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Last minute abstracts

New Opportunities for Lignin Conversion in the Biorefinery

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Keywords: lignin, biorefinery, economic evaluation, renewable

1. INTRODUCTION

The biorefinery is a valuable unifying concept for the conversion of agricultural and silvicultural feedstocks to chemicals and fuels (1). Although its strength is an ability to use renewable and sustainable raw materials, biorefinery infrastructure will likely model that of the existing petrochemical industry. This infrastructure will contain the components of *production* (i.e., farming), *separation* (obtaining the available building blocks from biomass, such as cellulose, hemicellulose, and lignin) and *conversion* (the transformation of the building blocks into useful products). Of these three components, *conversion* is the least developed for the biorefinery. While the petrochemical industry can describe a wide number of high yield, selective conversion paths for their primary building blocks (methane, ethylene, etc.) the same cannot be said for the emerging biobased products industry. The range of tools available to carry out conversions comparable in efficiency and breadth to the existing chemical industry is scarce. Nowhere is this technology gap more apparent than in the use of lignin as a chemical feedstock.

There is no doubt that lignin is readily available, as it is the second most abundant carbon source in nature. Agricultural residues, grasses and woody plants contain 15-25% lignin by weight, and ongoing biorefinery development is defining biomass separation technology that will make lignin an easily accessible process stream (2). The kraft process used for making cellulose pulp consumes over $100x10^6$ metric tons/yr of wood, generating a correspondingly large amount of lignin (3). For each of these biomass pretreatments, the cellulose and hemicellulose streams are allocated to production of fuel or chemicals. However, no general higher value use for lignin from any of these sources has been developed. In each case, the lignin can be burned as fuel, but this is the least effective and lowest value ($0.03 – 0.04/lb) use for an abundant and renewable carbon source. The ability to convert lignin to value added compounds selectively and in high yield would be an important step forward in the use of biomass as a chemical feedstock, and a key improvement for integrated biorefinery development. This presentation will present activities related to the development of lignin as a biorefinery feedstock including an overview of opportunities for lignin's use as a feedstock for new chemicals and materials and a summary of current activities at the University of Tennessee (UT) Forest Products Center directed at new conversion technology for biorefinery lignin.

2. RESULTS AND DISCUSSION

Although the rapidly increasing interest in the use of lignocellulosic raw material assures a continuous lignin process stream within the biorefinery, lignin faces several challenges as a feedstock, including its historic default use as a fuel, its greater structural complexity when compared to other biorefinery process streams, a wide range of potential lignin isolation techniques that each lead to material with different chemical and physical characteristics, and a lack of general technology applicable to the structural features offered by lignin. The U. S. Department of Energy (DOE) recently released a new "Top 10" report summarizing the potential utility of lignin as a higher value process stream within the biorefinery (5). The report describes near-, mid-, and long-term opportunities for lignin conversion in parallel with necessary technology development. Successful recognition of these opportunities is the goal of work at the UT Forest Products Center, which is directed at several aspects of lignin isolation and conversion technology. The presentation will include the following topics:

1) An overview of the recent DOE report. The final product portfolio described in the "Top 10" report results from a downselection process considering the degree of difficulty, the market and its risks, the potential for a given material to serve as a platform for other chemicals, and whether the material would be likely to be formed as a mixture. Near term opportunities include power, fuel and syngas by using lignin as a carbon source, and technologies designed to combust lignin or break it down into low molecular weight components. Mid-term opportunities include retaining lignin's high molecular weight, and using it as a source of lightweight carbon fiber, or as a component of thermoset resins. Long-term opportunities envision lignin as a fully functional raw material for the chemical industry, with both chemical and biochemical conversion technology directed at the selective production of low molecular weight aromatics, polymer components, and chemical intermediates.

2) Structural evaluation of biorefinery lignin. Effective use of lignin within the biorefinery will require an understanding of its structure after isolation, in order to tailor reaction processes appropriate for the substructural units present. We are using 2D HMQC NMR to investigate the changes in functional group profile for aspen lignins isolated under different conditions and severities. This profile information can be used to help define those chemical processes most effective for selectively transforming lignin into new chemical products.

3) Biochemical dihydroxylation of lignin. A barrier to lignin's use as a chemical feedstock is the relative stability of the network of aromatic rings comprising its structure. We are investigating the biochemical dihydroxylation (6) of lignin with *E. coli* or *P. putida* in order to disrupt this aromaticity and "activate" lignin to a much wider group of transformations. Our initial work is focusing on a two-step process shown in Figure 1.

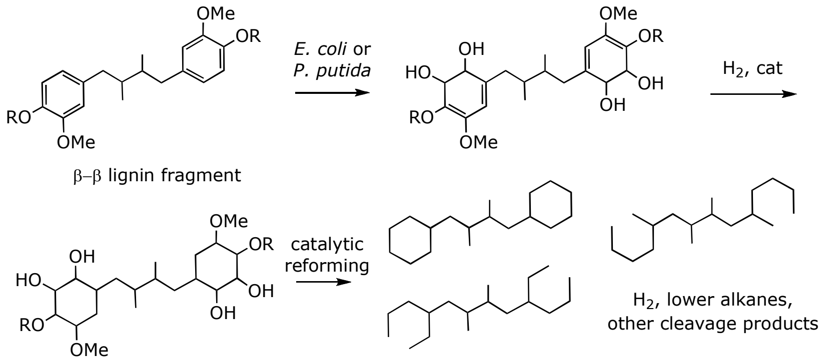

Figure 1. Schematic for converting technical lignin to alkanes via catalytic reforming

Treatment of lignin with dihydroxylation organisms will lead to the introduction of new functionality that renders lignin similar in structure to polyhydroxy compounds demonstrated to undergo catalytic hydrogenation or hydrogenolysis to alkanes (6). Our current work is directed at the biochemical transformation of a family of monomeric lignin models, and will be extended to the transformation of higher molecular lignin models, and ultimately, technical lignins expected to be available within the biorefinery.

4. CONCLUSIONS

Industrially, lignin is traditionally viewed as a source of fuel, and is assumed to be as a difficult, low-grade starting material because of its high level of structural heterogeneity. The recent DOE report has provided a framework for changing this perception by defining both product opportunities and technology needs for effective lignin conversion. Development of high yield lignin transformations based on this framework is a goal of research at the UT Forest Products Center, and will result in moving lignin from a low-value side product into a key raw material stream for the biorefinery.

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FTICR-MS Analysis of Cooperage Oak Wood Extracts: an Example of Metabologeography

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Oak from France but also from America and Eastern Europe is the major wood used for cooperage, because of its organoleptic properties. For instance, it is generally recognized that among the two predominant French oak species, *Quercus robur* L. (pedonculate oak) contains more tannins but fewer volatile compounds than *Quercus petraea* Liebl. (sessile oak). However, so far, all of the attempts to establish correlations between wood chemical properties and the origin, the species or even the position in the tree have relied on the targeted analysis of selected compounds. Here, we report the first FTICR-MS analysis of extracts of oak woods, which were initially selected from three French forests, traditionally supplying either pedunculate or sessile oak wood for cooperage. The aim of this study was to characterize these extracts in order to reveal metabolites or family of metabolites that could discriminate both the species and the geographical origins.

In agreement with existing studies, our results confirm that FTICR-MS analyses allow to identify selective compounds that unambiguously discriminate the two species commonly used in cooperage. However, we also show that it is possible to discriminate geographic origins thereby providing a striking new example of metabologeography.

Size-Exclusion Chromatographic Study of Humic-Like Substances from Oxidized Lignin

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1. INTRODUCTION

Biorefineries will receive and process massive amounts of lignin. For this reason, how lignin can be best used to support the economic health of the biorefinery must be defined. Different strategies for obtaining lignin-based value added products have been recently reviewed (1). Derivatization of lignin macromolecules have been proposed as one of the most promising approaches to obtain new products. Given that lignin is a cross-linked natural polymer composed of phenylpropanoid units (2), one of its promising transformations might be oxidation to humic-like substances (HLS). Humic substances are known to possess substantial biological activity (3). Hence, the HLS produced by lignin oxidation can start a new value chain of the lignin-based bioproducts. The important issue in establishing new products is developing quality control techniques. Size-exclusion chromatography (SEC) is a powerful technique traditionally used for quality control of HS (3-5). This technique was also applied to analyse native lignin (6).

The purpose of this study was to evaluate a role of non-exclusion effects in SEC analysis of the HLS obtained from lignin oxidation upon applying routine conditions used in HS analysis and to elaborate optimal conditions minimizing non-exclusion effects and maximizing column recovery.

2. MATERIALS AND METHODS

Humic and humic-like materials: Six samples of oxidized lignin were received from “Nobel” ltd (St. Petersburg, Russia). Two natural HS – humic and fulvic acids (HA and FA) isolated from leonardite and the IHSS standard fulvic acid (Suwannee River SRFA, USA) correspondingly – were used as comparison samples.

SEC: Liquid chromatographic system consisted of a solvent pump, a packed column and a UV-vis detector with variable wavelength was used as described (5). The UV-absorbance was measured at 254 nm. The SEC column was 15x250 mm packed with Toyopearl HW-55S (“Toso-Haas”, Japan). Phosphate buffer (PB) with different pH and ionic strength was used as a mobile phase at a flow rate of 1 ml/min. The following phosphate buffer pH and concentration values were applied: 0,03M and pH 6,8 (the standard conditions for HS analysis); 0,01M and pH 6,8; 0,03M and pH 8,0; 0,03M and pH 8,0 + 10% THF (vol.).

The column was calibrated using sodium polystyrenesulfonates (PSS): 4,48, 14,00, 20,70, 45,10 and 80,84 kDa ("Polymer Standard Service", Germany). Blue dextran (2000 kDa) served as a void volume probe (V_0), acetone – as a permeation volume probe (V_p).

RESULTS AND DISCUSSIONS

Given hydrophobic character of the HLS samples, the non-exclusion sorption effects were expected. To minimize these effects, three different modifications of the standard eluent were undertaken: a decrease in ionic strength, an increase in pH, and addition of hydrophobic solvent (THF). Typical chromatograms obtained are shown in Figure 1.

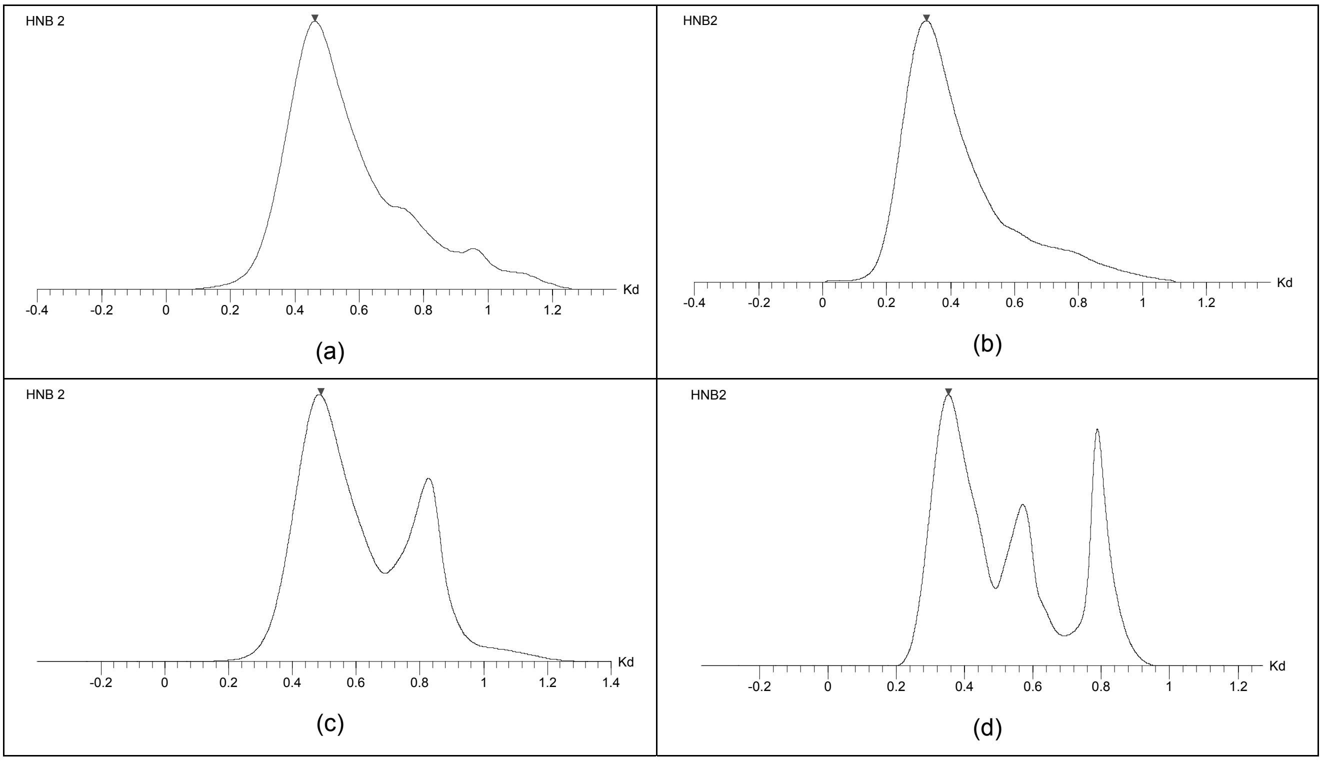

Figure 1. Typical chromatograms of HLS-2 studied: a) 0,03M PB, pH 6,8; b) 0,01M PB, pH 6,8; c) 0,03M PB, pH 8,0; d) 0,03M PB, pH 8,0 + 10% (vol.) THF.

The appearance of second peak at pH 8 (Fig. 1 c, d) may be caused by high HPO_4^{2-} ions concentration. Due to repelling forces of the column, these ions form the additional concentration front that carries along a portion of HLS. Appearance of the third peak in case of buffer modified with THF (Fig. 1 d) could be caused by the portion of HLS carried along with the organic solvent. As it can be seen from Figure 1, both a reduction in ionic strength and addition of organic solvent bring about lower elution volumes of HLS. In case of reduction in ionic strength (Fig. 1 b), this can be caused by an increase in electrostatic repulsion that results in shorter discharge time of HLS through the column. In the case of PB modification with THF (Fig. 1 d), the observed effect can be caused by a decrease in sorption. The molecular weight characteristics and column recovery values of the HLS and HS analyzed are shown in Table 1.

Table 1. The MW characteristics and column recovery values of the HLS and HS studied

Sample	Mpeak, kDa	Mn, kDa	Mw, kDa	Mz, kDa	**Mw/Mn**	Recovery, %
0,03M PB pH 6,8						
HLS1	3.6	0.6	3.2	5.9	5.5	12
HLS2	3.7	1.1	3.5	6.4	3.3	9
HLS3	3.4	0.7	2.9	5.3	4.0	10
HLS4	4.0	1.6	3.9	6.1	2.4	24
HLS5	4.5	1.3	4.0	6.6	3.1	9
HLS6	4.5	1.2	4.1	7.0	3.3	12
Coal HA	8.1	2.1	10.8	30.7	5.3	68
River FA	6.2	3.4	6.6	9.1	1.9	80
0,01M PB pH 6,8						
HLS1	17.3	32.1	15.7	2.6	6.0	12
HLS2	16.4	24.4	13.2	1.5	8.9	12
HLS3	16.7	23.6	13.2	1.7	7.7	14
HLS4	17.9	29.5	15.3	1.6	9.6	29
HLS5	17.1	26.1	13.7	1.3	10.5	15
HLS6	17.7	35.7	16.4	2.4	6.9	14
Coal HA	22.9	50.1	25.2	4.2	6.0	38
River FA	20.7	28.3	20.1	7.8	2.6	83
0,03M PB pH 8,0						
HLS1	5.5	1.4	4.8	9.1	3.3	21
HLS2	4.6	1.0	4.1	7.2	4.3	20
HLS3	4.5	1.0	3.8	6.3	3.9	18
HLS4	4.6	1.3	4.1	6.5	3.3	40
HLS5	5.1	1.2	4.7	8.2	3.8	21
HLS6	5.4	1.7	4.9	8.4	2.9	23
Coal HA	8.7	3.8	8.1	11.5	2.2	66
River FA	8.2	5.6	8.8	11.8	1.6	100
0,03M PB pH 8,0 + 10% (vol.) THF						
HLS1	6.1	0.7	4.6	8.4	6.9	24
HLS2	6.1	0.8	4.8	8.0	6.0	25
HLS3	6.6	1.0	5.3	9.4	5.3	29
HLS4	6.8	1.0	5.7	9.6	5.8	46
HLS5	6.8	0.6	5.6	10.4	9.0	39
HLS6	6.7	0.8	5.2	9.4	6.2	24
Coal HA	8.6	2.5	7.6	11.0	3.0	81
River FA	9.1	6.1	9.5	12.4	1.6	100

The molecular weights were calculated on the basis of the first major peak observed on the obtained chromatograms. It can be seen that sorption effects (recovery values) decreased with an increase in pH and upon adding THF. At the same time, the measured increase in molecular weights could be indicative of the fact that relatively high molecular weight fractions of the samples were preferentially sorbed on the gel under the standard elution conditions. Electrostatic repulsion in the case of the reduced ionic strength leads to the observed increase in molecular weights.

4. CONCLUSIONS

The decrease in non exclusion effects (sorption on column gel) was achieved under conditions of using phosphate buffer with higher pH (8.0) and its modification with THF.

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Aromatic Lignin Compounds in Soils of Different Ecosystems

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Keywords: humic, lignin, soil

1. INTRODUCTION

Lignin is one of the main components of plant litter (25% of the dry of dry weight). But despite the significance ascribed to lignin in the humus formation our knowledge on the really amount and degradation pathway of natural lignin in the different zonal soils of Russia is rather fragmentary. Such characteristics of soil organic matter as content and composition of aromatic lignin acids and aldehydes have never determined in them.

The aim of our investigation is to study in detail the composition and profile distribution of lignin parameters in soils under different types of vegetation and different biochemical landscape conditions.

2. MATERIALS AND METHODS

Amount and composition of lignin phenols (vanillin, syringaldehyde and vanillic, syringic, p-coumaric, ferulic acids) are investigated in Chernozems Haplic (Stavropol region) and Greyzems Haplic (Brjansk district) of arable land, Gleysols Mollic (Moscow district) of hydromorphic lower land, Cambisols Humic of juniper’s and pine’s forests (Dagestan), Leptosols Umbric of alpine meadow (Northern Caucas, 2200 m), Leptosols Mollic of subalpine meadow-steppe (Northern Caucas, 1200-1300 m). Ecosystems with different types of vegetation were studied: arable land, stepp, pine, spruce, birch, aspen, larch, cedar, junipers forests, alpine and subalpine meadows. Lignin phenols were determined in wood and plant tissues, root and plant falloff, litters, soils and humic acids.

The amount and stage of oxidative decomposition of lignin was estimated using the system of lignin parameters obtained from alkaline CuO oxidation (1). Samples of 100-300 mg were oxidized with CuO and 2 M NaOH for 2 h at 170 C° under N_2 atmosphere. Phenol CuO oxidation products were analyzed as trimethylsilyl derivatives by capillary gas chromatography (OV-1 fused silica column) using an HP 5890 instrument equipped with a FID detector. Phenol acetic acid was employed as internal standard. The sum (VSC) of vanilyl (V), syringyl (S), and cinnamil (C) units was adopted as an indicator of the amount of lignin. The mass ratios of acid-to-aldehyde in vanillic (v) and syringic (s) units and of syringyl-to-vanillyl (S/V), cinnamil–to-vanillyl (C/V), coniferyl-to-ferul (C/F) units were taken to assign the degree of microbial alteration of lignin within a sample.

3. RESULTS AND DISCUSSION

It was established, that the main source of lignin in ecosystems studied are wood and plant roots (Table 1). Actually, the content of lignin-derivated products strongly correlates with the amount of plant roots in soils under subalpine meadow (coefficient of correlation is 0,92) and alpine meadow (0,93), as well as under the juniper's forest (0,99).

Table 1. Lignin parameters of some plant tissues

Type of plant tissue	VSC, mg/g C_{org}	V	S	C	V:S:C
Pine					
Needle	24,34	15,29	0	7,30	2:0:1
Roots	30,99	24,70	3,50	2,83	8:1:1
Litter	16,31	7,07	0	5,81	1:0:1
Juniper					
Needle	6,06	2,00	0	4,06	1:1:2
Wood	23,01	12,96	5,38	4,67	2:1:1
Roots	65,45	62,40	1,43	3,05	41:1:2
Litter	18,60	15,30	2,87	3,30	5:1:1
Grasses					
Meadow cereals	24,30	8,59	8,63	22,21	1:1:3
Roots of cereals	36,23	10,52	14,12	21,20	1:1:2
Steppe cereals	42,09	15,26	15,61	33,35	1:1:2
Meadow herbage	25,59	5,79	10,46	19,69	1:2:3
Roots of herbage	48, 50	10,05	25,21	36,76	1:2:4

Sum of lignin-oxidative products in the soils studied reflects different biochemical conditions of humification (Table 2). The alpine-meadow Leptosols Umbric shows a decrease of VSC from 82,28 mg/g Corg in the OA horizon to about 1,12 – in the humus horizon.

Gleysols Mollic of Moscow region are characterized by the highest content of the aromatic lignin structures (to 16 mg/g Corg) in the humus horizon. The degree of side-chain alteration of the lignin molecules in it is also lower – 1-2 %. That is way the role of humic acids abiotic condensation from simple free monomers is more considerable in lowlands hydromorphic conditions and in high mountain soils, where microbial activity is more lower and ferment amounts is also low. High-molecule compounds preserve and gradually change into humic acids by the way of biopolymer degradation, the degree of lignin molecule oxidation increases.

The steppe type of accumulation and profile distribution of lignin-derived phenol compounds is characterized by low lignin content in the A horizon (0,66 mg/g Corg). VSC increases gradually along the profile of Chernozem from 0,66 mg/g Corg in the humus horizon to about 1,16-2,12 mg/g Corg in the AB horizon and can be explained by the input of root litter. The degree of side-chain alteration of the lignin molecules - to 22% in A horizon and to the 53 % on the 40-60 cm in buried middle Holocene humus horizon (Ab). That is way, in Chernozems, as in soils with high biochemical activity the more possible pathway of humification is the deep and quick fermentative degradation of high-molecular compounds to the monomers.

Table 2. Lignin transformation parameters in soils of different ecosystems

Soil	Horizon, depth, cm	VSC, mg/g C_{org}	$(\text{asids/aldehyde})_v$	$\frac{S}{V}$	$\frac{C}{V}$	$\frac{C}{F}$	V:S:C
Chernozems Haplic (n=6)	A 4-56	0,66	0,59	0,51	1,68	1,87	2:1:1
	AB 56-71	1,16	0,20	0,21	0,14	2,00	7:1:1
	B 71-83	2.12	0,22	0,63	0,21	0,86	3:3:1
Greyzems Haplic (n=10)	Ap 0-33	9,09	0,29	2,80	0,85	0,95	1:3:1
	Ab 33-63	0,72	2,92	0,91	0	0	6:5:1
Gleysols Mollic (n=12)	Ag 10-15	16,02	0,24	1,38	0,50	1,22	1:1:1
Cambisols Humic (n=6)	As 0-14	13,06	0,29	0,58	0,32	0,68	3:1:1
	A 14-30	3,91	0,46	0,51	0,26	0,60	4:2:1
	AB 30-60	8,23	0,39	0,69	0,53	0,23	20:12:1
Leptosols Umbric (n=6)	OA 0-5	82,3	0,54	2,73	0,77	1,08	1:2:1
	A 5-20	3,19	0,29	0,84	0,59	0,77	2:2:1
	20-50	1,12	0,46	0,84	0,55	0,62	2:2:1
	AC 50-70	2,71	0,37	1,08	0,78	1,47	1:1:1
Leptosols Mollic (n=8)	A 3-33	13,69	0,46	0,55	0,47	1,13	2:1:1
	Ab 33-52	0,96	0,34	0,46	0,35	1,00	3:1:1

The ratio $(\text{asids/aldehyde})_v$, what is the measure for the degree of side-chain alteration in the lignin remnant molecule in the Chernozems is about 0,59 in comparison to

0,24–0,29 in hydromorphic Greyzems Haplic and Gleysols Mollic. In the alpine Leptosols Umbric the $(\text{asids/aldehyde})_v$ ratio is about 0,54 with a distinct minimum in the intensively rooted horizon – 0,29, - produced by fresh root litter material. The S/V ratio in Chernozem profile increases from 0,51 in A horizon to 0,63 – in B. This fact reflects the degradation of siringyl alcohol-derived structures over coniferyl alcohol-derived structures. Actually, the C/V ratio in Chernozem decreases with depth from 1,68 in humus horizon to 0,14-0,20 – in AB and B horizons. The C/F ratio also decreases from 1,9 in A to 0,85 – in B horizon. These dates are result from high amount of coumaryl structures in remnant of grasses tissues.

Due to the biochemical specificity and high sustainability of plant lignin compounds it can be used as a molecular traces of terrestrial plants in the paleolandscape studies (2). Actually, proportions of syringic, vanillic and p-coumaric structures in organic matter of the arable Greyzems Haplic reflect the genesis of plant residues: grasses root litter in the plouth horizons – 1:2:1, leaves tissues at the depth 40-60 cm (buried humus horizon) – 6:5:1. This fact is the relict property of the forest genesis of these soils.

4. CONCLUSIONS

The main lignin source in the soils is found to be wood and plant roots.

The total production of lignin oxidation reflects biochemical conditions of humification in mineral soils.

The composition of lignin-oxidative products in the paleosoils may be used as molecula traces of ancient ecosystems.

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Detoxifying Ability of Coal Humic Acids and Their Hydroquinone Enriched Derivatives in relation to Copper under Field Conditions

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1. INTRODUCTION

Humic substances (HS) have been the subject of numerous scientific studies due to their mitigating effects on contaminants toxicity to biota. The detoxifying properties of HS are generally attributed to their capability for binding ecotoxicants of different classes including heavy metals. The binding of heavy metals to HS causes formation of less bioavailable complexes followed by lowering their toxicity and bioaccumulation. Therefore, development of approaches which are aimed to intensify binding ability of HS in relation to ecotoxicants are of ultimate importance. Oxygen containing functional groups of HS are supposed to be responsible for HS binding properties in relation to heavy metals. Thus, enrichment of HS with above mentioned moieties might provide increase in chelating properties of humics followed by increase in detoxifying ability of HS. The goal of this study was to estimate detoxifying ability of coal derived HA and their hydroquinone enriched derivatives in relation to copper.

2. MATERIAL AND METHODS

For this study humic acids isolated from leonardite was used (CHP). To synthesize hydroquinone enriched derivatives, polycondensation of CHP with hydroquinone has been carried out using HS : hydroquinone ratio as 1 g : 250 mg. Two initials concentration of CHP such as 5 and 12% were used and two corresponding derivatives assigned as CHP-HBQ250-5% and CHP-HBQ250-12% were obtained. The total and carboxylic acidity of the preparations was determined using standard barita and calcium acetate techniques, respectively. The obtained results demonstrated higher contents of both carboxylic and phenol acidic groups in the synthesized derivatives as compared to the initial humic material. That finding indicated successfulness of the performed modification.

Detoxifying activity of the humic preparations was estimated by two-years field experiments conducted under sod-podzolic soil conditions. Plants of wheat *Triticum aestivum* L. were used as a target object, and fresh weight of 10 plants was used as a response. To create contamination with copper, copper solution was applied in the first year of the study in the form of $CuSO_4 \times 5H_2O$ up to dosage of 3 t of $CuSO_4 \times 5H_2O$ per hectare. Humic preparations was introduced at the application rate of 100 kg/ha in the first year of the experiment as well. To determine mobile copper content in soil, samples were collected after plant harvesting and subjected to extraction with acetate-ammonium buffer (pH 4.7) at the soil : solution ration 1: 10 followed by AAS detection in propane-air flame.

3. RESULTS AND DISCUSSIONS

The obtained results demonstrated significant decrease in copper toxicity in the presence of humics (Fig. 1), and detoxifying ability of CHP-HBQ250-12% was the most pronounced that corresponded well to the highest contents of acidic groups in that preparation.

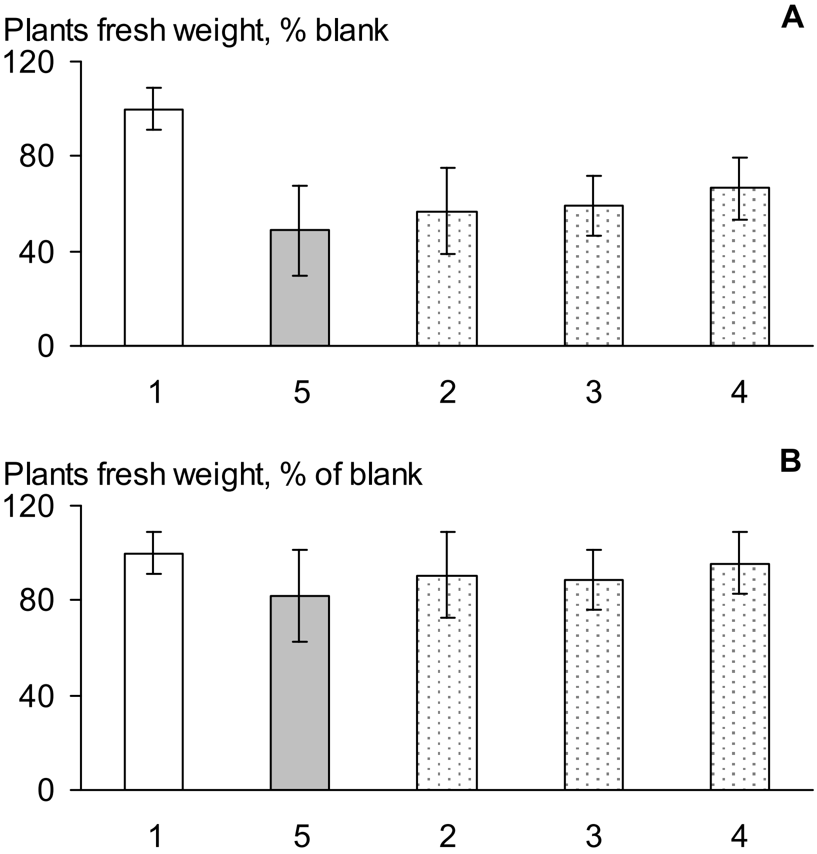

Fig. 1. Detoxifying ability of humic derivatives under study towards copper in the first (A) and second (B) year of treatment.

1 – blank; 2– CHP, 3 – CHP-HBQ250-5%, 4 – CHP-HBQ250-12%, 5 – $CuSO_4 \times 5H_2O$.

In the first year of treatment humics introduction into the copper-contaminated soil resulted in remarkable increase in plant biomass. However, even in case of CHP-HBQ250-12%, fresh weight of wheat plants did not exceed (67±13)%, i.e. was lower blank values. The latter meant that higher humic detoxicant : copper ration then 100 : 7 should be used for soil remediation.

In the second year of the study copper toxicity was less pronounced probably due to copper migration from the soil. Similarly to the first year, humic introduction resulted in significant decrease in copper toxicity to plants (Fig. 1B). When CHP-HBQ250-12% was applied to the copper contaminated soil, biomass of wheat did not differ statistically from that value for the blank variant. Therefore, prolonged detoxifying activity of humic preparations in relation to copper has been demonstrated.

According their detoxifying ability, the tested humic preparations can be put in the following descending order: CHP-HBQ250-12% > CHP-HBQ250-5% > CHP. Based on the conducted experiments, CHP-HBQ250-12% could be recommended as the most promising detoxifying agents towards heavy metals.

Contents of mobile copper in soil presented in Table 1.

Table 1. Content of mobile copper in sod-podzolic soil contaminated with copper and treated with humic detoxicants

Variant	Mobile copper content, mg/kg of soil	
	First year of treatment	Second year of treatment
Blank	10±4	8±3
Copper	600±5	75±10
Copper + CHP	580±5	79±10
Copper + HBQ250-5%	590±5	76±15
Copper + HBQ250-12%	406±10	73±11

Obtained results demonstrated that soil used could be characterized with content of mobile copper common for sod-podzolic soils. When copper was introduced into the soil, it concentration increased to 600 mg/kg. On the contrary to variants without added blue vitriol, treated of copper-contaminated soil with all the studied humics resulted in considerable decrease in content of mobile copper. Among the humics studied, the highest lowering in toxicant content in soil was observed for variant with HBQ250-12% which was shown to possess the highest detoxifying ability. That finding indicated that introduction of both parent humic material and humic-based detoxicants led to lowering of copper available for plants.

In the second year of the study mobile copper contents lowered considerably as compared to the year of treatment. Similar tendency of decrease in contents of mobile copper was observed for the variants with humics introduction. Taking into consideration, however, the fact that humics decreased contents of mobile copper in the year of treatment, it must be concluded that residual contents of copper in soil related to endogenous soil organic matter rather than introduced HS. So, humics introduction into copper-contaminated soil did not influence significantly on copper mobility in soil.

4. CONCLUSIONS

Our results demonstrated clearly that detoxifying ability of HS in relation to heavy metals could be increased by means of polycondensation with hydroquinone. The obtained detoxicant possessed prolonged activity for least two years after treatment and did not influence significantly on copper mobility in soil profile.

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Effective Algorithm of Charge State Determination in Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectra of Humic Substances

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Humic substances (HS) are complex mixtures formed during decay of plant and animal materials. Ultrahigh resolution of Fourier Transform Ion Cyclotron Resonance mass spectrometry (FTICR MS) makes it the most powerful analytical tool for molecular level understanding of HS structure. However, only low molecular weight HS fraction is available for FTICR MS with electrospray (ESI) ion source. ESI FTICR mass spectrum of HS contains about ten thousands of peaks in mass range 250–1200 Da (the most intensive peaks are usually located near 400 Da) with tenths of peaks, appeared at each nominal mass with high regularity. This unique peak density provides a significant challenge for data interpretation.

ESI source is known to produce ions with different charge states. The common way of charge-state determination in high-resolution mass spectrometry is based on the fact that the organic species containing one or more ^{13}C atoms (M + 1 ions) will appear 1/z above the m/z of the corresponding monoisotopic (e.g., all ^{12}C) ions. This approach shows the most intensive peaks in HS ESI FTICR mass spectra are singly charged (1). However, it is not necessary applicable to all peaks in the spectrum, because this approach can't be directly applied to all peaks in the spectrum.

At first, the abundance ratio between species with one ^{13}C atom and all ^{12}C is 0.0111n, where n is a total number of carbon atoms in the molecule. This means ^{13}C peak is expected to have lower intensity for species containing lesser than 90 carbon atoms. Most of the peaks in HS ESI FTICR mass spectrum have low signal-to-noise ratios. Thus, correspondent ^{13}C peak may be just absent in the spectrum making impossible to determine charge state by isotopologue mass. High peak density also increases difficulty of higher charge states determination because the expected location of isotopologues correspondent to high charge states is crossed with the location of monoisotopic peaks with higher fractional parts of their mass.

Recently, formation of doubly-charged ions from HS in ESI source was proved using ion mobility mass spectrometry (2). The goal of this work was to develop an effective algorithm of charge state charge state determination in HS ESI FTICR mass spectra, capable to determine charge states for monoisotopic peaks without ^{13}C isotopologues.

FT ICR mass spectra were acquired using 12 Tesla Apex Qe mass spectrometer with Apollo II electrospray ion source (Bruker Daltonics, Bremen, Germany) and 7 Tesla hybrid LTQ FT mass spectrometer with IonMax electrospray ion source (Finnigan, Bremen, Germany). Polymethacrylic acid (PAA) with M_w=3290 Da (Polymer Standard Service, Germany) was used as model compound for testing of the developed method. IHSS standard sample of Suwannee River fulvic acid (SRFA) was used as HS sample. The algorithm was implemented as a part of home-built FIRAN software developed for complex mixtures FTICR MS data treatment.

The developed algorithm is based on recently introduced Total Mass Difference Statistics (TMDS) approach (3, 4) for finding repetitive patterns in complex mixtures. This method finds a set of characteristic mass differences, which are frequently appearing between peaks in the mass spectrum, and corresponds to some repetitive structural units of the analyzed mixture. There are some series of peaks, which are differing one from each other by a number of a certain structural unit (e.g., a number of $–CH_2–$ groups in hydrocarbon skeleton). In this case, masses of these peaks will be differing one from each other by *d/z*, where *d* is mass of the structural unit, and *z* is the charge state of all peaks in series. The flowchart of the algorithm is presented on the Figure 1.

This algorithm was tested on model polymer compound (PAA, M_w=3290 Da). The major structural unit found using TMDS method is $C_4H_6O_2$ (PAA monomer unit). Charge states from –1 to –7 of 1901 peaks in mass range 197.1183...4025.6625 were determined successfully. The algorithm was also applied to FTICR mass spectra of SRFA sample acquired on different instruments. The structural units found in SRFA mass spectra were CH_2, CO, C_2H_2O, H_2, CH_2O, C_2H_4O and $C_3H_4O_2$. Peak series with more than 50 ions was found. Most of the species appeared to ionize in form of singly-charged or doubly-charged ions (or in both forms). The comparison between the number of peaks with found charge states determined by ^{13}C isotopologue masses and new algorithm are given in the table 1.

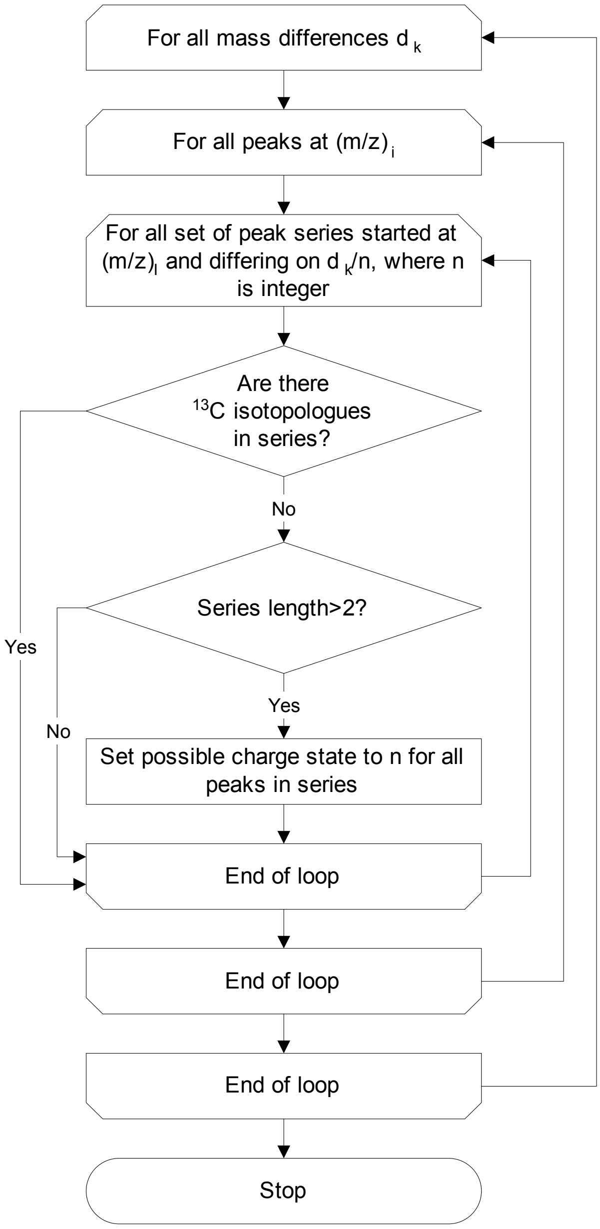

Figure 1. Flowchart of charge state detection algorithm based on Total Mass Difference statistics.

Table 1. Comparison between efficiencies of the traditional charge state determination algorithm based on ^{13}C isotopologue masses and new algorithm based on Total Mass Difference statistics.

Instrument	Algorithm	Peaks with charge state detected			Estimated Mn
		1–	2–	Total	
12T Apex Qe	^{13}C	1324	740	2161	528
	New	3423	5169	9574	672
7T LTQ FT	^{13}C	808	–	808	639
	New	2876	140	3016	668

The obtained results shown the developed algorithm can be used effectively for treatment of ESI FTICR mass spectra of humic substances. It helps to improve the detection of charge states for both singly- and doubly-charged ions and to increase the wideness of molecular mass range available for analysis.

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Technology of Waste Waters Purification Using Natural Sorbents Based on Humic Materials Derived from Brown Coals

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The prevention of pollution of water basins by waste waters is of great importance for national economy. Therefore, studies aimed to develop efficient and economically grounded technologies of waste water treatment are of great significance nowadays. To solve such environmental tasks, producing absorbents on the base of brown coals containing humic acids up to 70% are very promising. Humic acids are referred to weak acid polyelectrolytes and possess properties of sorbents, filtrants, ion-exchange materials, flocculants. By means of purposeful modification natural properties of coals may be enhanced.

Heavy metals are the most dangerous components polluting the environment. Fossil Fuel Institute (FFI) has developed the mode of waste waters purification and the plant for its implementing (1). Environmentally safe and economically efficient continuously working plant has a high productivity and high-quality technological characteristics due to the neutralization and purification of acid waste waters form metals cations and organic admixtures with a high level of purification reaching 90-100%.

The plant consists of the coal preparation block, natural sorbent development block (alkali solid of brown coal), water purification block where the neutralization of acid waste waters that have metals cations and organic purifications take place, separation block where the purified water is separated from the sorbent with purifications (Cu, Zn, Ni, Pb, Fe, Co, Hg, Mo, W, Cr, Al, As, U, etc.) and the worked off sorbent utilization block.

The purification of high volumes of technological solids in this plant goes with high speed by introduction in waste waters of solid alkaline Na-form carbon sorbent by stirring. As a result, non-solid forms of metal salts of humic acid are formed. The worked off sorbent separates from the purified solid by filtration and/or centrifugation.

The liquid phase goes into waste waters practically purified from the metal cations (except of alkaline). In this process the transition of metal cations to non-solid form goes in the same time with sorbtion stage.

The worked off sorbent is subjected to the thermal oxidation. In a homogenous surrounding humic acids forms rapidly non-solid salt of heavy metals directly during the purification process; the oil products can be separated using flocculation method.

It should be noted that natural sorbents may be used to purify waste water containing both metals and organic impurities and oil products.

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