Oxidation of Spruce Wood Sawdust by MnO_2 plus Oxalate: A Biochemical Investigation

Bernard Kurek* and François Gaudard

Institut National de la Recherche Agronomique (INRA), Unité de Physicochimie et Biotechnologie des Polymères (UPBP), équipe de Biochimie des Macromolécules Végétales, 2 esplanade Roland Garros, 51686 Reims Cedex 2, France

This paper reports the modification/degradation of lignin within spruce sawdust by manganese complexes formed by the association of MnO_2 and oxalate. The Mn oxidants formed are shown to modify both the chemical and physical properties of the wood cell wall. Scanning electron microscopy analysis of oxidized tracheids revealed a smoothing of the cell wall surface from the lumen side due to the removal of some material. Thioacidolysis analysis of the oxidized lignin showed reductions of up to 30% in the recovery of ether-linked guaiacyl monomers and up to 45% for the some dimers composing the polymer. The MnO_2/oxalate system also slightly modified polysaccharides, corresponding to a 10% loss in weight of arabinose and glucose in the oxidized sample. However, no delignification occurred, according to the acid insoluble lignin content of spruce. Oxalic acid at pH 2.5 did not induce detectable changes in the chemical structures of the lignin or of the polysaccharides.

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

INTRODUCTION

High-valence manganese species such as MnO_2 have been observed in connection with 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 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).

We have recently shown that the solubilization of MnO_2 by oxalate at pH 2.5 leads to the production of Mn chelates capable of oxidizing lignin within wheat straw and poplar cell wall (Lequart et al., 1998, 2000; Hames et al., 1998). Wood-destroying fungi accumulate large amounts of transition metals including manganese during decay (Illman et al., 1989; Jellison et al., 1997). They also produce significant levels of oxalic acid during the process, which can acidify wood to pH 2 (Micales, 1995; Dutton et al., 1993). We therefore propose at this time that MnO_2 and related Mn(IV) species could indeed be actively involved in the degradation of lignocellulose when associated with oxalates (Lequart et al., 1998, 2000; Hames et al., 1998). Still, such a mechanism is not demonstrated to occur in the natural environment, but all elements are in place within wood to promote this phenomenon.

This study on spruce wood degradation is the third in a series of three on the ability of the Mn/oxalate oxidative system to selectively modify lignocellulosic matrixes (Hames et al., 1998; Lequart et al., 1998). The results confirm the reactivity of MnO_2/oxalate toward the cell wall polymers, but also point to a specific degradation pattern of lignin, cellulose, and hemicelluloses different from those of wheat straw and poplar under the same experimental conditions.

EXPERIMENTAL PROCEDURES

Plant Material and Substrate. Extractive free spruce wood (*Picea abies*) sawdust was used throughout the study (Lapierre et al., 1986). Before oxidation a short 4-min ball milling was applied on the sample to reduce heterogeneity among particle sizes (<0.2 mm; Hames et al., 1998).

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

Oxidative Treatment of Spruce Sawdust. Two hundred and fifty milligrams of spruce sawdust containing 27.8% ± 0.2 sem Klason lignin (~300 μmol on the basis of MW = 230 for lignin monomers) was incubated with 5 lignin equiv of MnO_2 and 10 lignin equiv of oxalate buffer pH 2.5 under stirring and at room temperature (20−25 °C) in 50 mL total aqueous volume.

After ~20 h, the spruce sawdust was recovered by vacuum filtration and washed first with oxalate buffer (100 mM, pH 2.5) and then with hot water (Hames et al., 1998). The oxidized samples were freeze-dried before chemical analysis.

Statistical Analysis. All experiments were performed independently at least five times. Each set of experiments comprised two controls incubated in water, two samples incubated in oxalate buffer alone, and two samples incubated with MnO_2 in oxalate buffer. Statistical analyses were performed by comparing data obtained with two populations, defined as control spruce (incubated in water) and treated spruce (treated by MnO_2/oxalate or by oxalate buffer alone). The probability p for the two populations to be identical was determined using an unilateral Student test. The treated samples were considered to be different from the control samples for $p < 0.05$.

* Corresponding author (telephone +33 3 2677 3593; fax +33 3 2677 3599; e-mail kurek@reims.inra.fr).

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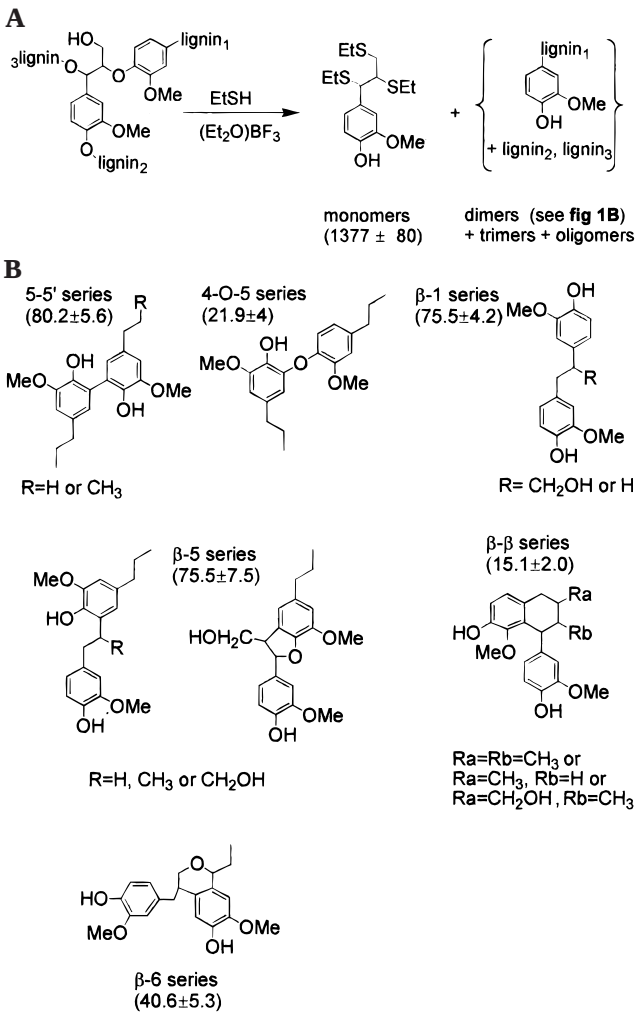

Figure 1. (A) Main lignin structures released by thioacidolysis from spruce wood; (B) main dimers recovered after Raney nickel desulfurization of thioacidolysis products. Abundance of monomer and dimers in nonoxidized lignins is shown in parentheses (in micromoles per gram of lignin ± sem at 95% confidence level).

Chemical Analysis. *Lignin Content.* The acid insoluble lignin content 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 on the lyophilized sample. The guaiacyl 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).

The total yields of the main guaiacyl (G) monomers reflect the amount of such 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 the G monomers and of the various dimers were determined relative to their content in the nonoxidized sawdust sample (Kurek et al., 1996; Kurek and Monties, 1994). The relative amount 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).

Polysaccharide Composition. Spruce polysaccharides were hydrolyzed by H_2SO_4 on 10 mg of samples according to the method of Blakeney et al. (1983). Fucose was added as an internal standard just after the prehydrolysis step. The monosaccharides released by the treatments were analyzed by high-performance anion-exchange chromatography, as described previously (Lequart et al., 1998).

Microscopic Analysis. Microscopic observations of samples were performed on freeze-dried samples with a LEO 980 scanning electron microscope without coatings.

RESULTS AND DISCUSSION

Analysis of Wood by Scanning Electron Microscopy (SEM). Microscopic analysis of wood sawdust was difficult due to the heterogeneity in size and structure of the particles. Therefore, only fragments with apparent wood structures were considered. The pieces selected here were groups of tracheids (Figure 2 A,D). The main effect of MnO_2/oxalate on such disrupted tracheids was a modification of the surface of the lumen-side cell wall (Figure 2B,C; Figure 2E,F). Some amorphous structures (Figure 2B,C) were removed by MnO_2/oxalate, leaving a smooth surface where a characteristic radial fibrillar organization could be seen (Figure 2E). Some needle-shaped oxalate crystals were also found, but only in oxidized samples treated with MnO_2 despite extensive washing performed (Figure 2D, arrows Ox).

Chemical Analysis of Oxidized Wood. *Composition of Wood*. Despite visual changes in the oxidized wood by SEM, no lignin removal could be quantified, confirming that MnO_2/oxalate is not a delignifying system, as previously reported with wheat straw and poplar samples treated under similar conditions (Lequart et al., 1998; Hames et al., 1998) (Table 1).

As shown in Table 1, the quantitative analysis of the carbohydrate portion of the wood revealed no significant loss of either xylose and mannose, but a ~10% loss of arabinose, galactose, and glucose occurred in samples oxidized by MnO_2/oxalate. No degradation was, however, observed when oxalate buffer alone was used (data not shown). Thus, hemicelluloses and possibly cellulose were slightly modified, but no free monomeric sugars were detected in the reaction medium. No attempt to determine whether the degraded glucose was part of cellulose or hemicellulose was made here. The polysaccharide degradation pattern obtained here was not observed with Mn-oxidized wheat straw and poplar and may reflect the anatomical differences existing between these last two species containing vessels and fibers (Kurek et al., 1998). In particular, access of the reactives to the macromolecular structures within the tracheids of spruce wood could be different from those within hardwoods and graminae (Singh et al., 1999).

Lignin Structure. The major impact of MnO_2/oxalate treatment on wood was found to be on lignin structure. Indeed, the bonding pattern between the constitutive guaiacyl units was severely altered during oxidation. A marked reduction in the monomers as well as in the various dimers recovered after the selective chemical cleavage of β-O-4 bonds by thioacidolysis is observed (Figure 1A,B; Table 2), showing that all of these structures were oxidized by the Mn complexes.

The dimeric structures were degraded, but at different rates, according to the type of intermonomer bonds (Table 2). As previously observed with lignin-oxidizing enzymes acting on spruce lignin and MnO_2/oxalate on poplar or wheat straw sawdust, the β-1 structures are here again more easily oxidized than the others (Table 3). On the other hand, the main β-5 and the minor 4-O-5 structures were not significantly degraded by MnO_2/

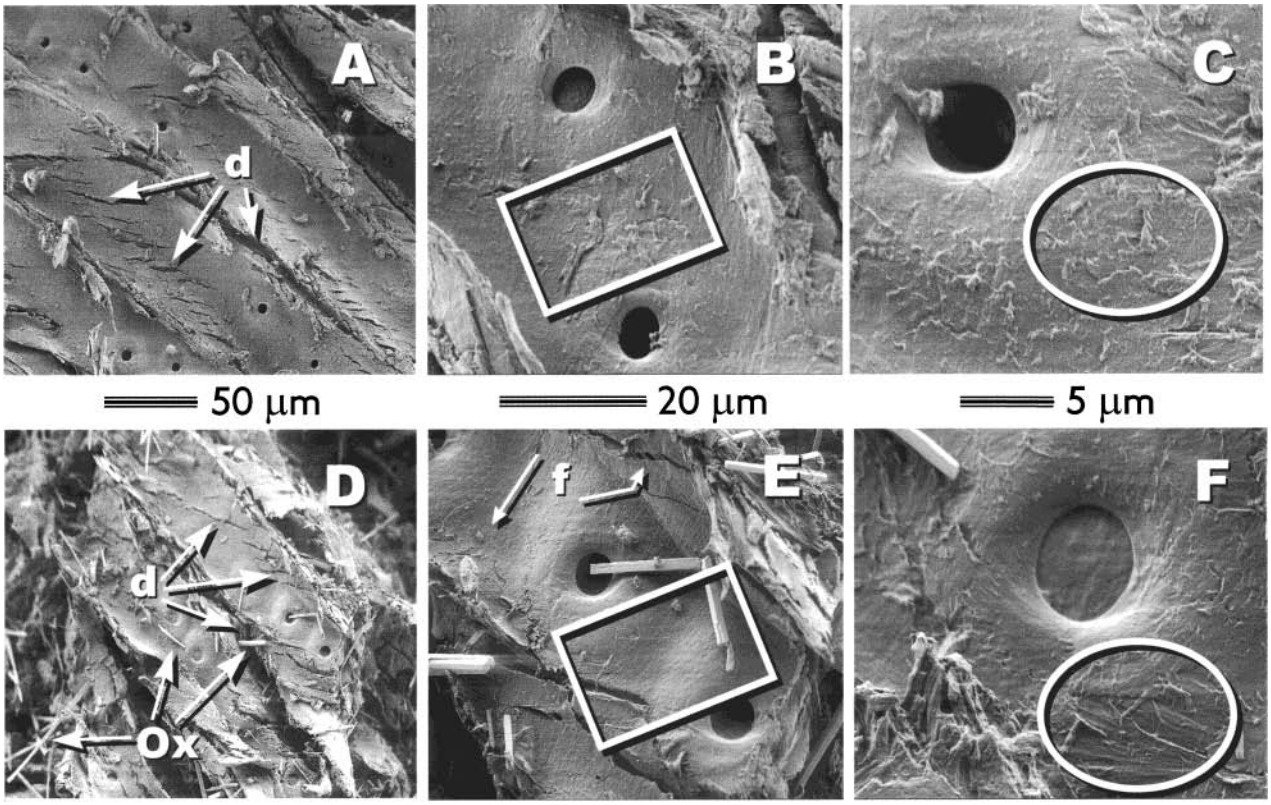

Figure 2. SEM of spruce wood tracheids in sawdust incubated in water (A−C) and after oxidation by MnO_2/oxalate (D−F). A mechanical disruption zone in cell wall can be observed in all samples (A and D, arrows d); Ox, needle crystal of oxalate; B and C, delimited areas, the lumen side wall of tracheid appears as a rough surface bearing amorphous structures; E and F, delimited areas, the tracheids treated by MnO_2/oxalate present a clean and smooth surface; some underlying radial (E, arrows f) or unordered (F, encircles area) fibrillar organization is also observed.

Table 1. Spruce Sawdust Composition before and after Oxidation by MnO_2/Oxalate[a]

	control spruce[b]	MnO_2/oxalate-treated spruce
lignin	278 ± 2.1[c]	279 ± 8.3 (NS)[d]
glucose	444 ± 14.9	398 ± 31 (S)[e]
mannose	113 ± 17.8	105 ± 17.1 (NS)
xylose	46 ± 11.3	45 ± 13.6(NS)
galactose	14 ± 0.9	13 ± 1.1 (S)
arabinose	9.8 ± 0.6	8.8 ± 0.5 (S)

[a] In mg/g of sample. [b] Incubated in water. [c] sem at 95% confidence level. [d] (NS), difference with control not significant at $p < 0.1$ for $n = 10$. [e] (S), difference with control significant at $p < 0.05$ for $n = 10$.

Table 2. Degradation Extent Determined by Thioacidolysis of Monomer and Dimeric Structures in Oxidized Spruce[a]

monomer[b]	dimers[b]					
guaiacyl	β−1	β−5	β-β	β−6	4-O-5	5−5
29 (S)[c]	40 (S)	18 (NS)[d]	18 (S)	45 (S)	18 (NS)	22 (S)

[a] In percent of the content in nonoxidized sawdust. [b] Refer to Figure 1 for structures and content in control sample. [c] (S), value significant at $p < 0.05$ for $n = 10$. [d] (NS), value not significant at $p < 0.1$ for $n = 10$.

oxalate in spruce, as opposed to previous results obtained during oxidation by MnO_2 of poplar, but in accordance with wheat straw experiments (Hames et al., 1998; Lequart et al., 1998) (see Table 3). These differences may again be due to topochemical factors related to the supramolecular organization of the cell wall matrix.

Oxidation of Phenolic and Nonphenolic Structures in Lignin. Spruce wood contains ~30% G structures, which

Table 3. Extent of Degradation of Dimers in Poplar and Wheat Straw by MnO_2/Oxalate

	dimers[a]					
	β−1	β−5	β-β	β−6	4-O-5	5−5
poplar + MnO_2/oxalate[b]	78	78	54	nd	58	51
wheat straw + MnO_2/oxalate[c]	25	17	12	nd	++[d]	0

[a] In percent of the content in nonoxidized sawdust. [b] From Hames et al. (1998). [c] From Lequart et al. (2000). [d] A higher recovery yield was obtained, relative to control; nd, not determined.

are phenolic in the β-O-4 domain of lignin (Lapierre and Rolando, 1988). In this study, the decrease in monomer recovery after thioacidolysis of the oxidized sample was never in excess of these proportions. As the MnO_2/oxalate system is unable to directly promote oxidation of nonphenolic structures, only a portion of the lignin polymer that contains free phenolic hydroxyl groups could be modified in spruce (Lequart et al., 1998). However, even if phenolic structures are necessary for efficient Mn/oxalate catalysis, nonphenolic structures were also attacked via indirect routes, as indicated by the percentage of loss of the various dimers in β-O-4 domains of lignin after reaction. Indeed, syringaresinol (β−β) structures (Figure 1) are mostly nonphenolic in lignin. This is also the case for biphenyl (5−5′) structures, which could exclusively comprise G units involved in dibenzodioxocin cycles in spruce wood (Karhunen et al., 1995). Their degradation (>18%) would then only proceed after preliminary oxidation of an attached lignin phenolic domain by MnO_2/oxalate reagent. Confirming the importance of phenolic structures in the degradation process, the more easily degraded structure is of the β-1 type, which represents lignin end-groups with free

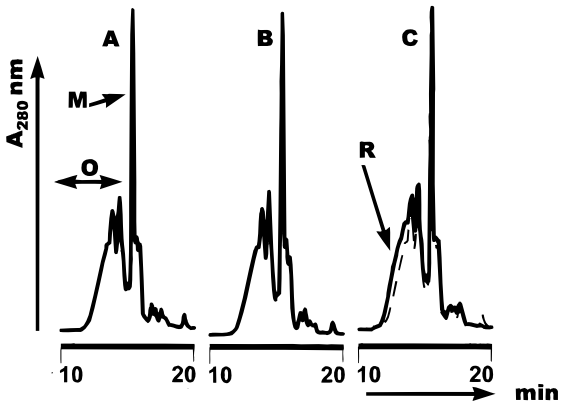

Figure 3. HP-SEC profile of thioacidolysis products released from spruce wood sawdust incubated in water (A), in oxalate buffer (B), and in the presence of MnO_2 and oxalate (C). O, elution zone of dimers, trimers, and oligomers (see also Figure 1); M, peak of G monomers [refer to Kurek et al. (1996)]; R, elution zone with increased amount of condensed structures; dashed line in profile C, superposed profile A, for purposes of comparison.

phenolic hydroxyl groups, located at the interface between lignin and polysaccharides (Gellerstedt and Zhang, 1991).

Repolymerization and Depolymerization of in Situ Lignin. HP-SEC analysis of thioacidolysis products of the oxidized samples shows a slight increase in the lignin content in high molecular weight structures (Figure 3, elution area "O", arrow R). This indicated that secondary in situ radical coupling reactions yielding to oligomeric C−C structures could occur during the MnO_2/oxalate oxidation in situ. It therefore seems possible that some solubilized material could be recondensed with lignin in some easily accessible domains.

Conclusion. This study is the last in a series demonstrating the degradative action of a new abiotic oxidative system composed of MnO_2 and oxalate on wood and graminaceous species (Lequart et al., 1998, 2000; Hames et al., 1998).

The strong modifications in the bonding pattern of lignin and the slight but significant degradation of hemicelluloses and possibly cellulose confirm that abiotic Mn chelates could be part of an efficient system that pretreats lignocellulosic material before or during microbial and/or enzymatic attack of cell walls. Compared with the results obtained with poplar, wheat straw, and spruce as substrates for MnO_2/oxalate, it appears that a specific degradation pattern exists for each plant considered at the lignin and/or polysaccharides level. This is probably related to the specific supramolecular organization of the cell wall matrixes and the particular interactions existing between the constitutive polymers.

ABBREVIATIONS USED

G, guaiacyl; SEM, scanning electron microscopy; sem, standard error of the mean; MW, molecular weight; GC-MS, gas chromatography−mass spectrometry; HP-SEC, high-performance size exclusion chromatography.

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