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Structural features of lignohumic acids

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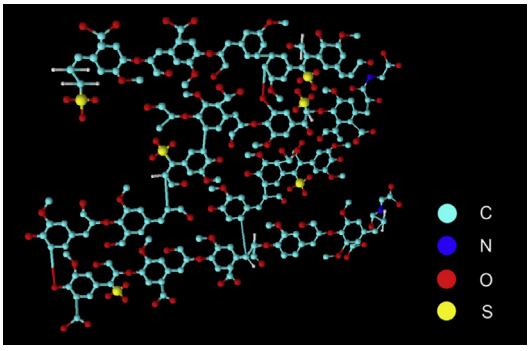
HIGHLIGHTS

- Humic-like substances can be prepared by thermal conversion of technical lignosulfonate.
- Resulting lignohumate contains humic acids (HA) whose properties and structural features are close to young soil HA.
- Hypothetical chemical structure of "average" lignohumate HA was proposed.

GRAPHICAL ABSTRACT

Average structural formula of lignohumate humic acid.



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ABSTRACT

The composition and structure of humic acids isolated from lignohumate, which is produced by hydrolytic–oxidative conversion of technical lignosulfonates, were characterized by chemical and spectral methods (UV/VIS, FTIR, and ^{13}C NMR spectroscopy). As comparative samples, humic acids (HA) were isolated also from lignite and organic horizon of mountain spruce forest soil. When compared with other HA studied, the lignohumate humic acids (LHHA) contained relatively few carboxyl groups, whose role is partly fulfilled by sulfonic acid groups. Distinctive ^{13}C NMR signal of methoxyl group carbons, typical for lignin and related humic substances, was found at the shift of 55.9 ppm. Other alkoxy carbons were present in limited quantity, like the aliphatic carbons. Due to the low content of these carbon types, the LHHA has high aromaticity of 60.6%.

Comparison with the natural HA has shown that lignohumate obtained by thermal processing of technical lignosulfonate can be regarded as an industrially produced analog of natural humic substances. Based on the chemical and spectral data evaluation, structural features of lignohumate humic acids were clarified and their hypothetical chemical structure proposed, which described typical "average" properties of the isolated fraction.

Introduction

Humic substances (HS) are extremely complex amorphous mixtures of highly heterogenous molecules, chemically reactive, while substantially resistant to decomposition, produced in the nature during litter decomposition. Many researchers tried to accelerate

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the long-term natural processes of humification and/or coalification with the aim to convert waste from industrial processing of biomass into humic or humic-like substances usable as soil conditioners or stimulants of plant growth. One of the few successful processes is the hydrothermal carbonization of biomass to industrial lignite [1]. This conversion can be stopped at humus as an intermediate product, if the process is suspended after a few hours.

Another process which allows to produce HS analogous to natural ones was developed by Gladkov et al. [2]. Their technology, based on the thermal hydrolytic–oxidative conversion of technical lignosulfonates under high pressure, produces so called lignohumate or lignosulfonate-humate [3]. The technical lignosulfonate arises from pure wood mass as byproduct in the production of sulfite wood pulp using sulfite pulping. Delignification in sulfite pulping involves acidic cleavage of ether bonds, which connect many of the lignin constituents. The primary site for the ether cleavage is a carbon atom attached to the aromatic ring of the propyl side chain. The electrophilic carbocations produced during the ether cleavage react with hydrogen sulfite ions to form sulfonates.

The conversion of technical lignosulfonates to HS runs under regulated and strictly controlled conditions in continuous oxidative mode typically for 1.5–2 h. The whole technological process is thus greatly accelerated in comparison with analogous humification in the nature. Resulting lignohumate is a dark brown powder or a concentrated alkaline (pH 8–10) solution, containing mixture of lignohumic acids, fulvic acids, and a smaller proportion of low-molecular organic compounds. The lignohumate exhibits a hormone-like activity on plants. Its application increases growth of roots and leaves, chlorophyll content, and activity of glutamine synthetase, glutamate synthase and rubisco enzymes [3]. Being environmentally friendly product, it is recommended to use for increasing crop growth [4,5].

This work has been aimed at characterizing by physicochemical methods the fraction of humic acids (HA) isolated from the "crude" lignohumate, comparing it with HA of other origin, and clarifying lignohumate structural features and proposing its hypothetical structural formula based on evaluation of chemical and spectral data.

Experimental

Materials

Samples

HA were isolated from following samples: lignohumate A extra (Lignohumate Ltd., Saint-Petersburg, Russia), lignite (Mír mine near Mikulčice, Czech Republic [6]), and soil from the Podzol O_f-horizon (mountain spruce forest soil, Alžbětínka stand, Giant Mts., Czech Republic [7]). The technical lignosulfonate (Biotech Paskov, a.s., Czech Republic) and sodium salt of lignosulfonic acid, p.a. (Sigma) served for comparison. For FTIR measurement, lignosulfonic acid was prepared from the sodium salt in a column packed with Dowex 50W × 4 (Aldrich) and freeze-dried.

Humic acid isolation

Lignite and litter from the O_f soil horizon of mountain spruce stand were air-dried, moderately crushed, and sieved (<2 mm). Before HA extraction, both samples were decalcified by 0.1 M HCl for 20 h and then washed by distilled water. HA were extracted from these samples and lignohumate powder by 0.1 M NaOH at a solution/sample ratio of 20:1 for 20 h, then purified by repeated re-precipitation followed by ultrafiltration desalinization (Amicon filtration cell, Millipore YM 1 K filter, 1 kDa cut-off, nitrogen pressure). The sodium humates were converted into HA (H^+ form) in a column packed with the strong cation-exchange resin Dowex 50W × 4, freeze-dried, and stored in the dark until analyzed. In order to distinguish between HA isolated from lignohumate and the initial material, we denote the preparation "lignohumate HA" (LHHA).

Physico-chemical characterization

Elemental (CHNS) composition

Elemental (CHNS) composition of the freeze-dried samples was determined using an Elementar vario EL III analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Samples were analyzed twice, sample weight was 5 mg. Results were referenced to 4-amino-benzene sulfonic acid as a standard. Ash content was determined in 100 mg of sample burnt at 550 °C for 4 h in a muffle furnace.

UV/VIS spectra

UV/VIS spectra were measured in a 0.01 m quartz cell at a concentration of 80 mg l^{-1} on a Varian Cary 3E UV/VIS spectrophotometer (Agilent Technologies, Santa Clara, USA) at wavelengths over the range of 200–900 nm. The studied compounds were dissolved in 0.05 M $NaHCO_3$. The so-called color quotient $A_{4/6}$ was calculated as the ratio of absorbances A_{465}/A_{665}.

The molecular weight

The molecular weight distribution was determined by low pressure size exclusion chromatography (SEC) using Britton–Robinson buffer solution at pH 9.0 as a mobile phase (0.04 M phosphoric acid, 0.04 M acetic acid, and 0.04 M boric acid in 1:1:1 mixture, pH adjusted with 0.2 M NaOH). The column was filled with Sephadex G-50 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). To characterize the HA molecular weight we used a set of sodium polystyrene sulfonates calibration standards (Scientific Polymer Products, Inc., New York). Blue dextran (MW 2000 kDa, Sigma) and acetone (MW 58 Da, p.a. grade, Lachema, Czech Republic) were used as standards for determination of the columns void volume (V_0) and total permeation volume (V_t). A HA sample (5 mg) was quantitatively dissolved in 1 ml of mobile phase. The injection volume was 0.5 ml and the flow rate 1 ml min^{-1}. Relative concentrations of standards and samples in the eluate were measured by a Libra S 22 (Biochrom Ltd., Cambridge, UK) UV/VIS spectrophotometer in a 0.01 m quartz cell at a wavelength of 280 nm. The average molecular weights M_w were calculated according to Kudryavtsev et al. [8].

The infrared spectra

The infrared spectra of dry samples homogenized in an agate mortar were recorded using a Nicolet 6700 FTIR spectrometer equipped with a Smart Miracle Si crystal attenuated total reflectance (ATR) accessory and a DTGS KBr detector in the wavenumber range of 4000–630 cm^{-1} (at wavelengths of 2.5–15.9 μm). Total number of scans averaged for each spectrum was 64, with a resolution of 4 cm^{-1}. Spectra analysis, involving atmospheric suppression, advanced ATR correction, and baseline correction were performed using the OMNIC operating system (Thermo Nicolet, Madison, USA).

^{13}C NMR spectra

^{13}C NMR spectra were recorded using a Bruker Avance 500 DRX NMR spectrometer (Bruker, Karlsruhe, Germany) with working frequency of 125.758 MHz (^{13}C). Samples dissolved in 0.1 M NaOD in deuterium oxide were measured with following parameters: temperature 298.15 K, NMR-tube diameter 5 mm, number of scans 12 000, excitation pulse 5.7 μs (90 °), acquisition time 0.43 s, spectral width 37 538 Hz, pulse repetition delay 5 s, and ^1H inverse