MACROMOLECULAR COMPOUNDS AND POLYMERIC MATERIALS

Chemical Structure and Physicochemical Properties of Oxidized Hydrolysis Lignin


Kirov State University of Forestry Engineering, ul. Mirgorodskaya 26-28, St. Petersburg, 191024 Russia
Institute of Chemistry, St. Petersburg State University, Universitetskii pr. 26, Petrodvorets, St. Petersburg, 198504 Russia
Institute of Nanobiotechnologies, St. Petersburg Polytechnic University, ul. Politekhnicheskaya 29, St. Petersburg, 195251 Russia
E-mail: edward-evst@mail.ru

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Abstract—The structure of hydrolysis lignin oxidized with hydrogen peroxide in acid medium was studied by NMR spectroscopy and by matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry. The sorption, ion-exchange, and surfactant properties of oxidized hydrolysis lignin were studied, and possible application areas of this substance were suggested.

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Oxidation of hydrolysis lignin (HL) with hydrogen peroxide in acid medium was studied previously [1]. The lignin product obtained under these conditions is soluble in dilute alkali.

In this work, we studied the chemical structure and physicochemical properties of oxidized hydrolysis lignin.

EXPERIMENTAL

Experiments were performed with hydrolysis lignin from Bobruisk Hydrolysis Plant (Belarus), recovered from softwood. The oxidation procedure and methods for analysis of hydrolysis lignin and oxidized hydrolysis lignin (OHL) are described in detail in [1]. The analytical characteristics of the initial and oxidized hydrolysis lignin are given in Table 1.

The OHL yield was 75.1%. Thus, the HL oxidation with hydrogen peroxide in acid medium yields approximately 25% low-molecular-mass compounds soluble in the reaction medium.

The sorption properties of OHL were determined by the procedure accepted for evaluating the sorption properties of enterosorbents [2].

The ion-exchange properties of OHL were determined as follows. A 250-mL flask was charged with 0.1 g of OHL and 80 mL of H₂O₂, and the contents were stirred for 5 min on a magnetic stirrer. After that, 20 mL of a 0.01 M AgNO₃ solution was added, the mixture was stirred for

Table 1. Analytical characteristics of the initial and oxidized hydrolysis lignin [1]

<table>
<thead>
<tr>
<th>Lignin sample</th>
<th>Content, %</th>
<th>Solubility in alkali, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lignin</td>
<td>carboxy groups</td>
</tr>
<tr>
<td>Initial</td>
<td>81.5 (0.3)ᵃ</td>
<td>0.8</td>
</tr>
<tr>
<td>Oxidized</td>
<td>88.1 (2.8)ᵇ</td>
<td>8.9</td>
</tr>
</tbody>
</table>

ᵃ The content of acid-soluble lignin is given in parentheses.
1 h, and the precipitate was filtered off on a glass frit and washed with water (100 mL). The content of Ag⁺ ions in solutions was determined potentiometrically with a KRITUR 47-17 ion-selective electrode.

The surfactant properties were determined by Rebinder’s method [3].

Elemental analysis of lignin was performed with an EA-300 EuroVektor device. The content of elements was as follows (%): HL, C 60.38, H 5.12, O 34.5; OHL, C 57.5, H 5.0, O 37.5. The oxygen content was determined from the difference.

The elemental composition of the ash was studied by inductively coupled plasma atomic emission spectrometry (ICP OES) with an OPTIMA-4300DV device (Perkin–Elmer). The following components (%) were determined in the HL ash: SiO₂ 94.4, K₂O 0.94, Na₂O 0.5, Fe₂O₃ 0.44, CaO 0.33, MgO 0.046, MnO 0.0052, and P₂O₅ 0.0038. In addition, 81.7 ppm Cu was found.

The UV spectra were recorded with a UV-2400PC Series spectrophotometer (Shimadzu).

Analysis by gas chromatography–mass spectrometry was performed with a G 2570A GC/MSD device (Agilent Technologies).

The solid-phase ¹³C NMR spectra were recorded with a Bruker Avance III 400 WB device (operation frequency 100 MHz) at 25°C using the cross-polarization technique for proton decoupling. The chemical shifts were measured relative to external TMS.

The matrix-assisted laser desorption/ionization (MALDI) mass spectra were taken with a Varian 902-MS MALDI ion cyclotron mass spectrometer equipped with a 9.4 T superconducting magnet. The sample desorption/ionization were performed using the third harmonic (355 nm) of a Nd:YAG laser. 2,3-Dihydroxybenzoic acid was used as matrix; the solvents were dimethyl sulfoxide, acetone, or alkaline aqueous solution.

Electrospray ionization mass spectrometry (ESI MS) was performed with Waters Xevo TQD (TOF) or MaXis (ESI-QTOF) liquid chromatographs–mass spectrometers or with a Bruker Daltonics high-resolution mass spectrometer with direct solution inlet into the ionization chamber.

RESULTS AND DISCUSSION

Today, NMR spectroscopy is one of the most widely used methods for studying the chemical structure of lignin and other wood components [4]. Being a polyfunctional, polydisperse, and irregular heteropolymer, lignin is a difficult object for studying. The lignin spectra contain a large number of overlapping bands, which considerably complicates their interpretation. Numerous papers summarized in a review [5] and in a monograph [6] deal with the signal assignment in the ¹³C NMR spectra of lignin.

Solid-state NMR spectroscopy (CP/MAS NMR) appeared to be a more informative method for studying the chemical structure of OHL. Until recently, the method had limited use in the lignin chemistry because of relatively low resolution compared to the NMR spectroscopy of solutions. Earlier studies using this method have been summarized in [7]. The development of the equipment, experimental procedure, and software gives grounds to hope that solid-state NMR spectroscopy will find wider use in analysis of lignin and other wood components [4, 8].

The solid-state NMR spectra of OHL and initial HL are shown in Fig. 1. The signal assignment in these spectra, based on published data [5, 6], is given in Table 2.

Comparison of these spectra allows the following conclusions concerning the chemical transformations of HL under the conditions of oxidation with hydrogen peroxide in acid medium. The breakdown of a part of aromatic rings and the lignin demethoxylation occur under these conditions. Simultaneously, the content of carboxy groups of different types appreciably increases. Of
particular interest is the fact that the content of structures with $\beta$-O-4 bonds changes insignificantly. Specifically these bonds are the main type of bonds in natural softwood lignin (49–51 bonds per 100 phenylpropane units [9]).

In other words, the HL degradation resulting in approximately 25% transfer of lignin into the reaction medium does not noticeably involve alkyl aryl ether bonds between phenylpropane units.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is successfully used in analysis of natural and synthetic polymers [10, 11]. It opens new possibilities for studying lignin; however, as noted in

Table 2. Signal assignment in solid-state $^{13}$C NMR spectra of hydrolysis lignin and oxidized hydrolysis lignin (chemical shifts, ppm, relative to TMS)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Assignment$^a$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>196.9</td>
<td>196.9</td>
<td>210–220: Nonconjugated $\text{C}=\text{O}$ in ketones</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>194: $\text{CHO}$ in cinnamaldehyde</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>191.6: $\text{CHO}$ in benzaldehyde</td>
<td></td>
</tr>
<tr>
<td>174.6, 174.3</td>
<td>172.7, 170.1</td>
<td>171–173: $\text{C}=\text{O}$ in aliphatic esters</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>165–167: $\text{C}$ in aromatic acids</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>165–172: $\text{C}=\text{O}$ in aliphatic acids</td>
<td>[5]</td>
</tr>
<tr>
<td>146.8</td>
<td>146.6</td>
<td>149.1: C-3 in G-e</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>147.4: C-4 in G-e</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>146.9: C-3 in G-ne</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>145.3: C-4 in G-ne at $\beta$-O-4 bond</td>
<td></td>
</tr>
<tr>
<td>130.4</td>
<td>128.9</td>
<td>128–129: $\text{C}<em>\beta$ in Ar–$\text{CH}=\text{CH}$–CHO and $\text{C}</em>\alpha$ and $\text{C}_\beta$ in Ar–$\text{CH}=\text{CH}$–$\text{CH}_2\text{OH}$</td>
<td>[6]</td>
</tr>
<tr>
<td>124.1</td>
<td>124.5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>114.7</td>
<td>114.9</td>
<td>115–115.6: C-5 in G, G'</td>
<td>[6]</td>
</tr>
<tr>
<td>106.0</td>
<td>106.0</td>
<td>106–107: C-2, C-6 in S,S' with $\alpha$-$\text{C}=\text{O}$</td>
<td>[6]</td>
</tr>
<tr>
<td>89.2</td>
<td>89.3, 88.3</td>
<td>84.5: $\text{C}_\beta$ in G $\beta$-O-4 three</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>83.7: $\text{C}_\beta$ in G $\beta$-O-4 erythro</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>81–84: $\text{C}_\beta$ at $\beta$-O-4 bond with $\alpha$-$\text{C}=\text{O}$ group</td>
<td>[6]</td>
</tr>
<tr>
<td>75.3</td>
<td>75.1</td>
<td>75: $\text{C}<em>\alpha$ at $\alpha$-O-4 and $\text{C}</em>\alpha$OH</td>
<td>[6]</td>
</tr>
<tr>
<td>72.7</td>
<td>72.8</td>
<td>72.5: $\text{C}_\alpha$ in G and S $\beta$-O-4 erythro</td>
<td>[5]</td>
</tr>
<tr>
<td>71.8</td>
<td>71.9</td>
<td>71.8: $\text{C}_\alpha$ in G and S $\beta$-O-4 three</td>
<td>[5]</td>
</tr>
<tr>
<td>65.3</td>
<td>65.3</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>55.9</td>
<td>55.9</td>
<td>55.5–56: OMe in Ar–OMe</td>
<td>[6]</td>
</tr>
<tr>
<td>47.6</td>
<td>47.6</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>38.0</td>
<td>38.0</td>
<td>20–40: $\text{CH}_3$ and $\text{CH}_2$ in saturated aliphatic compounds</td>
<td>[5]</td>
</tr>
<tr>
<td>30.4</td>
<td>30.3</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>18.7</td>
<td>18.7</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ (G) Guayacylpropane unit, (S) syringylpropane unit, (e) esterified unit, and (ne) nonesterified unit.
review [12], low efficiency of lignin ionization under the MALDI conditions prevents obtaining high-quality mass spectra and restricts the possibility of obtaining information on the lignin structure. Nevertheless, it is possible to approximately estimate from the spectrum (Fig. 2) $M_n$ of OHL, which is about 2000. It is impossible to compare this value with the molecular mass (MM) of the initial HL, because the latter is insoluble in all the known lignin solvents.

The structure of the OHL degradation products soluble in the reaction medium was studied by liquid chromatography–electrospray ionization mass spectrometry (ESI MS). For this purpose, the filtrate after the OHL separation was preliminarily neutralized with a NaOH solution to pH 3.5. The ESI MS method was used previously for studying the oligomeric fractions of lignin and certain lignans [13–15].

The results obtained are presented in Fig. 3 and Table 3. As can be seen, the mass spectrum consists of a series of peaks in a wide MM range. The majority of them form a sequence with a difference of 142 Da.

![Fig. 2. MALDI mass spectrum of oxidized hydrolysis lignin.](image)

![Fig. 3. ESI mass spectrum of the oxidized hydrolysis lignin filtrate (direct inlet).](image)
Thus, the HL oxidation with hydrogen peroxide in a strongly acidic medium is accompanied by the breakdown of a part of aromatic rings and by lignin demethoxylation. Simultaneously, the content of carboxy groups of different types appreciably increases. The content of structures with β-O-4 bonds varies insignificantly. The oxidation product is OHL, a part of which (25%) passes into the solution in the form of oligomers containing a repeat unit of mass 142 Da. For clearness of the subsequent discussion, the results obtained can be presented in the form of a hypothetical scheme of a molecular fragment for the insoluble OHL fraction (Scheme 1). It should be emphasized that this is not a structural formula but just a scheme reflecting the alternation of unoxidized and oxidized units in the molecule.

This scheme requires comments. First, they concern the topological structure and MM of lignin. In accordance with traditional views [16], natural lignin is a three-dimensional network polymer. This viewpoint is traditional but not the only one. In Freudenberg’s opinion [17], MM of lignin is low, but its macromolecule is branched and three-dimensional. In addition, for three-dimensional network polymers the notion of MM loses physical sense.

Wide use of modern physical methods of investigation in the lignin chemistry led recently to obtaining the results that do not fit in traditional concepts. It is not occasional that recent publications demonstrate evolution of views on the lignin structure. As noted in review [18], lignin is characterized by two different types of macromolecules, namely, by linear and branched macromolecules. Finally, it is stated that lignin consists of aggregates of linear oligomers [19, 20]. The existence of linear molecules in the low-molecular-mass fraction of eucalypt lignin has been experimentally proved by mass spectrometry [21]. The capability of lignin, in contrast to polymers, to exhibit diffusion waves in electrochemical reduction or oxidation on different electrodes also allows lignin to be classed with oligomers [22].

The results obtained in studying the chemical structure of OHL are, on the whole, consistent with the modern views. The number-average MM of OHL is about 2000, i.e., it is an oligomer. Unfortunately, we failed to obtain high-quality mass spectrum of OHL with high signal/noise ratio. Therefore, it is impossible to determine the mean MM of the elementary units. However, a study of the soluble fraction of OHL allows certain assumptions concerning it.

**Table 3.** Molecular masses of peaks in the ESI mass spectrum of oxidized hydrolysis lignin filtrate (direct inlet)

<table>
<thead>
<tr>
<th>Peak</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z</td>
<td>165</td>
<td>307</td>
<td>449</td>
<td>591</td>
<td>733</td>
<td>875</td>
<td>1017</td>
<td>1159</td>
<td>1300</td>
<td>1442</td>
<td>1584</td>
<td>1726</td>
</tr>
</tbody>
</table>

**Scheme 2.** Assumed structure of the molecular fragment of the soluble OHL fraction.
The molecular masses of the oligomeric components of the soluble OHL fraction differ by 142, or, more precisely, by 142.11 Da (Fig. 3, Table 3). This value coincides with MM of muconic acid. At first glance, these fractions are muconic acid oligomers, but this is not quite true. Analysis of the $m/z$ values in Table 3 shows that each fraction contains one unit with MM 165 Da and one or several units of muconic acid derivative (Scheme 2).

The UV data count in favor of such structure of the soluble OHL fraction. In the UV spectrum of the UHL filtrate, there is a broad band with $\lambda_{\text{max}} = 200$ nm (Fig. 4). Carboxy and ester groups absorb in this region of the spectrum (195–210 nm) [23]. Muconic acid under these conditions has an absorption band with $\lambda_{\text{max}} = 261$ nm and $\varepsilon = 19\,070$.

The above-indicated groups (carboxy and ester) are also present in the soluble OHL fraction, as indicated by the broad signal in the solid-state $^{13}$C NMR spectrum in the range 165–173 ppm (Fig. 1, Table 1).

As already noted, ESI MS studies of the OHL filtrate were performed after preliminary neutralization with a NaOH solution to pH 3.5. A large amount of sodium sulfate formed in the process. To eliminate the effect of the electrolyte on the analysis results, the neutralized filtrate was also examined after its desalination using G-10 gel. The results obtained (Table 4) show that, under the ESI MS conditions, OHL molecules decompose into fragments whose molecular masses differ by 70.97 Da. We believe that such constancy in MM variation suggests certain ordering of the OHL structure (Scheme 2).

The insoluble and soluble parts of OHL apparently differ in the extent of oxidation, namely, in the content of oxidized units. As found previously, the insoluble OHL fraction contains one carboxy group per three phenylpropane units [1]. This product is soluble in dilute alkali but insoluble in acid. On the other hand, the soluble OHL fraction, amounting to 25%, readily dissolves in concentrated sulfuric acid, and this is specifically due to considerably higher content of carboxy groups (Scheme 2).

These differences are well consistent with the previously suggested concept that the functionalization plays the crucial role in the lignin dissolution [24, 25]. By functionalization is meant formation of functional groups in lignin (phenolic OH, $-\text{SO}_3\text{H}$, or $-\text{COOH}$) to the level ensuring the lignin dissolution under the given conditions.

The soluble fraction of OHL (filtrate) contains, along with the above-discussed muconic acid derivatives, also other products of lignin degradation. Their content is low, as indicated by the following data. The extract of the filtrate (diethyl ether, $5 \times 50$ mL) exhibits no peaks (exceeding the noise level) of organic compounds in the chromatogram. The chromatogram obtained after the extract evaporation by a factor of 50 is shown in Fig. 5.

As shown by chromatography–mass spectrometry, the major compounds in the extract are pyrocatechol, 4-hydroxybenzoic acid, and 3-hydroxy-4-methoxybenzoic acid. HL prior to oxidation was treated to remove extractable substances [1]; therefore, these compounds are lignin degradation products. Thus, the HL oxidation with hydrogen peroxide in acid medium leads not only to aromatic ring opening, but also, to a small extent, to other reactions: introduction of hydroxy groups into the aromatic ring, oxidative demethylation, replacement of propane chains, and cleavage of alkyl aryl ether bonds.

The presence of carboxy (8.9%) and phenolic (2.9%) functional groups [1] gives grounds to anticipate that OHL will exhibit sorption, ion-exchange, and surfactant

| Table 4. Molecular masses of peaks in the ESI mass spectrum of oxidized hydrolysis lignin filtrate after desalination (direct inlet) |
|---|---|---|---|---|---|---|---|---|---|
| Пик | 1 | 2 | 3 | 4 | 5 | 6 |
| $m/z$ | 164.92 | 235.88 | 306.85 | 377.82 | 448.78 | 519.75 | 590.72 | 661.68 | 732.65 | 803.61 | 874.58 |
| *a* Additional peaks appearing in the mass spectrum after desalination of the neutralized filtrate.

| Table 5. Physicochemical properties of oxidized hydrolysis lignin |
|---|---|---|
| Sorption capacity, mg g$^{-1}$ | 97.8 |
| Ion-exchange capacity, mg-equiv g$^{-1}$ | 1.1 |
| Surface tension, mN m$^{-1}$ | 43.0 |
properties. The results of studying these properties are given in Table 5.

To determine the potential OHL application fields, it is necessary to compare the results obtained with published data. The sorption capacity of OHL was determined from the amount of the adsorbed Methylene Blue, following the procedure accepted for evaluating the sorption of ability of Polyphepanum enterosorbent [2]. As noted in [2], for HL-based sorbents the acceptable level of the sorption capacity is 40 mg g⁻¹, although for certain sorbents higher values in the range 48–78 mg g⁻¹ were obtained. The sorption capacity of OHL is as high as 97.8 mg g⁻¹; therefore, it is a promising raw material for preparing new enterosorbents.

In the ion-exchange capacity (1.1 mg-equiv g⁻¹), OHL is appreciably inferior to commercially produced ion-exchange resins [26] whose sorption capacity is 1.6–10 mg-equiv g⁻¹.

The surfactant properties of OHL were evaluated by the surface tension of aqueous alkaline solutions using the maximum bubble pressure method (Rebinder’s method) [3]. The concentration dependence (Fig. 6) shows that, at c > 30 mg mL⁻¹, σ varies insignificantly. In other words, the critical micelle concentration (CMC), at which a large number of micelles occurring in thermodynamic equilibrium with solute molecules are formed in the solution, is above this boundary.

To compare OHL with other surfactants, we determined σ of aqueous solutions of sodium oleate (traditional component of detergents) and sodium lignosulfonates (LSTNa). All the measurements were performed under equal conditions: c = 30 mg mL⁻¹, pH 9.1.

The surface tension of solutions of sodium oleate, OHL, and LSTNa was 29, 43, and 52 mN m⁻¹, respectively. Thus, in surfactant properties OHL is inferior to sodium oleate but surpasses LSTNa. The relatively low surface activity of LSTNa was also shown in [27]. In that study, the surface tension of the LSTNa solution in the region of CMC was determined by the stalagmometric method and Du Noüy ring method to be 57–58 mN m⁻¹.

Nevertheless, lignosulfonates as surfactants are widely used in many branches of industry as dispersants, emulsifiers, flotation reagents, and additives to drilling fluids, concrete, cement, detergents, etc. [28, 29]. For various reasons, including environmental reasons, the world’s production of lignosulfonates decreased from

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**Fig. 4.** UV spectrum of the oxidized hydrolysis lignin filtrate. (D) Optical density and (λ) wavelength.

**Fig. 5.** Chromatogram of the diethyl ether extract of the oxidized hydrolysis lignin filtrate. (I) Intensity and (τ) time.

**Fig. 6.** Surface tension σ of aqueous solutions of oxidized hydrolysis lignin as a function of concentration c.
20 million tons in 1980 to 7 million tons in 2006, and this trend continues [29]. Because the surfactant properties of OHL and lignosulfonates are close, prospects are opened for using OHL as surfactant. The stock of HL in Russia is estimated at 95 million tons [30].

CONCLUSIONS

(1) Oxidation of hydrolysis lignin with hydrogen peroxide in acid medium leads to degradation of a part of aromatic rings and to lignin demethoxylation; simultaneously, the content of carboxy groups of different types appreciably increases. The content of structures with \( \beta -O-4 \) bonds under these conditions changes insignificantly.

(2) Oxidation of hydrolysis lignin yields oxidized hydrolysis lignin. A part of it (25%) passes into the solution in the form of oligomers containing a repeat unit of mass 142 Da, which can be tentatively identified as muconic acid derivatives.

(3) High sorption ability of oxidized hydrolysis lignin allows using it as a raw material for preparing sorbents, including enterosorbents.

(4) The surfactant properties of oxidized hydrolysis lignin and lignosulfonates are similar; therefore, oxidized hydrolysis lignin can be used in the same branches of industry in which lignosulfonates are traditionally used.

ACKNOWLEDGMENTS

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REFERENCES


