Preparation and hydration characteristics of carbodiimide crosslinked lignite humic acids

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ABSTRACT

Polar and apolar moieties of humic acids are spatially separated forming domains of different polarity. In this work, we tested the procedures to crosslink functional groups in polar domains of humic acids by using carbodiimide coupling and analyzed to which extent influenced the modification their hydration properties and stability. For this reason, we prepared eight derivatives of lignite humic acids using either water-soluble N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide (EDC) or water-insoluble N,N′-dicyclohexylcarbodiimide (DCC) under various conditions. Characterization of prepared derivatives showed that both methods lead to formation of crosslinked humic structures. Using of EDC resulted in lower degree of crosslinking, but better hydration properties. Higher moisture uptake and water holding capacity were observed in humic acids, which were pre-wetted prior to crosslinking for at least 24 h. Although the EDC derivatives of humic acids contained only between 60 and 85% of original free carboxylic groups, they showed similar moisture uptake as parental humic acids by equilibration at relative humidities of both 94% and 76%. Under water-saturated conditions, the EDC derivatives showed faster swelling kinetics and reached almost the same water holding capacity as the original sample after 18 days. However, both the EDC and DCC derivatives began to degrade already after 3–9 days during swelling tests, which subsequently decreased their hydration. The results suggested that water holding capacity, swelling kinetics and moisture uptake of humic acids were not influenced significantly by the amount of polar groups, but also by their spatial arrangement and distribution.

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1. Introduction

Applications of carbonaceous amendments such as humic acids (von Wandruszka, 2000), biochar (Conte, 2014) and composts (Scotti et al., 2013) have been gaining growing attention due to their (mostly) positive effect on the productivity of soil organic matter. The progress in analytical instrumentation enabled a detailed molecular analysis of these amendemnts (Grasset and Ambles, 1998; Grasset et al., 2002; Knicker et al., 2013; Nebbioso and Piccolo, 2011; Stenson et al., 2003), but the knowledge on relationship between their chemical composition, physical structure and biological activity is still incomplete. This relationship is, however, beneficial for their appropriate use in terms of dosage, effects on soil biota, structure and water holding capacity.

Humic substances (HS) are ubiquitous organic materials developed as decomposition products of plant and animal residues (MacCarthy, 2001). Humic acids (HAs) represent a fraction of HS, which are insoluble under acidic conditions, but become soluble and extractable in alkali solutions. HAs are a mixture of molecules rich in carboxyl groups (–COOH), hydroxyl groups (–OH), amino groups (–NH$_2$), quinonyl groups (–C$_6$H$_3$O$_2$) and others (Stevenson, 1994), which results in versatile properties and multitude of environmental functions. Currently, HAs are mostly extracted from peats and low rank coals and commercialized for industrial and agricultural purposes (Pena-Mendez et al., 2005). In most applications are used native HAs, but sometimes, they are also chemically modified to improve their physicochemical properties (Ctvrtnickova et al., 2011; David et al., 2014; Kovalenko et al., 2006; Perminova et al., 2005; Ryabova and Mustafina, 2003; Schneckenburger et al., 2012). Crosslinking the molecular segments is one of the possibilities to enhance and/or control their reactivity, thermal and chemical resistance, sorption properties and solubility (Ryabova and Mustafina, 2003). Crosslinking increases molecular weight, creates a network structure, reduces solubility, inhibits biodegradation, increases matrix rigidity, decreases free volume and influences sorptive properties towards organic compounds (Pignatello, 2012). Indeed, crosslinking is a natural process in stabilization of soil organic matter (Smejkalova et al., 2006) also playing a role in diagenesis of organic geopolymers (Grasset and Ambles, 1998; Grasset et al., 2002; Pignatello, 2012; Schneckenburger et al., 2012) and it is believed to protect soil organic matter against mineralization (Monreal et al., 2010).

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In HAs, the molecules of different polarity are separated forming hydrophobic and hydrophilic domains or "hotspots" stabilized by non-covalent interactions (Kucerik et al., 2012). Accordingly, the hydrophilic domains are formed by polar functional groups, which are too far from each other to form intermolecular crosslink (Schulten et al., 1998). The most abundant groups in humic acids are carboxylic groups; in lignite HAs studied in this work alcoholic OH (Valkova et al., 2009; Vlckova et al., 2009), phenolic OH and NH_2 groups (Doskocil et al., 2015) can also be expected, but to a lesser extent. On the contrary, the hydrophobic hotspots consist of apolar aliphatic and aromatic moieties. It is our working hypothesis that stability of these domains can be enhanced by introduction of covalent bonds between individual moieties.

In our previous work (Cihlar et al., 2014), we crosslinked lignite humic acids by using formaldehyde to create the $-CH_2-$ bridges between substituted aromatic rings. The study showed that this modification slightly increased moisture uptake, but did not result in formation of "gel-like" structure that is typical of crosslinked polymers.

In this work, the polar groups in hydrophilic domains of humic acids were crosslinked by using carbodiimide. The carbodiimides are zero-length crosslinkers, because they conjugate directly the carboxylates ($-COOH$) with primary amines ($-NH_2$), or with esters without becoming part of the final crosslink (amide or esters bond) between targeted molecules (Wong, 1991). As the arrangement of polar groups in HA hotspots is determined by the used solvent, we employed two strategies of crosslinking. First, we used water-soluble N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide (EDC) for crosslinking HAs in aqueous solutions and water-insoluble $N,N′$-dicyclohexylcarbodiimide (DCC) for crosslinking HAs in non-aqueous solutions (Dombekova et al., 2008; Wong, 1991). We assume that in aqueous solutions polar domains can be penetrated by EDC easily, while in non-aqueous solutions, the crosslinker DCC can be distributed via apolar domains. Therefore, using these crosslinkers, we would like to (i) find whether these strategies are suitable for production of HAs with enhanced properties in terms of water holding/uptake capacity and stability, and to (ii) contribute to the fundamental understanding the relationship between crosslinking of humic acids and their physicochemical properties that is still not well understood (Schneckenburger et al., 2012). For this reason, the HAs were crosslinked using carbodiimide under different conditions and tested with respect to their chemical properties, water holding capacity, hydration mechanisms and moisture uptake.

2. Materials and methods

2.1. Extraction and crosslinking of humic acids

Humic acids (HAs) were isolated from South Moravian lignite, kindly provided by Lignit s.r.o., Mír mine in Mikulčice, located nearby Hodonín in Czech Republic. HAs were extracted by alkaline extraction followed by their precipitation and purification (Cihlar et al., 2014). The obtained product was freeze-dried and stored. Details can be found in the Supplementary information (SI) in the chapter "Extraction of humic acids".

A part of HAs was crosslinked using an agent composed of a mixture of EDC and N-hydroxysuccinimide (NHS) and dissolved in a mixture of ultrapure water and ethanol. As HAs dissolve in aqueous solutions only partially and slowly, we tested also role of soaking time. For this reason, part of HAs was hydrated for 24 h and a part for 5 days. Another part was crosslinked by DCC in dichloromethane (DCM). DCM does not swell HAs, therefore, the mixing time was only 2 h during which only a negligible part of HAs was dissolved. The reaction mechanisms of both syntheses are reported in Fig. 1. Details can be found in the SI in the chapters "Crosslinking using EDC" and "Crosslinking using DCC".

The abbreviation of samples was chosen in the following way: original sample without any treatment is abbreviated as "ORIG". The crosslinked samples are marked either as "DCC" or "EDC" with respect to the used crosslinker. Furthermore, in the case of DCC, the presented number indicates a molar ratio between crosslinker and relevant functional groups in HAs, in the case of EDC the number represents the period of pre-hydration. The suffix "_DMF" and "_w" denote HA samples crosslinked in dimethylformamide and ultrapure water, respectively. Finally, the suffix "_w_C" represents sample crosslinked without using catalysis. The products and their names are summarized in Table 1.

2.2. Chemical characterization

To evaluate the changes induced by crosslinking with respect to the original structure, samples were characterized using FTIR spectroscopy, elemental analysis, thermogravimetry (TG) and pH titration.

FTIR spectroscopy was performed on Bruker Tensor 27 with a diamond ATR crystal in the range between 4000–600 cm^{-1}. Spectra were recorded at laboratory temperature and evaluated by OPUS software. As the ATR technique allows the quantitative analysis in solid state (Gulley-Stahl et al., 2009), the intensities of selected bands were compared to assess the relative changes in chemical structure induced by crosslinking. Details can be found in the SI in the chapter "Attenuated total reflectance Fourier transformed infrared spectroscopy".

The content of carbon, hydrogen, nitrogen and sulfur were determined by using an Elementar Vario Micro cube. The content of oxygen was taken as the difference from 100% (total content of determined elements). Measurements were carried out in triplicates and the deviations never exceeded 0.2%. Detailed description and results can be found in the SI in the chapter "Elemental analysis".

Simultaneous Thermal Analyzer NETZSCH STA 449 F3 Jupiter coupled with a Quadrupole Mass Spectrometer QMS 403C Aëolos (Selb, Germany) was used to determine the thermo-oxidative stability of produced materials. The obtained thermogravimetry curves show the mass change in dependency on temperature (Figs. S1 and S2, SI). The samples were measured in triplicates, the standard deviation of mass losses never exceeded 3%. The details and exemplary records can be found in the SI in the chapter "Stability test carried out using thermogravimetry (TG)".

As the carboxylic groups are involved in all crosslinking scenarios (Fig. 1), the pH titrations of HAs were conducted to assess the amount of residual COOH groups that were not involved in crosslinking reactions. For this reason, NaOH was used for titration of HAs (Masini et al., 1998). Equivalent point of the titration of carboxyl groups was recorded at pH 7.0 (Khil'ko et al., 2011). The pH values of solutions were measured using a multi-parameter analyzer Consort C 863, (Belgium) equipped with a glass combined electrode. All titrations were performed in the absence of background electrolyte. Each solution was titrated at least three times, the reported data representing the average values. The standard deviations of the consumption of NaOH never exceeded 7%. Details are described in the SI in the chapter "Determination of carboxylic group contents".

2.3. Hydration analyses

The ^1H NMR relaxometry was carried out to monitor the progress in moisture uptake and hydration connected with swelling. For this purpose, two subsequent experiments were conducted. In the first step, the kinetics of moisture uptake was studied, i.e. we studied the hydration of polar domains and their environment. In the second step, water was added by a pipette to hydrate whole structure and induce its swelling. ^1H NMR measurements were performed using a Minispec mq 7.5 NMR Analyzer (Bruker Co., Germany) instrument, operating at a proton Larmor frequency of 7.5 MHz. Two T2 (spin–spin) transverse relaxation times were determined to assess the properties of bound (short T2) and loosely bound (long T2) water fractions. The relaxation times were obtained by fitting the transverse relaxation decay curves using a biexponential function employing the RIWinFit software Version 2.4, Resonance Instrument Ltd., Oxfordshire, United Kingdom.