MICROSCOPIC STUDY OF LIME WOOD DECAYED BY *CHAETOMIUM GLOBOSUM*

CARMEN-MIHAELA POPESCU, CARMEN MIHAELA TIBIRNA,[*] AL. MANOLIU,[**] PETRONELA GRADINARIU[**] and CORNELIA VASILE

Romanian Academy, "Petru Poni" Institute of Macromolecular Chemistry, Department of Physical Chemistry of Polymers, Iasi, Romania
[*]Centre de Recherche sur le Bois (CRB), Départment des Sciences du bois et de la forêt, Laval University, G1V 0A6, Québec, Canada
[**]Biological Research Institute, Iasi, Romania

Received January 20, 2011

Wood is degraded by microorganisms, when moisture, oxygen and other environmental factors favour microbial growth. In this study, biodegradation of lime wood was artificially induced for evaluating the damage of lime wood caused by *Chaetomium globosum*. SEM analysis showed that the fungal hyphae grow almost exclusively in the cellulosic part of the wood cell wall, known as the S2 layer. Initial colonization is caused by the hyphae that enter the tubular wood cells and grow inside them, frequently occurring as distinct decay cavities revealed in longitudinal sections. These cavities are elongated, with sharp pointed ends, occurring as either singular cavities or in chains. The latter are helically oriented, following the microfibrillar angle. Microscopic examination of the interior part of the wood samples showed generalized thinning and erosion of the cell walls, without any localization of decay around the hyphae. The overall effect is that the wood loses its strength and shrinks, developing longitudinal and transverse cracks, which eventually join the wood breaks.

***Keywords*:** SEM, biodegradation, lime wood, *Chaetomium globosum*

INTRODUCTION

Biodeterioration represents a complex of natural physical and chemical spoilage processes in various materials, caused by the growth of very different organisms. These are generically called biodeteriogens, but they are all characterized by the saprotrophic ability of using substrates to sustain their growth and reproduction.[1,2]

The main components of woody cell walls – cellulose, hemicelluloses and lignin – are degraded to different extents by various groups of organisms. Several types of biodegradation have been recognized in wood, *e.g.* fungal decay, bacterial degradation and insect attack, but the greatest damage results from the fungi action.

The ability of rot fungi to degrade wood varies among fungal species and depends on the chemical properties of wood and on its structural features. Wood decay is initiated by fungal enzymes, acting on the cell wall components of the wood. Although most of the wood-rotting fungi are able to degrade both cellulose and lignin, they exhibit different degradation rates for these substances. The growth characteristics of the microorganisms in wood and the type of degrading system produce different decay patterns.[3] Depending on the type of decay, different physical, chemical and morphological changes occur in wood.[4,5] According to the macroscopic differences of their substrate utilization, wood rotters are classified into three specific decay groups: white rot, brown rot, and soft rot fungi.[6-8]

The reactive oxygen species (ROS) are involved in all these major types of wood rotters. White rot basidiomycetes degrade both wood polysaccharides and lignin and, in

some cases, delignify wood selectively. There are reports that white rot fungi produce extracellular -OH, but they also secrete peroxidases, laccases and cellulases, which undoubtedly participate in lignocelluloses degradation. Brown rot basidiomycetes degrade wood polysaccharides, while leaving behind an oxidized, polymeric lignin residue. Brown rotters lack most of the oxidative enzymes that white rot fungi have, and most of them lack complete cellulase systems, but many studies report that these fungi produce -OH. Soft rot ascomycetes and deuteromycetes resemble brown rotters in that they degrade wood polysaccharides more readily than lignin. Little is known about their decay mechanisms, but there is evidence that some produce ROS.[9]

A large part of the wood degradation on land is carried out by aerobic white rot and soft rot fungi. Both classes degrade cellulose and hemicelluloses to soluble carbohydrates by secreted synergistic enzymes. White rot fungi are *Basidiomycetes* and can totally mineralize lignin. Soft rot fungi, often *Ascomycetes*, do affect and modify the lignin, but do not mineralize it.[10-14]

The decay pattern caused by soft rot fungi is either a chain of cavities following the microfibril angle in the secondary cell wall (Type 1), or erosion from the cell lumen towards the middle lamella (Type 2).[15] Soft rot is considered to be of the first decay type found in a general sequence of wood colonization,[16] possibly occurring in dry environments,[4,17] but it is mostly present where brown and white rot fungi are inhibited by factors such as high moisture content, low aeration and presence of preservatives or high temperatures.[15,16] Soft rot fungi were found able to degrade cellulose, but not lignin. These fungi cause substantial losses in wood properties in early stages of attack and in various environments.

What type of deterioration occurs and how these processes impact on wood are important questions that need consideration, if the properties of wooden artifacts are to be studied and properly preserved. It is essential to improve our understanding on the fungi and on the processes that affect wood, and to deeper develop our knowledge of the structural and chemical changes produced in wood by degradation. Previous papers of ours evaluated the structural changes[18] and thermal properties[19] of the fungal degraded lime wood, having established that *C. globosum* is able to reduce wood strength by acting preferentially upon the wood constituents. It was assumed that the mode of attack on the lignified cell wall is characterized by cavity formation in the secondary wall. The present study, based on SEM examination, brings new evidence on these aspects. Knowing the morphological and structural changes[18] of the wood decayed by this fungus and its deterioration potential is essential both for understanding the ageing of wooden cultural heritage objects and for conducting the antiquating process of wood by various techniques.[20]

EXPERIMENTAL

Materials

The lime wood samples were cut from a 70 year-old tree, at a 1.30 m height above the ground, and let to air-dry thoroughly (TAPPI T 257 cm-02). The wood blocks (50×50×3 mm) were oven-dried at 105 °C (TAPPI T 264 cm-97), until constant weight was reached. The samples were sterilized and exposed (by contact method) to *Chaetonium globosum* in Petri dishes containing 2% malt extract, 2% dextrose, 2% agar, in distilled water, pre-inoculated 7 days prior to the test, and then incubated at 28 °C for 133 days.

During the 133-day period, at each 7-day interval, three lime wood samples were taken up from the exposure medium, washed repeatedly with twice-distilled water to remove mycelia from their surface, and then oven-dried to constant weight. The mass loss of each individual sample was calculated and used to determine the mean weight percentage losses.

The fungus action was manifested by a continuous decrease in the sample mass, of 0.49 wt%/day in the first 70 days, and of 0.29 wt%/day in the next 63 days. The average mass loss of the lime wood blocks after 133 days of exposure to *C. globosum* was of 50.4%.

By visual observations and mass loss determinations confirmed by spectral studies[18] and thermogravimetric analysis,[19] it has been established that the period of fungus growth on the wood sample surface is of about 28 days, after which the fungus reaches maturity and forms spores, its attack becoming more aggressive. The visual aspect of the fungus colony on the wood surface is the same for all samples exposed from 70 to 133 days.

Method

Scanning electron microscopy (*SEM*). The lime wood samples were analyzed with a SEM JEOL-JSM-840 A. The acceleration voltage was

of 15 kV. To improve the conductivity of the samples and the quality of the SEM images, the samples were coated with a very thin layer (18 nm ± 0.2 nm) of Pt-Au alloy, using a covering SEM device.

The SEM micrographs were taken at a magnification of 1000 X in the longitudinal direction of the stem.

RESULTS AND DISCUSSION

Lime wood, whose pores are uniform in size and distributed across the growth ring, is considered to be diffuse-porous. Fibres are characteristically long and needle-like, with tapering, pointed ends and relatively thick walls (Fig. 1).

The presence of the soft rot decay caused by *Chaetomium globosum* has been revealed by scanning electron microscopy. The cell walls showed hyphal tunnelling along the cellulose microfibrils of the S2 layer, resulting in the formation of holes in transverse sections. This mode of attack is typical of a Type 1 soft rot.[21] Even at advanced stages, the persistence of a "lignin-rich" skeleton, representing the most lignified components of wood, preserved stiffness, so that the wood became brittle. The persistence of lignin-rich regions of the wood matrix in trees decayed by *C. globosum* was not only due to their high lignin content *per se*, but also to their high percentage of guaiacyl lignin.[22]

Especially fibres with a narrow lumen were colonized uniformly by solitary hyphae. However, they frequently occurred within the S2 layers with distinct decay cavities, as revealed in the longitudinal sections.

These cavities were elongated, with sharp pointed ends, occurring either singularly or in chains. The latter were helically oriented following the microfibrillar angle.

Initial colonization by the hyphae entering the tubular wood cells and growing inside them was observed. A lateral branch then penetrated the thin inner lignified S3 layer within the lumen of the tube. As soon as it entered the cellulosic S2 layer, it branched into two hyphae growing up and down the cell wall – presumably aligned with the cellulose microfibrils – giving the appearance of a T junction. Fine hyphae exhibiting branching continued to extend for a short time, but then stopped apical growth. At this stage, a cavity was formed within the cell wall, following the orientation of the cellulose microfibrils, which was followed by a further phase of apical growth at the hyphal tip, producing a needle-like proboscis hypha. This process was repeated for many times, leading to the formation of a spiral chain of cavities within the wood cell wall, all oriented to the angle of the cellulose microfibrils and each showing different stages of cavity expansion (Fig. 2).

The repetitive start-and-stop pattern of apical hyphal growth resulted in the gradual breakdown of the wood cell wall layer of the secondary wall (Type 1 degradation),[23] which is a characteristic mechanism of cell wall degradation. Discrete notches of cell wall erosion by hyphae growing within the lumina, in addition to the cavities formed by hyphae within the cell wall, are also frequently found in wood degraded by soft rot fungi. These erosion troughs (Type 2), which are indistinguishable from those of the white rot fungi, have been attributed to a category of soft rot known as Type 2 attack.[22] It is known that *Chaetomium globosum* produces both types of attack.

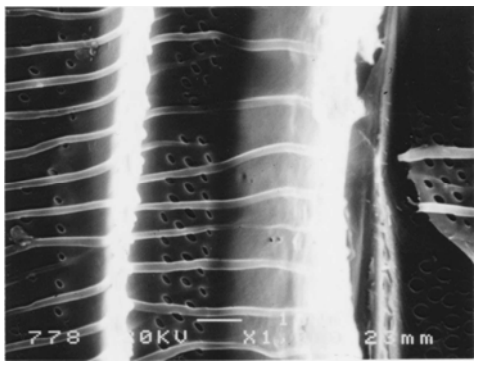
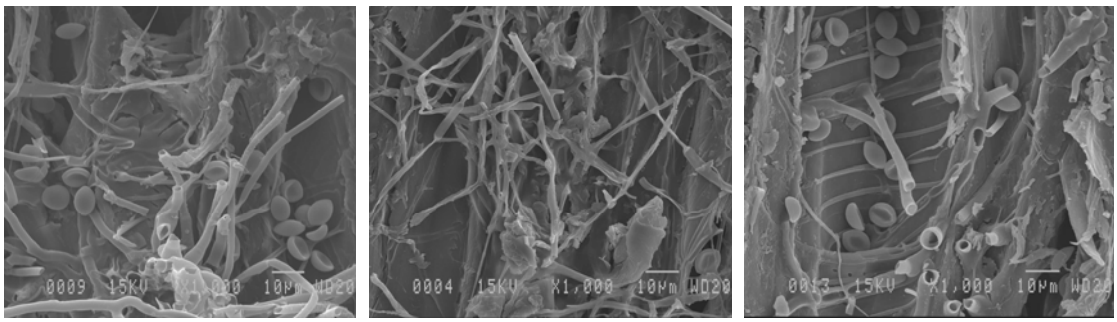
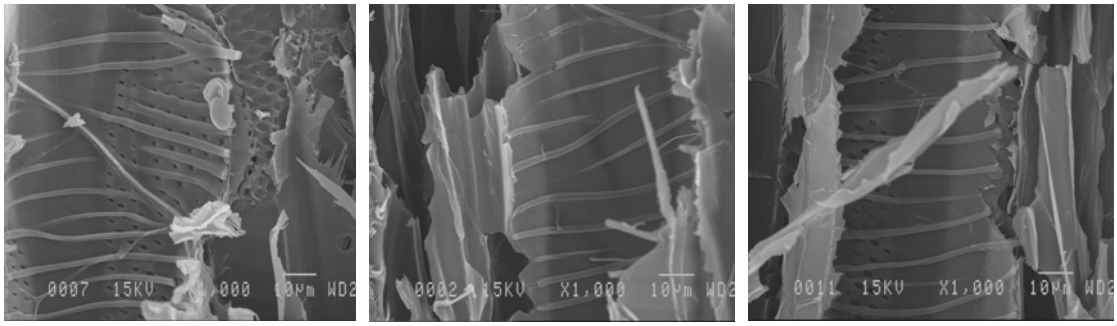


Figure 1: SEM micrographs of reference lime wood, magnification 1000 X



(a) (b) (c)

Figure 2: SEM micrographs of the surface of the lime wood samples decayed by *Chaetonium Globosum* for: 49 days (a), 91 days (b) and 133 days (c), magnification 1000 X



(a) (b) (c)

Figure 3: SEM micrographs of the inner part of lime wood samples decayed by *Chaetonium globosum* for: 49 days (a), 91 days (b) and 133 days (c), magnification 1000 X

Discrete notches of cell wall erosion by hyphae growing within the lumen, in addition to the cavities formed by hyphae within the cell wall, were found in the wood degraded by *C. globosum*.

The penetrating hyphae are narrow where they first advance, after which they get larger, probably because they have eroded a wider channel. As biodegradation advances, the hyphae are strongly interconnected with the structure of wood.

Microscopic examination of wood after decay by a soft rot fungus in the inner part of the samples shows generalized thinning and erosion of the cell walls, without any localization of decay around the hyphae (Fig. 3). The accepted explanation of this appearance is that cellulose enzymes are released from the hyphae and diffuse freely within the wood. The overall effect is that the wood loses its strength and shrinks.

In the micrographs plotted in Figure 3, degradation is evident as fibres gradually become more disrupted. *C. globosum* is a typical soft rot fungus capable of forming cavities aligned along the orientation of the cellulose microfibrils. Also, the hyphae erode from the lumen of the tertiary wall and penetrate up to the middle lamella/primary wall.

As previously established by detailed spectral and thermal methods,[18-20] within the cell wall, soft rot fungi degrade cellulose and hemicelluloses. Compared to the brown rot fungi, the cellulolytic agents diffuse, however, not so deep into the cell wall, but remain in the direct proximity of the hyphae. Lignin is little attacked, mainly by demethylation, so that, with regard to the decay type, this soft rot resembles the brown rot.

Due to intensive carbohydrates degradation, soft rot fungi, exactly like brown rot fungi, cause an about 50% decrease of the impact, bending to only 5% mass loss, and cracks occurring due to the reduction of dimensional stability.

CONCLUSIONS

The morphological characteristics of wood decay by a soft rot fungus, *Chaetomium globosum*, were studied.

SEM showed that the hyphae grow more intensely and are more connected to the

wood structure with increasing the time of exposure to fungi. Also, the formation of cavities aligned along the orientation of the cellulose microfibrils was observed. In the micrographs, degradation is evident as fibres gradually become more disrupted and eroded.

Knowledge on these specific decay signs provide important information about the organic substrate, necessary for understanding the wood condition and for planning appropriate accelerated ageing for antiquating and/or conservation methods, as well as for selecting or developing specific consolidation procedures or other treatments for each decay situation.

REFERENCES

[1] F. Pinzari, G. Pasquariello and A. de Mico, *Macromol*. *Symp*., **238**, 57 (2006).
[2] G. Gilardi, L. Abis and A. E. G. Cass, *Enzyme Microb*. *Technol*., **17**, 266 (1995).
[3] A. Blanchette, in "The Structural Conservation of Panel Painting", edited by K. Dardes and A. Rotne, Getty Conversion Institute, Los Angeles, 1998, p. 55.
[4] R. A. Blanchette, *Int*. *Biodeter*. *Biodegr*., **46**, 189 (2000).
[5] A. Pournou and E. Bogomolova, *Int*. *Biodeter*. *Biodegr*., **63**, 371 (2009).
[6] C. A. da Silva, M. B. Bacellar Monteiro, S. Brazolin, G. A. Carballeira Lopez, A. Richter and M. R. Brag, *Int*. *Biodeter*. *Biodegr*., **60**, 285 (2007).
[7] A. Istek, H. Sivrikaya, H. Eroglu and S. K. Gulsoy, *Int*. *Biodeter*. *Biodegr*., **55**, 63 (2005).
[8] G. Henriksson, G. Johansson and G. Pettersson, *J*. *Biotechnol*., **78**, 93 (2000).
[9] K. E. Hammel, A. N. Kapich, K. A. Jensen Jr. and Z. C. Ryan, *Enzyme Microb*. *Technol*., **30**, 445 (2002).
[10] K. E. Eriksson, R. A. Blanchettete and P. Ander, "Microbial and Enzymatic Degradation of Wood and Wood Components", Springer Verlag, Berlin, 1990.
[11] A. Leonowicz, A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wojtas-Wasilewska, M. Hofrichter and J. Rogalski, *Fungal Genet*. *Biol*., **27**, 175 (1999).
[12] B. Mohebby and H. Militz, *Int*. *Biodeter*. *Biodegr*., **64**, 41 (2010).
[13] G. Daniel, *FEMS Microbiol*. *Rev*., **13**, 199 (1994).
[14] H. Tanaka, S. Itakura and A. Enoki, *Holzforchung*, **53**, 21 (1999).
[15] G. Daniel, in "Wood Deterioration and Preservation: Advances in our Changing World", edited by B. Goodell, D. D. Nicholas and T. P. Schultz, American Chemical Society, Washington, DC, 2003, p. 34.
[16] R. A. Eaton and M. D. C. Hale, in "Wood-Decay, Pest and Protection", Chapman & Hall, London, 1993, p. 546.
[17] R. A. Blanchette, B. W. Held, J. A. Jurgens, D. L. McNew, T. C. Harrington, S. M. Duncan and R. L. Farrell, *Appl*. *Environ*. *Microbiol*., **70**, 1328 (2004).
[18] C.-M. Popescu, M.-C. Popescu and C. Vasile, *Microchem*. *J*., **95**, 377 (2010).
[19] C.-M. Popescu, G. Lisa, A. Manoliu, P. Gradinaru and C. Vasile, *Carbohydr*. *Polym*., **80**, 78 (2010).
[20] http://www.woodweb.com/knowledge_base/Artificially_Aging_and_Weathering_Wood.html
[21] N. H. Corbett, *J*. *Inst*. *Wood Sci*., **14**, 18 (1965).
[22] F. W. M. R. Schwarze, *Fungal Biol*. *Rev*., **21**, 133 (2007).
[23] M. D. C. Hale and R. A. Eaton, *Mycologia*, **77**, 594 (1985).