white rot are known based on the manner in which the wood cell wall components are oxidized. "Simultaneous white rot" is the most common type of white rot of wood and wood products in nature and typically, cellulose, hemicellulose and lignin are all degraded at some relatively uniform rate with this type of white rot. "Selective white rot" occurs when hemicellulose and lignin are attacked preferentially to cellulose, in some cases and with some fungal species, allowing the cellulose to remain relatively undegraded. Fungi that produce this later type of degradation have been studied for several years in selective delignification systems for biotechnological applications (Akhtar, Blanchette, Myers, & Kirk, 1998; Hatakka, 1994; T. Kent Kirk, Tien, & Faison, 1984). In nature there is often an intergradation of white rot types and both simultaneous and selective white rot decays have been reported to be produced by the same fungus (Messner et al., 2003; Otjen & Blanchette, 1986).

Overview of Brown Rot Fungal Degradation of Wood

Brown rot fungi were previously considered to be more primitive than other species of wood decay fungi because the brown rots have an incomplete enzymatic system for the degradation of both lignin and cellulose. However, it is now known that the true brown rot fungi (and also some ectomycorrizal fungal lineages which are closely related), have evolved relatively recently from predecessors of the white rot wood degrading fungi (D. C. Eastwood, 2014). White rot fungi are therefore evolutionarily more primitive but, as reviewed above, they have a complete complement of extracellularly secreted enzymes that are capable of attacking and depolymerizing all cellulosic components (cellulose and hemicellulose) as well as lignin in woody materials (D. C. Eastwood, 2014; Daniel C. Eastwood et al., 2011). The brown rot and ectomycorrizal fungi have abandoned many of their extracellular enzymes, and this has allowed these fungi to establish themselves in broad ecological niches, including for the brown rot fungi, wood used in construction. The brown rot fungi in particular have evolved an efficient non-enzymatic mechanism that generates reactive oxygen species (ROS) (Arantes, Jellison, & Goodell, 2012) or "free radicals" that are capable of directly oxidizing lignocellulose cell walls and depolymerizing both cellulose and lignin (Yelle, Wei, Ralph, & Hammel, 2011). These are the same "free radicals" that are discussed in television commercials that can cause damage in the human body, and that are related to ailments ranging from heart disease to skin melanomas.

This ROS generating system that the brown rot fungi use is known as the "chelator mediated Fenton" (CFM) system, and it permits the generation of oxygen radicals at a distance from the fungus. The CMF mechanism was previously reported by our group in the journal *Science* and elsewhere (Arantes & Goodell, 2014; Daniel C. Eastwood et al., 2011; Goodell et al., 1997). By generating the ROS at a distance from the fungus and within the wood cell wall, it helps the fungus to avoid attack by the oxygen radicals generated. Figure 1 presents a schematic overview of the CMF system, which involves two different types of chelators.

Because the brown rot lignocellulose degrading fungi are more recently evolved, their species numbers are relatively small compared to the earlier white rot fungal species. Only 6% of all lignocellulose degrading fungi are considered to be brown rots (Daniel C. Eastwood et al., 2011). Despite the small number of species, the brown rot fungi have exploited important ecological niches and they are now the dominant species found (relative to white rot fungi) in the coniferous forests of the northern hemisphere (Arantes & Goodell, 2014). This also means that they also are the dominant fungal type that attacks softwood construction timber. This does not mean that the brown rots will not attack hardwoods, and they can readily do this as well.

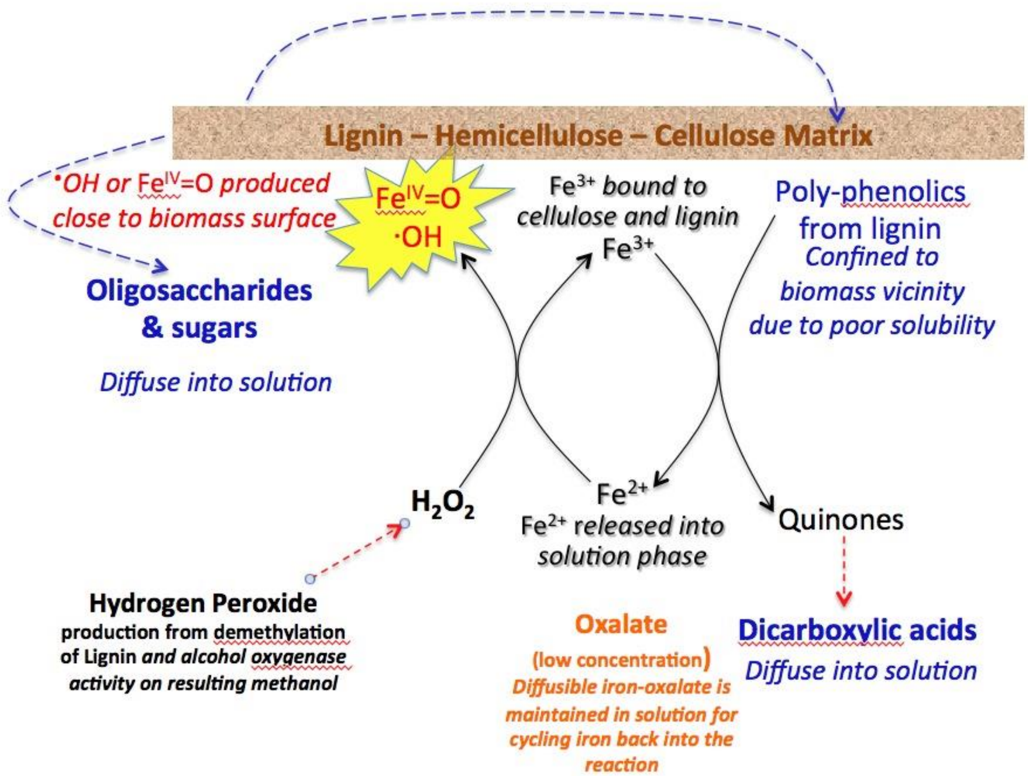

Figure 1. The roles of two chelators for: 1) iron solubilization and sequestration, and 2) iron-reduction with other components of the Chelator-Mediated Fenton (CMF) reaction.

It has been postulated that, by jettisoning the physiologically expensive production of many extracellular enzymes that white rot fungi have, and instead adopting a non-enzymatic unique CMF mechanism which permits deconstruction of cellulose and lignin substrates, that the brown rot fungi have become more efficient organisms relative to their ability to extract energy from woody biomass and that this has permitted their expansion in broader environmental niches. Evolutionarily therefore, we might expect brown rots to continue their expansion into attacks on other wood species, and recent evidence now suggests that related fungal species may be expanding their substrate/host capacity significantly by exploiting chemistries similar to that of the CMF.

Can we Develop New Wood Protection Systems Based on this Knowledge?

New details about the CMF mechanism have emerged since the initial reports of the mechanism by our laboratories approximately 20 years ago (Chandhoke, Goodell, Jellison, & Fekete, 1992) (Goodell & Jellison, 1998) (Goodell et al., 1997), and this talk will provide insight into how enhanced wood protection systems may potentially be developed specifically to counteract this mechanism. Most biocides on the market function in preventing decay and deterioration of wood because of their overall toxicity to fungi and insects. Although this is an effective method for treatment, broad-spectrum biocides often run afoul of environmental concerns, and metallic biocide systems, including copper-based systems are under regulatory pressure now. This is particularly true in Europe where copper and other metal-based biocidal systems are being phased out because of the new EU biocide directive. Because the CMF system relies on a pH differential being developed at the nanoscale, systems that disrupt this differential can potentially be both environmentally appropriate and also effective in controlling brown rot decay. Similarly, systems that scavenge free radicals, and that chelate metals can also be effective. However, like most of life, and single-target biocide may not be effective simply because fungi are highly evolved to adapt to roadblocks they may encounter. Therefore understanding decay processes, in conjunction with learning how to develop multi-target biocides combined with non-biocidal protection systems, may be the best approach to the exploration of future protection systems.

Summary and Conclusions

White rot, soft rot and brown rot fungi remain as the primary agents of destruction for wood in-service. Recent evidence shows however that brown rot fungi have evolved from the predecessors of white rot fungi, and in this process the actually lost many of their enzymatic systems to become highly efficient degraders of wood. As the brown rot fungi lost many of their enzymes, they evolved a unique oxygen radical generating system that does not involve extracellular enzymes and that is responsible for much of damage that occurs in brown rotted wood. The unique CMF oxygen radical mechanism has not been reported in any other biological system, and allows the oxygen radicals (free radicals) to deconstruct wood during decay without damage to the brown rot fungus.

Brown rot is the dominant decay type in Canada and throughout the northern hemisphere, and even though the relative number of brown rot species is quite small, these organisms are very important relative to the damage caused to wood. Understanding the mechanisms of how these fungi decay wood is vital for developing the next wave in wood protection systems especially as new regulations and public perceptions limit options for utilization of more traditional biocides. A multi-pronged approach that takes advantage of new knowledge may help in the development of new protection methods and systems.

Literature Cited

Akhtar, M., Blanchette, R., Myers, G., & Kirk, T. (1998). An overview of biomechanical pulping research.

Arantes, V., & Goodell, B. (2014). Current Understanding of Brown-Rot Fungal Biodegradation Mechanisms: A Review. In *Deterioration and Protection of Sustainable Biomaterials* (Vol. 1158, pp. 3-21): American Chemical Society.

Arantes, V., Jellison, J., & Goodell, B. (2012). Peculiarities of brown-rot fungi and biochemical Fenton reaction with regard to their potential as a model for bioprocessing biomass. *Applied Microbiology and Biotechnology, 94*(2), 323-338.

Blanchette, R. A., Abad, A. R., Farrell, R. L., & Leathers, T. D. (1989). Detection of Lignin Peroxidase and Xylanase by Immunocytochemical Labeling in Wood Decayed by Basidiomycetes. *Appl Environ Microbiol., 55*, 1457-1465.

Chandhoke, V., Goodell, B., Jellison, J., & Fekete, F. A. (1992). Oxidation of 2-keto-4-thiomethylbutyric acid (KTBA) by Iron-binding Compounds Produced by the Wood-decaying Fungus *Gloeophyllum trabeum*. *FEMS Microbiology Letters, 90*, 263-266.

Daniel, G. (2003). Microview of Wood under Degradation by Bacteria and Fungi. In *Wood Deterioration and Preservation* (Vol. 845, pp. 34-72): American Chemical Society.

Eastwood, D. C. (2014). *The evolution of fungal wood decay*: American Chemical Society - Oxford University Press, Book Series.

Eastwood, D. C., Floudas, D., Binder, M., Majcherczyk, A., Schneider, P., Aerts, A., et al. (2011). The Plant Cell Wall-Decomposing Machinery Underlies the Functional Diversity of Forest Fungi. *Science, 333*(6043), 762-765.

Goodell, B. (2003). Brown rot degradation of wood: Our evolving view. (Eds): Goodell, B., D. Nicholas, and T. Schultz. . . In B. Goodell, D. D. Nicholas & T. P. Schultz (Eds.), *Wood Deterioration and Preservation: Advances in Our Changing World* (pp. 97-118): American Chemical Society Series. Oxford University Press.

Goodell, B., & Jellison, J. (1998). Role of biological metal chelators in wood biodeterioration. In A. Bruce & J. Palfreyman (Eds.), *Forest Products Biotechnology* (pp. 235-250). London: Taylor and Francis Publishers.

Goodell, B., Jellison, J., Liu, J., Daniel, G., Paszczynski, A., Fekete, F., et al. (1997). Low Molecular Weight Chelators And Phenolic Compounds Isolated from Wood Decay Fungi and their Role in the Fungal Biodegradation of Wood. *Journal of Biotechnology, 53*, 133-162.

Greaves, H. (1977). An Illustrated Comment on the Soft Rot Problem in Australia and Papua New Guinea. *Holzforschung, 31*(3), 71-79.

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Reviews, 13*(2-3), 125-135.

Hatakka, A., Lundell, T., & Jeffries, T. (2002). Preface. *Enzyme and Microbial Technology, 30*(4), 423-424.

Kirk, T. K., & Cowling, E. B. (1984). Biological decomposition of solid wood. In R. M. Rowell (Ed.), *Chemistry of Solid Wood,* (pp. 455-487). Washington, DC: American Chemical Society Press.

Kirk, T. K., Tien, M., & Faison, B. D. (1984). Biochemistry of the oxidation of lignin by Phangerochate chrysosporium. *Biotechnology Advances, 2*(2), 183-199.

Messner, K., Fackler, K., Lamaipis, P., Gindl, W., Srebotnik, E., & Watanabe, T. (2003). Overview of White-Rot Research: Where We are Today. In (pp. 73-96): ACS Symposium Series, Vol. 845.

Otjen, L., & Blanchette, R. A. (1986). A discussion of microstructural changes in wood during decomposition by white rot basidiomycetes. *Canadian Journal of Botany, 64*(5), 905-911.

Uzcategui, E., Ruiz, A., Montesino, R., Johansson, G., & Pettersson, G. (1991). The 1,4-β-d-glucan cellobiohydrolases from Phanerochaete chrysosporium. I. A system of synergistically acting enzymes homologous to Trichoderma reesei. *Journal of Biotechnology, 19*(2–3), 271-285.

Yelle, D. J., Wei, D., Ralph, J., & Hammel, K. E. (2011). Multidimensional NMR analysis reveals truncated lignin structures in wood decayed by the brown rot basidiomycete Postia placenta. *Environmental Microbiology, 13*(4), 1091-1100.

Zabel, R. A., Lombard, F. F., Wang, C. J. K., & Terracina, F. (1985). Fungi associated with decay in treated southern pine in the eastern United States. *Wood and Fiber Science, 17*, 75-91.