Interaction between MnO_2 and Oxalate: Formation of a Natural and Abiotic Lignin Oxidizing System

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This paper reports for the first time the efficient modification/degradation of in situ lignin by manganese complexes formed by the association of MnO_2, accumulated as black deposits during wood decay, and oxalic acid, produced by fungi or resulting from oxidative degradation of cell wall components. The Mn oxidants formed are shown to modify both the chemical and physical properties of the wood cell wall. Microscopic analysis revealed the disruption of ray cell in oxidized wood and the formation of amorphous globular material not yet characterized. Thioacidolysis analysis of the oxidized lignin showed a reduction up to 25−30% in the recovery of ether-linked guaiacyl (G) and syringyl monomers and up to 50−80% of several diarylpropane and phenylcoumarane dimers composing the polymer. Preferential oxidation of G moieties in all structures examined was observed during catalysis. The MnO_2/oxalate system also appears to selectively oxidize the lignin macromolecule, as no xylose or glucose loss was observed in all treated samples. The oxidation process catalyzed by MnO_2 and oxalate may play an important role in the general pathway of lignin degradation and also in the transformation of the lignin polymer into humus and/or its precursors.

Keywords: *Manganese dioxide; oxalate; abiotic oxidation; poplar wood; lignin; cellulose; hemicellulose*

INTRODUCTION

Manganese is an active component of the enzymatic systems involved in the fungal degradation of wood (Kirk and Farrell, 1987). In particular, chelates of Mn(III) generated by the manganese-dependent peroxidase (MnP) in the presence of Mn(II) and H_2O_2 have been implicated in lignin substrate oxidation and depolymerization (Glenn et al., 1986; Tuor et al., 1992; Wariishi, 1991).

High-valence manganese species such as MnO_2 have also been observed in connection with many examples of fungal white-rot degradation. Indeed, the insoluble manganese(IV) species is deposited at the tip of new fungal hyphae in the early stages of infestation and growth (Blanchette, 1995; Daniel and Bergman, 1997), and black MnO_2 deposits are frequently observed on fibers in severely delignified wood (Blanchette, 1984, 1991). However, the role of manganese(IV) as MnO_2 species in the white-rot degradation and the reasons for its accumulation in extensively delignified wood remain unknown (Roy et al., 1994; Jellison et al., 1997; Shimada et al., 1989, 1994).

Indeed, MnO_2 is insoluble in aqueous solutions in neutral conditions and is therefore incapable of permeating the wood matrix and accessing the imbedded lignin in concentrations necessary for heterogeneous reactions (Blanchette, 1995; Kern, 1989). However, many low molecular weight molecules present in physiological conditions of biodegradation, such as oxalates and malonates, are capable of binding to manganese as Mn(IV) complexes that are soluble in aqueous media (Fatiadi, 1976; Perezbenito et al., 1996; Chrétien et al., 1960). Oxalate is of particular interest as being reported (i) to depolymerize cellulose through acid hydrolysis and (ii) to be involved in the chelation and reduction of Fe(III) to Fe(II) to generate Fenton reaction in the presence of hydrogen peroxide, although this latter possibility has been recently disputed (Schmidt et al., 1981; Shimada et al., 1994, 1997; Hyde and Wood, 1997).

We propose here that MnO_2 and related Mn(IV) species could be actively involved in the degradation of lignocellulose by forming complexes with oxalates which are able to oxidize lignin and participate directly in the overall biodegradation process.

This study describes for the first time the formation of oxidants issued from the interaction between MnO_2 and oxalate under near physiological conditions of wood degradation and reports the ability of this oxidative system to selectively modify the lignocellulosic wood matrix of poplar sawdust.

EXPERIMENTAL PROCEDURES

Plant Material and Substrate. Extractive-free poplar wood (*Populus trichocarpa* cv. Fridzi Pauley) sawdust was used throughout the study (Lapierre and Rolando, 1988). Before

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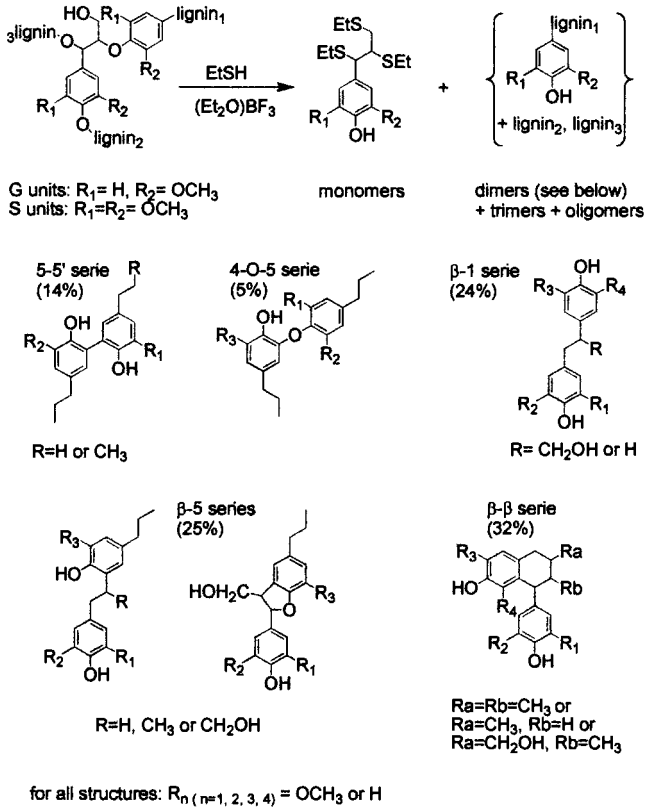

Figure 1. Main monomers and dimeric structures released from thioacidolysis; relative abundance of dimers in nonoxidized lignins shown in brackets.

oxidation, a short ball milling of 5 min was applied on the sample to reduce heterogeneity between particles size.

Chemicals. Activated MnO_2 (85%, particle size < 5 μm), sodium oxalate, and oxalic acid were purchased from Sigma Aldrich Chemicals (France). Other reagents and solvents used were of analytical grade.

Oxidative Treatment of Poplar Sawdust. Fifty milligrams of poplar sawdust containing 20 ± 2% Klason lignin (~45 μmol on the basis of MW = 220−240 for lignin monomers) was incubated under stirring and at room temperature (20−25 °C) in 10 mL of solvent. Reaction vessels were sealed with rubber septa and purged with nitrogen gas. The following conditions were investigated: (A) 1 equiv of lignin, 1 equiv of MnO_2, 2 equiv of oxalate buffer, pH 2.5; (B) 1 equiv of lignin, 5 equiv of MnO_2, 10 equiv of oxalate buffer, pH 2.5; (C) 1 equiv of lignin, 10 equiv of MnO_2, 20 equiv of oxalate buffer, pH 2.5; (D) 1 equiv of lignin, 20 equiv of MnO_2, 20 equiv of oxalate buffer, pH 2.5.

Control experiments consisted of wood incubated in condition D but without manganese.

After ~20 h, the poplar sawdust was recovered by vacuum filtration and washed first with oxalate buffer (100 mM, pH 2.5) to remove excess of MnO_2 and then with hot water to remove a white precipitate, believed to be a low-valent Mn/oxalate complex. The oxidized samples were freeze-dried before chemical analysis.

Chemical Analysis. *Lignin Content.* The content of acid insoluble lignin in the samples was estimated according to the method of Effland (Effland, 1977; Monties, 1984).

Lignin Characterization. The content of β-*O*-4-linked monomers and dimeric structures was determined by thioacidolysis as described by Lapierre et al. on the lyophilized sample (Lapierre et al., 1995). The absence of artifacts during the thioacidolysis reaction due to residual traces of MnO_2 and/or oxalate in wood was tested beforehand. The various monomers and dimers released (Figure 1) were separated by capillary gas chromatography as trimethylsilyl derivatives and identified by gas chromatography/mass spectrometry (GC/MS; electronic impact, ion-trap instrument) (Lapierre et al., 1995; Rolando et al., 1992).

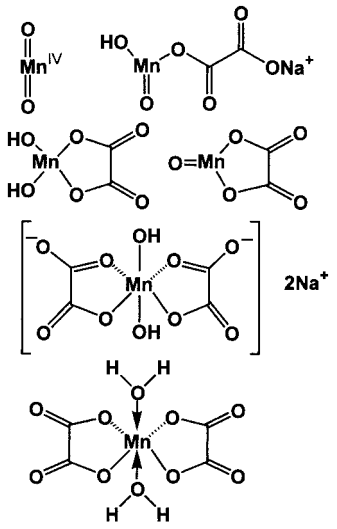

Figure 2. Putative Mn(IV) species generated during the reaction of MnO_2 and oxalate in aqueous medium.

The total yields and relative distribution of the main guaiacyl (G) and syringyl (S) lignin-derived monomers reflects the amount and ring type of lignin units only involved in β-*O*-4 bonds. The main dimers recovered after thioacidolysis and then Raney nickel desulfurization of lignins are representative of the various carbon−carbon and diaryl ether bonds in the polymer, referred to as the "condensed" bonds.

The degradation yields of G and S monomers and of the various GG, GS, and SS dimers were determined relative to their content in the nonoxidized sawdust sample (Kurek et al., 1996; Kurek and Monties, 1994). The content of C−C-linked structures in the oxidized lignin was also estimated qualitatively by high-performance size exclusion chromatography (HP-SEC) analysis of the thioacidolysis products (Suckling et al., 1994; Kurek et al., 1996).

Sugar Analysis. Poplar samples were subjected to a two-stage pre- and advanced-sulfuric acid hydrolysis (Blakeney et al., 1983). The monosaccharides liberated were analyzed as their alditol acetates derivatives by capillary GC (Englyst and Cummings, 1984).

Microscopic Analysis. Microscopic observations of samples were made without staining using a Zeiss optical microscope at a magnification of 40×.

RESULTS AND DISCUSSION

Formation, Decomposition, and Nature of Manganese Complexes. The addition of black MnO_2 powder to the oxalate buffer induces the rapid formation of a very dark burgundy red solution in which poplar wood particles are incubated. The color is characteristic of the presence of Mn(IV)/oxalate complexes, which are unstable to light and temperature (putative structures shown in Figure 2) (Chrétien et al., 1960). Such Mn(IV)/oxalate chelates would then rapidly disproportionate in aqueous media, forming both Mn(III) and Mn(II)/oxalates as indicated by the progressive appearance of a pink color in the reaction medium, which gradually fades (Perezbenito et al., 1996; Fatiadi, 1976; Whelan and Sims, 1995). As the reaction proceeds, MnO_2, probably formed during the release of oxalate from high-valent Mn/oxalate complexes and/or from dismutation of Mn(III), begins to precipitate in the reaction flask and the wood particles become coated with black manganese oxides.

Decomposition of oxalate into CO_2 by high oxidation state Mn species is also likely to occur (Xyla et al., 1992).

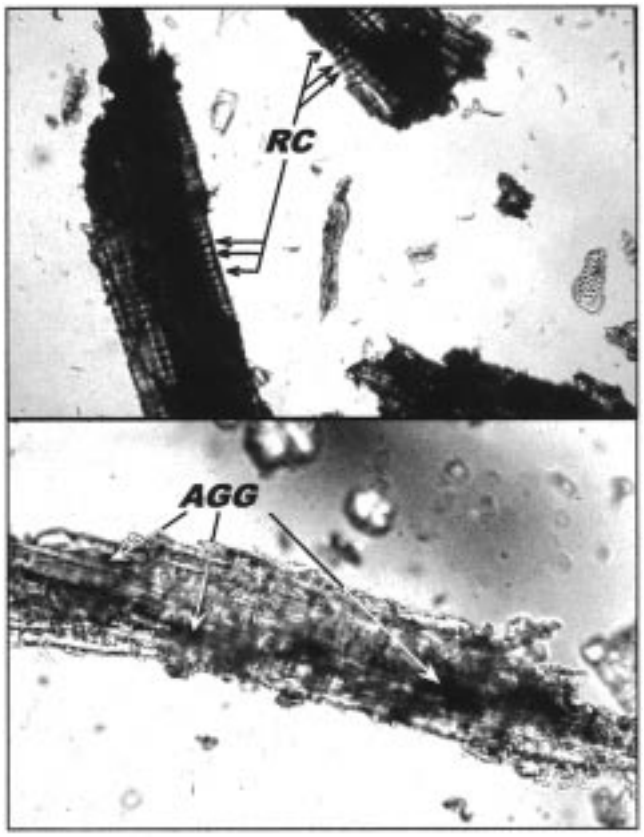

Figure 3. Microscopic photographs of poplar wood sawdust before (top) and after (bottom) oxidation using MnO_2/oxalate in condition D. Magnification = 40×; RC, ray cells; AGG, unidentified aggregates.

Indeed, a gentle and constant bubbling is observed as the reaction proceeds with a concomitant increase of pH from 2.5 to 6, indicating that the oxalate buffer would be progressively degraded and titrated by the newly formed carbonate buffer.

Manganese chemistry is exceptionally complex. Manganese forms stable complexes in the 2+, 3+, 4+, 6+, and 7+ oxidation states, and oxidative systems containing manganese clusters with the metal in more than one oxidation state are common (Chrétien et al., 1960; Hage et al., 1994; Zondervan et al., 1997). Therefore, at room temperature and in the presence of water, the formation of a single manganese/oxalate species in our reaction mixture is unlikely. No further attempts were made at this point to characterize the many different manganese/oxalate complexes that could be present and active in the system described here.

Microscopic Analysis of Wood. Microscopic analysis of wood sawdust particles oxidized by MnO_2/oxalate in condition D shows significant disruption of ray cell structures. As shown in Figure 3, the regular pattern of these structures (RC) perpendicular to the fibers and vessels is replaced by agglomerates of small particles (AGG) stuck to the surface of the wood pieces in an irregular manner. This modification is visible even in the center of large sawdust particles.

Chemical Analysis of Oxidized Wood. *Composition of Wood*. Despite striking visual changes in the oxidized wood, no significant lignin removal could be quantified in any of the conditions tested, suggesting that MnO_2/oxalate is not a delignifying system, at least in the conditions described here (Table 1). Also, reactions run in two-phase solvent systems [methyl isobutyl ketone (MIBK) and water] or extracted with MIBK after oxidation show no evidence that free vanillin, syringaldehyde, or guaiacol is released during the oxidation process. This may indicate either that the MnO_2/oxalate system does not depolymerize the lignin polymer extensively or that cleavage products remain trapped in the wood cell wall matrix.

Table 1. Poplar Sawdust Composition before and after Oxidation by MnO_2/Oxalate

	poplar (control)[a]	oxidized poplar[a]
lignin (mg/g)	223.8 ± 4	221.7 ± 4
xylose (mg/g)	145.4 ± 7	142.9 ± 7
glucose (mg/g)	471.2 ± 23	465.3 ± 23

[a] Mean values for conditions A, B, C, or D.

Table 2. Degradation Extent of Dimeric Structures Released by Thioacidolysis in Oxidized Poplar (Condition C)[a]

	GG[b]	SS[b]	GS[b]	total for each bonding type
β-1	82	74	78	78
β-5	80	*c*	75	78
4-*O*-5	45	66	*c*	58
β-*β*	*c*	54	*c*	54
5-5′	51	*c*	*c*	51

[a] In percent of the content in nonoxidized sawdust; variation between duplicates is <10% except for 4-*O*-5 structures (<18%). [b] Refer to Figure 1 for structures. [c] Structures present in trace amount in poplar lignins.

As shown in Table 1, the quantitative analysis of the carbohydrate portion of the wood also revealed no significative loss of either glucose or xylose during the oxidation. No monomeric sugars were released freely into the reaction medium (data not shown). This analysis, however, does not eliminate the possibility that oxalate buffer alone may cause some minor hydrolysis of the carbohydrate polymers or reduces the cellulose fiber length which can still remain in place within the cell wall (Shimada et al., 1994, 1997).

Lignin Structure. The major impact of MnO_2/oxalate treatment on wood was found to be on lignin structure. Indeed, severe and remarkably specific alteration of the bonding pattern between the constitutive G and S units occurred during oxidation. A marked reduction in the monomers as well as in the various GG, GS, and SS dimers recovered after the selective chemical cleavage of *β*-*O*-4 bonds by thioacidolysis is observed (Figures 1 and 4; Table 2), suggesting that all of these structures were oxidized by the Mn complexes.

Main Features of Lignin Oxidation by MnO_2/Oxalate. *Preferential Oxidation of G Structures.* The loss of *β*-*O*-4-linked G monomers was always more pronounced than the loss of the corresponding S structures, and the S/G ratio increased from 1.2 in controls to 1.5 with increasing MnO_2 charge (Figure 4). The same pattern was observed when the more complex lignin-derived dimers were evaluated, for which the ratio between the gross content in S and G moieties shifted from 1.0 to 1.3 after oxidation (condition D; recalculated from raw data of Table 2). Also, higher degradation extents are observed for dimers comprising two G units and increase from SS, to SG, and then to GG structures for the *β*-1 pattern (Table 2). An exception is the 4-*O*-5 series, in which SG dimers are more degraded than GG ones. However, these structures are minor components of native lignin (5% of the total), compared to the main *β*-1 and *β*-5 major dimers (each ∼25% of the total; see Figure 1). The molecular basis of the observed dif-

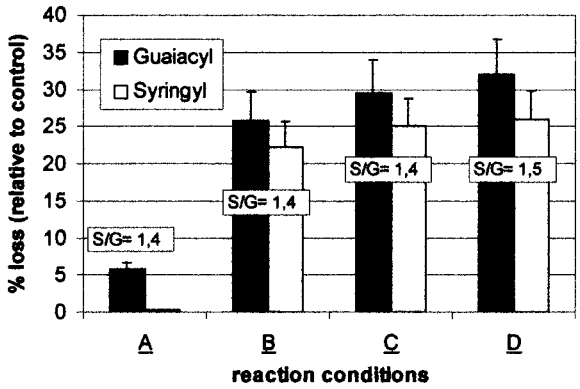

Figure 4. Percentage loss of the β-*O*-4-linked monomers in the oxidized samples. Reaction conditions A−D are described under Experimental Procedures; monomer content in the nonoxidized sample (μmol/g of lignin): G = 963 ± 83, S = 1129 ± 84; S/G ratio = 1.2.

ferential reactivity of G and S moieties in ether-linked monomers and dimer structures remains unknown. However, attack of phenols by MnO_2/oxalate seems to be a key step in the overall oxidation process, as discussed below.

Oxidation of Phenolic and Nonphenolic Structures in Lignin. Poplar wood contains ~30% G structures and ~5% S structures, which are phenolic in the β-*O*-4 domain of lignin (Lapierre and Rolando, 1988). Therefore, any decrease in monomer recovery after thioacidolysis of the oxidized sample in excess of these proportions implies that a portion of the lignin polymer that does not contain free phenolic hydroxyl groups was modified. Accordingly, our data suggest that only phenolic G could be oxidized by MnO_2/oxalate, whereas nonphenolic S structures had to be oxidized in conditions B, C, and D (Figure 4). However, complementary studies revealed that this MnO_2/oxalate system was unable to promote lignin oxidation within wheat straw in which the phenolic group have been extensively permethylated (Lequart and Kurek, 1997). Phenolic structures are therefore necessary for efficient catalysis, and new ones must be formed during the oxidation to allow the alteration of nonphenolic S domains.

Confirming the importance of phenolic structures as a preferred target for MnO_2/oxalate oxidation, the percentage loss of the various dimers in β-*O*-4 domains of lignin correlates well with their phenolic nature. Indeed, syringaresinol (β-β) structures (Figure 1) are essentially nonphenolic in lignin. This could also be the case for biphenyl (5-5′) structures, which exclusively comprise G units involved in dibenzodioxocin cycles in spruce wood (Karhunen et al., 1995). According to the above discussion, their degradation would proceed only after preliminary oxidation of an attached lignin phenolic domain by MnO_2/oxalate reagent. Sustaining this hypothesis, there is compelling evidence that β-1 structures (Figure 1**)**, strongly degraded by the MnO_2/oxalate system, belong to the most reactive portion of lignin as lignin end-groups with free phenolic hydroxyl groups, located at the interface between lignin and polysaccharides (Gellerstedt and Zhang, 1991). In this respect, the degradation of such highly reactive structures should efficiently promote the recurrent degradation of nonphenolics in lignin by MnO_2 (Martinez-Inigo and Kurek, 1997). Finally, the β-5 structures show a degradability similar to that of β-1. However, these structures are not as phenolic as the β-1 ones, at least in pine wood (Lapierre et al., 1991). Their pronounced degradability cannot therefore be accounted for only on the basis of a high content in free phenolic groups, as was done for β-1 structures. The most likely hypothesis relies on the lability of α-*O*-4 ether bonds (Figure 1). The degradation of the benzylic ether bonds in phenylcoumaran structures could generate reactive intermediates (quinone methides) that could subsequently evolve into secondary structures not characterized in this study.

Topochemical Effects. Another particular aspect of lignin oxidation in situ is the arrangement of the different macromolecular constituents in the various cell wall types which might impose topochemical constraints. It is almost impossible to delineate in our case which one of the chemical aspects or topochemical aspects is prevalent in determining structural modification by MnO_2 catalysts. Nevertheless, microscopic comparison of the poplar wood before and after oxidation shows nearly complete destruction of parenchyma ray cells (Figure 2). Because these structures are the liquid access sites to the wood matrix, they would be the first to be exposed to the oxidants in the MnO_2/oxalate system. As they have higher G contents compared to the rest of the wood, the preferential oxidation of these structures could explain some of the large drop in G units recovered from thioacidolysis of the oxidized sample (Terashima et al., 1993; Vallet et al., 1996; Chesson et al., 1997).

Biochemistry of Lignin Degradation. The oxidative action of MnO_2/oxalate on lignin has revealed several features of its reactivity toward the oxidants. However, the biochemistry at the origin of these modifications is unclear. As Mn/oxalate chelates are formed in this process, some common reactions, observed in oxidations using manganese-dependent peroxidases or their related biomimetic systems on model compounds, are presumably taking place (Hammel et al., 1989; Shimada et al., 1989; Musel et al., 1997; Tuor et al., 1992; Wariishi et al., 1989). These includes phenoxy radical formation, which can further undergo the various alkyl−phenyl cleavage, ether cleavage, and carbonyl formation reactions.

Side-Chain and Ether Bond Cleavage Reactions. Extensive cleavage of β-*O*-4 bonds during oxidation should release either propyl−aryl (C_6-C_3) monomers (Figure 1) or lower molecular weight methyl−aryl (C_6-C_1) degradation products such as vanillin or syringaldehyde. These low molecular weight compounds were not observed in the reaction medium. Also, no catechol moieties, thioethylated vanillin, syringaldeghyde, or corresponding acids were observed in the monomeric thioacidolysis products recovered from oxidized samples, indicating that cleavage of Cα−Cβ bonds does not occur in the ether-linked part of the lignin polymer. Confirming this feature, no increase in thioacidolysis derived dimers with shortened side chains was obtained (e.g., $C_6C_1-C_6C_3$ structures, refer to Figure 1). It is nevertheless possible that the released monomeric products could have been trapped through rapid formation of new C−C bonds into trimeric or oligomeric condensed structures, which could not be analyzed by the thioacidolysis method used here (Rolando et al., 1992).

Repolymerization and Depolymerization of Lignin. HP-SEC analysis of thioacidolysis products of the oxidized samples did not show an increase of the lignin content in high molecular weight structures (Figure 5, elution area O). Also, no increase in dimers indicative

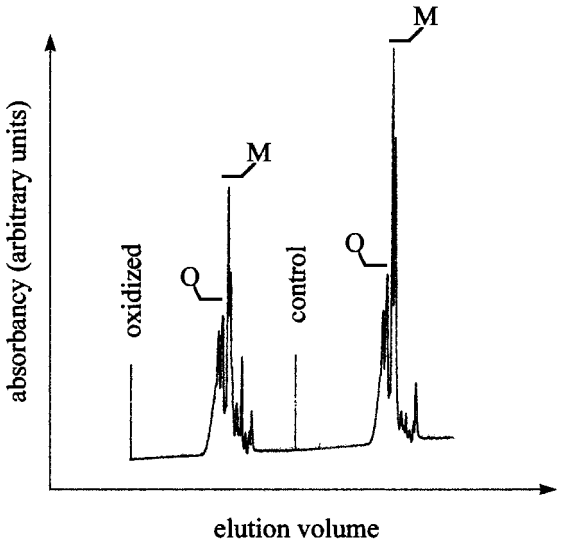

Figure 5. HP-SEC profile of thioacidolysis products released from nonoxidized (control) and oxidized poplar wood sawdust (condition C); M, elution area of monomers; O, elution area of dimers, trimers, and oligomers, respectively (see also Figure 1).

of recondensation reactions such as β-6 dimers found in Kraft lignins could be evidenced by GC/MS in thioacidolysis products of oxidized samples (condition C; data not shown). This confirms that secondary in situ radical coupling reactions yielding oligomeric C−C structures were minor during the MnO_2/oxalate oxidation, in accordance with previous observations made during lignin oxidation in spruce wood by synthetic porphyrins (Kurek et al., 1996).

α-*Carbonyls Compounds in Lignins.* Solid MnO_2 has been shown to be highly selective for the oxidation of benzylic alcohols to the corresponding carbonyl compounds (Fatiadi, 1976). Furthermore, nonphenolic β-*O*-4 lignin model compounds possessing benzylic alcohol groups do not undergo Cα−Cβ bond cleavage in the presence of solid MnO_2 but are converted in high yield (>80%) to α-ketones (Adler and Becker, 1961). However, mass spectrometry analysis of thioacidolysis products from oxidized samples did not reveal any increase in Ar−CSEt=CHSEt monomers typically released during thioacidolysis from α-carbonylated G and S structures and no accompanying residual β-*O*-4 dimers could be evidenced (data not shown; Ralph and Grabber, 1996). This result does not rule out the possibility of ketone formation but rather suggests that carbonyl formation may not be the only structural modification responsible for the observed decrease in monomers released by thioacidolysis.

CONCLUSION

This study has demonstrated for the first time that an abiotic oxidative system is capable of the selective oxidation of hardwood lignin in situ. This simple system is formed from association of MnO_2 and oxalate, suggesting an important role for these compounds in the wood decay process.

The MnO_2/oxalate system does not appear to depolymerize the lignin in the sense that free monomeric and oligomeric structures are released from cell wall matrix. However, the changes in the anatomy of oxidized wood as well as the strong modifications of the bonding pattern of lignin indicate that abiotic Mn chelates would be part of an efficient system that pretreats lignocellulosic material before or during microbial and/or enzymatic attack of lignin. How microorganisms (bacteria and/or fungi) participate in the formation of Mn(IV) species and coordinate the action of such abiotic complexes and enzymes for efficient lignin degradation remains to be determined.

Finally, the chemistry at the origin of the modifications evidenced here as well as the structure and oxidation state of the active manganese oxidant formed when MnO_2 and oxalate are combined remains unknown. Additional studies with lignin model compounds are therefore necessary to understand the mechanism and mode of oxidation of this new manganese/oxalate system.

ABBREVIATIONS USED

G, guaiacyl; S, syringyl; GC/MS, gas chromatography/mass spectrometry; HP-SEC, high-performance size exclusion chromatography; MIBK, methyl isobutyl ketone.

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