

The Nature of Lignin from Steam Explosion/ Enzymatic Hydrolysis of Softwood

Structural Features and Possible Uses

Scientific Note

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Abstract

Effective utilization of the lignin by-product is a prerequisite to the commercial viability of ethanol production from softwood wastes using a steam explosion (SE)/enzymatic hydrolysis (EH)/fermentation process. Changes in the chemical composition of Douglas fir wood on SO₂-catalyzed SE followed by EH were assessed using conventional analytical methods and new halogen-probe techniques. A significant solubilization of hemicelluloses was observed in the SE stage, the severity of which affected subsequent fermentation of cellulose and sorption of enzymes. SE of softwood resulted in dramatic changes in the chemical structure of lignin in the residual material involving chemical reactions via the benzyl cation. This leads to a more condensed lignin with partly blocked α -reaction centres. Possible uses for this lignin are discussed.

Index Entries: Lignin; steam explosion; enzymatic hydrolysis; softwood.

Introduction

For the efficient production of ethanol from softwood wastes by carbohydrate hydrolysis/fermentation, the lignocellulosic substrate must be pretreated to make the cellulose more accessible. Steam explosion (SE) in the presence of an acid catalyst such as sulphur dioxide has proven to be a successful pretreatment procedure (1). However, for the whole process to be commercially viable, a high value use is required for the lignin by-product

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(2). A prerequisite for the development of high-value uses for the lignin is knowledge of its composition, structure, and physical properties.

In this article, we report the results of an investigation into the composition and structure of several lignins obtained during steam explosion (SE)/enzymatic hydrolysis (EH)/fermentation of Douglas fir (*Pseudotsuga menziesii*).

Background

Based on our previous findings (3,4), when using hardwoods as the substrate, an excellent place to remove the lignin is immediately after SE and water washing by subsequent alkali and/or alkaline-peroxide extraction. This provided a recovery of more than 90% of the lignin, while at the same time providing a substrate that was more amenable to enzyme hydrolysis and enzyme recycle. A completely different situation exists for isolation of lignin from steam-exploded softwoods. For softwoods, using present technology, it is impossible to use just alkali to extract the lignin because this will remove only 50% of the lignin and give a substrate that is still recalcitrant to enzymatic hydrolysis. The use of an alkaline extraction followed by alkaline-peroxide extraction is feasible because this will remove approx 80% of the lignin and improve the susceptibility of the substrate to hydrolysis (2). However, the 20% of lignin that remains with the cellulose will effectively prevent recycle of the hydrolytic enzyme (5). Thus, the process will be expensive, with high costs for alkali, hydrogen peroxide, and enzyme. To minimize the overall costs of the process for ethanol production by SE of softwoods, it is probably more efficient to leave the lignin with the insoluble substrate and remove it from the process stream after hydrolysis of the cellulose. Different characteristics and properties can be expected for lignin recovered at different stages in the process. For example, the lignin remaining after hydrolysis/fermentation will be contaminated by media components, residual carbohydrates, proteins, and so on. For softwoods, the lignin obtained through alkali and alkaline-peroxide extraction will probably be more reactive and of lower molecular weight than the lignin fraction that is not extractable.

Most of the past investigations on the structure of lignins from steam explosion have been conducted on lignins from hardwoods. Early studies on methanol extracted lignins from steam-exploded aspen (*Populus tremuloides*) indicate that β -O-4 aryl ether linkages were cleaved causing a decrease in molecular weight and increase in phenolic content (6). Work on beech-wood lignin also showed that the molecular weight decreased with increasing temperature of treatment but suggested that, at temperatures above 215°C, condensed structures began to form (7). A considerable increase in phenolic content was also observed, but some of the aliphatic hydroxyl groups were lost, especially with longer treatment times. The phenolic content increase was consistent with severance of β -O-4 lignin interunit linkages. In general, the syringaldehyde/vanillin ratio was much

higher in steam-exploded lignin than in milled-wood lignin, consistent with our work showing the resistance of guaiacyl lignin to removal in the SE process (1). Similar observations have been made for steam-exploded aspen (8), where condensation reactions have also been reported (9).

Very little work has been done on the characterization of lignins from steam-exploded softwoods. Although some work has been reported on lignin released through SE of *Pinus radiata* under various pretreatment conditions (10), this work only examined a small portion of the 27–30% lignin potentially available from steam-exploded softwoods. Steam-exploded wood was extracted with acetone to give yields of about 8% of lignin based on the total water solubles plus insolubles (10). The lignin was contaminated with extractives consisting primarily of resin and fatty acids. A significant portion of the acetone-extracted lignin was soluble in ether (40%) and this fraction showed low depletion of β -O-4 and β -5 linkages. Use of SO_2 during the steam pretreatment cleaved more of the β -O-4 and β -5 linkages. The ether fractions showed substantial amounts of reactive sites including unsaturation, carbonyl groups, side-chain, and phenolic hydroxyl groups (10).

Possible high-value uses for SE lignins include adhesives for wood composites. In these composites, it may be possible to replace the high-cost phenolic component of the phenol-formaldehyde adhesive with SE lignin. The most desirable type of lignin would be one that is high in phenolic content with reactive 5-positions on the C-9 unit. Other uses, such as dispersants, would require a high degree of sulphonation, whereas a high extent of crosslinking would hinder the production of low molecular-weight chemicals from SE lignins.

In the following report, we describe the results of our recent investigations into the structure of lignins obtained from the SO_2 -catalyzed SE of Douglas fir and discuss possible end-uses.

Methods

The SE procedure was applied to the chips of Douglas fir (*Pseudotsuga menziesii*) sapwood (A), heartwood (B) and mixture of sapwood and heartwood 2:1 (B,C) under conditions of increasing severity 1 (7.5 min, 175°C, 4.5% SO_2 to O.D. wood), 2 (4.5 min, 195°C, 4.5% SO_2), 3 (2.4 min, 215°C, 2.4% SO_2) (Table 1). The level of severity of the treatment is represented by the severity parameter $R_o = t \cdot \exp[(T - 100)/14.75]$, which combines time, t (min) and temperature, T (°C), to express the severity of a given pretreatment (11). The corresponding levels of thus defined severity are for 1, $\log_{10} R_o = 3.08$, 2, $\log_{10} R_o = 3.45$ and for 3, $\log_{10} R_o = 3.77$.

The water-insoluble material was water-washed at 20% (A), 5% (B), or 10% (C) consistency. Sample C was further washed with 0.1 N NaOH at 5% consistency. Enzymatic hydrolysis was performed using cellulase at 60 FPU/g cellulose, supplemented with β -glucosidase (Celluclast and Novozym 188, respectively; Novo-Nordisk, Bagsvaerd, Denmark) for

75 (A), 165 (B), and 96 h (C). Alkaline washes from the SE substrates were acidified, the precipitated lignin separated, washed with distilled water, and freeze-dried. All solid materials were washed with distilled water and freeze-dried prior to analysis. For the analyses, wood samples (sapwood and heartwood) and other solid samples, when necessary, were milled to 40 mesh and freeze-dried.

In the bromination experiments (12), 50 mg of a solid sample was treated with the solution of 100 mg bromotrimethylsilane (Aldrich, Milwaukee, WI) in 5 mL pentane for 4 d with shaking. The product was washed with an excess of pentane and dried under vacuum. In the experiments with dry hydrogen iodide (13), a steady current of hydrogen iodide in a nitrogen flow was allowed to pass through a nuclear magnetic resonance (NMR) tube with 0.7 mL CDCl_3 and 8.5 mg dispersed solid sample for 1 h. After that, the internal standard (1,1,2,2-tetrachloroethane in CDCl_3) was added and the ^1H NMR spectrum recorded.

NMR spectra were recorded on a Bruker WH400 instrument; FTIR spectra on a Perkin-Elmer 1600 instrument in KBr pellets; elemental analyses were performed by the Canadian Microanalytical Service (catalytic oxidation, accuracy $\pm 0.3\%$). Insoluble and acid-soluble lignins were determined according to modified procedures outlined in TAPPI standards T222om-88 and UM250, respectively; monomeric sugars were determined using a Dionex DX300 high-performance liquid chromatography (HPLC) instrument. Acid-condensation lignin (Klason) was obtained using 18 h treatment with 72% sulfuric acid at room temperature followed by 1.5 h hydrolysis with 3% sulfuric acid at 120°C . The longer time compared to the standard protocol was chosen to provide full depolymerization of carbohydrates; in separate experiments, the modification has shown no significant effect on the measured Klason lignin content.

Results and Discussion

Composition of Steam-Exploded Douglas-Fir

Consistent with our prior work (1), the SE of softwoods results in an increase in lignin content of the water-insoluble component as the carbohydrates are dissolved and very little lignin is solubilized (Table 1). The effect of SE on the composition of the residue is controlled by the severity parameter R_0 (11). Under less severe treatment conditions ($\log_{10} R_0 = 3.08$, 175°C for 7.5 min), most of the hemicelluloses (77%) are removed, resulting in a corresponding 20% increase in lignin content (Table 1, A1 and B1). Under more severe conditions ($\log_{10} R_0 = 3.77$, 215°C for 2.4 min) the cellulose begins to degrade while pseudo-lignins are formed, resulting in a further increase in lignin content (A3). As found previously with spruce (2), alkali extraction removes an insignificant amount of lignin from the steam-exploded substrate (B1 vs C1).

Enzymatic hydrolysis of the insoluble residue remaining after SE and washing/extraction converted part of the cellulose to soluble glucose, leav-

Table 1
Lignin Content in the Materials
After Steam Explosion and Enzymatic Hydrolysis^a

Sample	% Klason	% Acid-soluble	% Total
Sapwood	26.4	0.3	26.7
Heartwood	28.8	0.3	29.1
Residue after steam explosion			
A1	36.6	0.8	37.4
B1	35.6	0.3	35.9
C1	35.3	0.3	35.6
A3	57.3	0.5	57.8
C3	53.6	0.2	53.8
Residue after steam explosion and enzymatic hydrolysis			
A1/EH	49.0	0.6	49.6
B1/EH	42.9	0.4	43.3
C1/EH	41.4	0.5	41.9
B2/EH	80.8	1.3	82.1
A3/EH	93.3	0.8	94.1
C3/EH	77.5	0.5	78.0

^aFor sample identification, see *Methods*.

ing an insoluble residue with even higher lignin content (Table 1). The enzymatic hydrolysis of the cellulose is facilitated by more severe pretreatment conditions as can be seen from a higher increase in the lignin content (12%, A1 to A1/EH vs 36%, A3 to A3/EH). It is noteworthy that, even though the alkali-extracted sample C3 had a lower lignin content (54%) than the water-washed sample A3 (58%), after enzymatic hydrolysis the lignin content of the latter (A3/EH, 94%) was considerably higher than the former (C3/EH, 78%). This is consistent with our previous observation that for spruce (14), alkali extraction hinders cellulose hydrolysis, resulting in an insoluble residue with lower lignin content.

Analysis of the SE/EH samples for component sugars by hydrolysis/anion-exchange liquid chromatography indicated that almost no hemicelluloses were present. Mannose content of the lignin-rich residues were less than 4% of the glucose content.

Structural Changes in Lignin

Under the high temperature and acidic conditions of the steam pretreatment, changes in the chemical structure of lignin can be expected. The elemental analyses in Table 2 show a gradual enrichment in carbon consistent with the increasing lignin content.

Very little sulphur was incorporated into the lignin and, in separate experiments, we found no residual sulphur dioxide after SE. However, a

Table 2
Elemental Composition of the Materials
After Steam Explosion and Enzymatic Hydrolysis^a

Sample	% C	% H	% N	% S
Residue after steam explosion				
A1	52.0	6.0	0.2	0.1
B1	51.4	5.9	0.1	0.1
C1	50.4	5.8	0.1	<0.1
B2	54.3	5.6	0.2	0.2
A3	56.2	5.7	0.1	0.2
C3	52.4	5.5	0.2	0.1
C1/AW ^b	58.1	5.5	0.6	nd ^c
C2/AW ^b	59.4	5.0	0.5	nd
C3/AW ^b	60.8	5.2	0.4	nd
Residue after steam explosion and enzymatic hydrolysis				
A1/EH	58.0	5.5	1.4	0.1
B1/EH	55.0	5.8	1.8	0.1
C1/EH	52.9	5.8	2.0	0.3
A2/EH	63.0	5.5	1.2	nd
B2/EH	61.4	5.5	1.5	0.1
C2/EH	58.6	5.4	1.9	nd
A3/EH	64.6	5.3	0.8	<0.1
C3/EH	59.6	5.2	1.0	0.1

^aFor sample identification, see *Methods*.

^bAlkaline-soluble lignin.

^cnd, not determined.

small amount of sulphuric acid, 4–8% based on original sulphur dioxide, was detected. It is apparent that the sulphur dioxide is providing the acid for hydrolysis of the hemicelluloses at least in part through oxidation to sulphuric acid.

The nitrogen contents of the steam-exploded samples were very low, approx 0.1%, increasing on enzymatic hydrolysis to 1.5–2.0%. This increase corresponds to absorption of enzyme. The nitrogen contents after EH were higher for wood steam-exploded under the milder conditions. These same samples also had higher cellulose contents. Possible explanations for the correlation between nitrogen and cellulose content include preferential binding of the enzymes to cellulose rather than lignin, or a decrease in absorption capacity of the lignin with increasing severity of treatment (15).

The Fourier transform infrared (FTIR) spectra (not shown here) revealed dramatic changes in the 1500–1700/cm region ascribable to the changes in the aromatic and aryl ketone moieties of lignin. This strongly suggested significant changes in the chemical structure of the lignin during the

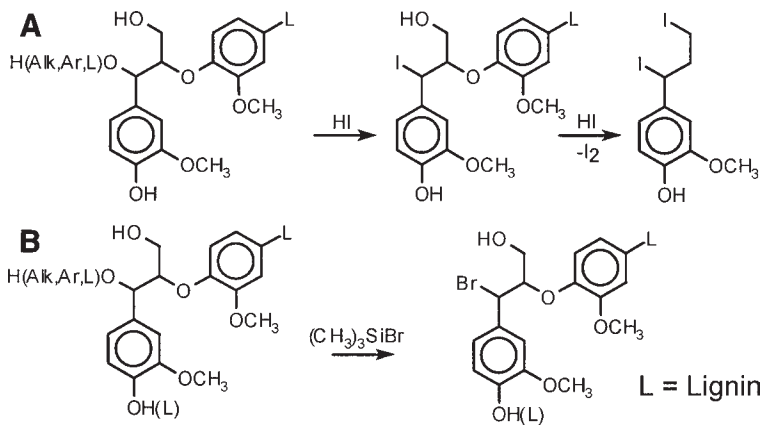


Fig. 1. Reactions of softwood lignin with (A) hydrogen iodide; (B) bromotrimethylsilane.

Table 3
Bromine Content in the Materials Treated
with Bromotrimethylsilane^a

Sample	% Br	% Br in lignin
Sapwood	10.4	39.0
Residue after steam explosion		
A1	8.4	22.4
A3	7.5	12.9
Residue after steam explosion and enzymatic hydrolysis		
A1/EH	10.2	20.5
A3/EH	7.6	8.1

^aFor sample identification, see *Methods*.

SE/EH process. Possible changes include acid condensation and oxidation. To probe further the structural changes, we applied the techniques of selective halogenation and reductive cleavage.

In selective bromination with bromotrimethylsilane, lignin is brominated at the α -position via benzyl cations (12). Without a base, the reagent does not silylate lignin, and the reaction is highly selective. On the other hand, dry hydrogen iodide initially reacts with lignin in a similar way, but in this case, halogenation is followed by reductive cleavage (16). These reactions are shown in Fig. 1.

Treatment of Douglas-fir sapwood with bromotrimethylsilane resulted in a high degree of bromine incorporation (Table 3). After SE, the treatment led to considerably lower levels of lignin bromination. The extent of bromination achievable decreased as the severity of the steam explosion increased

(A1 vs A3). Enzymatic hydrolysis reduced the extent of bromination by an additional, but smaller, amount. The reduction in reactivity of the lignin suggests that reactive groups at the α -position, such as hydroxyl and ethers, are being oxidized to carbonyls or generating benzylic cations, which form carbon-carbon bonds with the electron rich centers of lignin. The extent of oxidation and/or condensation increases as the steam pretreatment conditions become more severe.

The results obtained with dry hydrogen iodide are consistent with the results from bromination (Fig. 2). Treatment with dry hydrogen iodide depolymerized 40% of the lignin in Douglas-fir sapwood to the monomeric diiodide. After SE/EH, the degree of conversion was in the range of 0.1–0.2%. This is consistent with loss of reactivity at the α -position plus restructuring and condensation of the of β -O-4 linear fragments of native lignin during SE.

Implications for Possible Uses

Changes in the chemical composition of Douglas-fir wood upon SO_2 -catalyzed SE followed by EH result in the formation of materials with a high lignin content and a noticeable amount of incorporated nitrogen and a low degree of sulphonation. SE of softwood results in dramatic changes in the chemical structure of lignin present in the residual material. This leads to the recondensed products being both less susceptible to alkaline removal than similarly treated hardwood lignin, and probably less reactive. Most likely, their use will require chemical modification. The high extent of crosslinking will probably hinder the production of low molecular-weight chemicals from SE(SE/EH) lignins. On the other hand, numerous uses and ways of chemical modification developed for acid-hydrolysis lignins are obviously applicable to the materials after SE/EH (17). In particular, oxoammoniation is considered as an effective way to obtain value-added products for an agricultural use. This and other uses of the SE/EH lignocellulosic residues in agriculture are the most consistent with our knowledge of their structure and properties (18,19). Sulphonation of SE/EH lignins will open possible uses as a dispersant, while adhesive application in wood composites could also be considered. The use in such systems would require a lignin high in phenolic content with reactive 5-positions on the C-9 unit. Further studies are needed to assess fully the potential of SE/EH lignin residues in this application.

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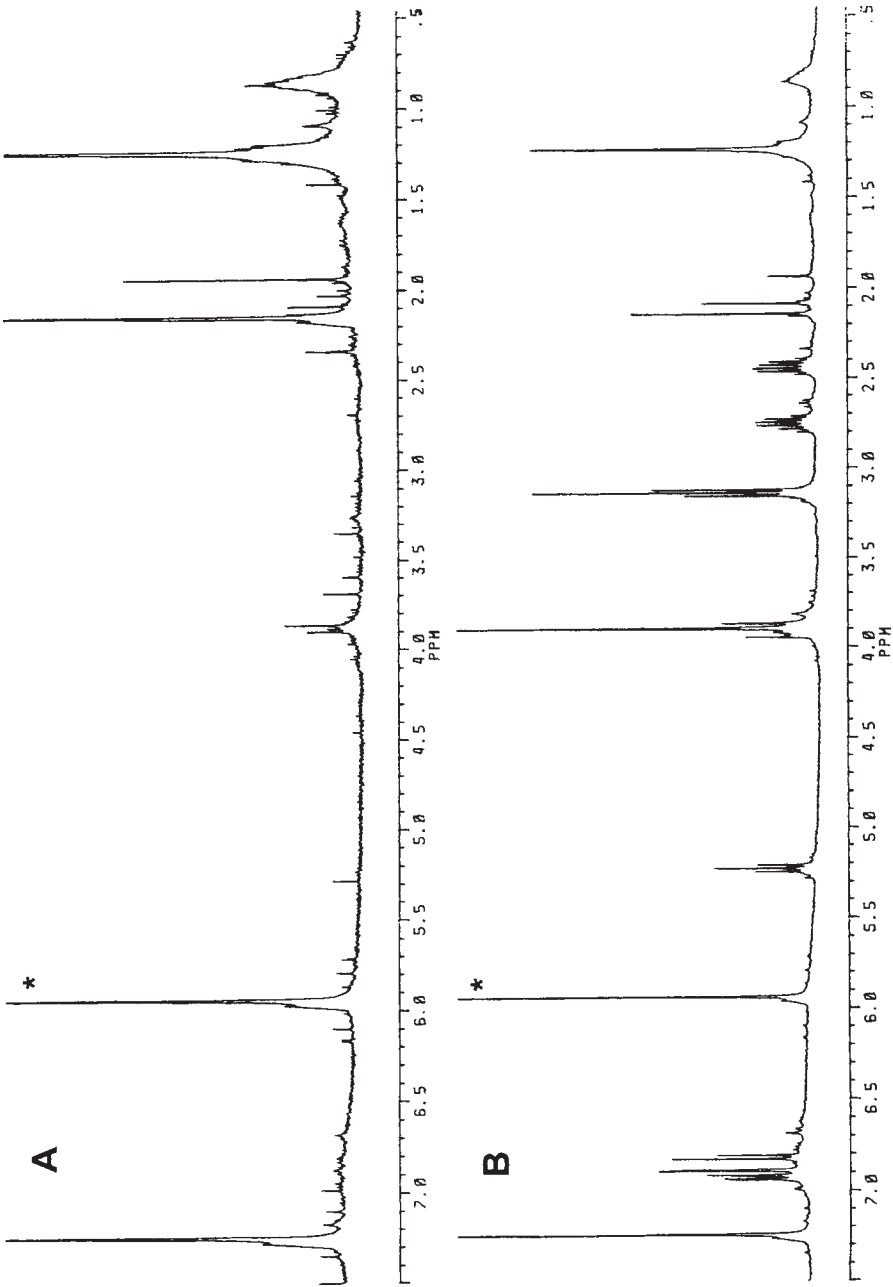


Fig. 2. ¹H NMR spectra of the solution in CDCl₃ after treatment of the samples with dry hydrogen iodide: (A) Douglas fir, sapwood, after SE/EH (sample A3/EH); (B) Douglas fir, sapwood, before the procedure. The signal of an internal standard (1,1,2,2-tetrachloroethane) is marked with an asterisk.

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