Structural Study of Oxidized Lignin Samples using Size-Exclusion Chromatography, NMR Spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Oxidized lignin is seen as the promising source of humic-like substances which can be used for many biomedical applications. To verify this concept the structural studies on similarity of the structures of oxidized lignin and humic substances (HS) are needed. The objective of this work was to compare the structures of oxidized lignin with those of HS from different sources. For this purpose, both low and high resolution structural analytics was used including size exclusion chromatography (SEC), 1D and 2D NMR spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy (FTICR MS).

Three samples of oxidized lignin (Nobel ltd, Russia) and two HS (one coal HA from Sakhalin Island, Russia, and one aquatic FA from Suwannee River, USA) were studied.

SEC analysis was conducted using HPLC system consisted of a solvent pump, a packed column, a UV-detector and a PC. The UV-absorbance was measured at 254 nm. The SEC column was 15x250 mm packed with Toyopearl HW-55S (Toso-Haas, Japan). 0,03M phosphate buffer with pH 6,8 was used as a mobile phase at a flow rate of 1 ml/min. The column was calibrated using sodium polystyrenesulfonates.

Quantitative ^{13}C solution state NMR spectra were acquired using Avance 400 spectrometer (Bruker, Germany) operating at 100 MHz carbon-13 frequency. The spectra were recorded on the samples dissolved in 0,3 M $NaOD/D_2O$ at concentration of 80 mg/ml. Two-dimentional COSY, TOCSY and HSQC NMR spectra were acquired using DMX-500 spectrometer (Bruker, Germany) operating at 500 MHz proton frequency. The spectra were recorded on the samples dissolved in 0,1 M $NaOD/D_2O$ at concentration of 12 mg/ml. All NMR spectra were acquired with a 5 mm broadband probe.

FTICR MS analysis was conducted on 12 Tesla spectrometer (Bruker, Germany). The samples were dissolved in methanol and diluted to 10 mg/l. All mass spectra were acquired in negative ion mode.

The NMR data on the content of aromatic and aliphatic carbon have shown the close resemblance between structures of oxidized lignin and those of chernozem soil humic acids. But the size exclusion chromatographic studies have shown that the oxidized lignin samples have much higher hydrophobicity as opposed to all HS samples including those from coal: the recover extent from the column did not exceed 12% while for HS it was not less than 68%. Therefore CH_nO structural fragments of the oxidized lignin samples detected using NMR spectroscopy do not refer to hydrocarbon fragments and belong to ether and ester groups.

The molecular formulas were for the first time determined for oxidized lignin materials using FTICR MS data treated with FIRAN software. It was unambiguously shown that C_3H_2O fragment is a structural block in the analyzed samples as well as $C_6H_4O_2$ and $C_9H_6O_3$. For the first time the stoichiometric coefficients were determined in the formulas assigned to the samples studied. It was shown that the formula can be written as $(C_3H_2O)_x(C_2H_2O)_y(CH2)_z$: where $x \leq 18$, $y \leq 13$, $z \leq 48$.

The conclusion was made that the samples of oxidized lignin analyzed contained two very different classes of compounds: one was of a humic nature containing mostly aromatic compounds, while the other was mostly represented by lipid components like fatty acids.

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