



Molecular characteristics of humic acids extracted from compost at increasing maturity stages

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ARTICLE INFO

Article history:

Received 6 November 2008

Received in revised form

16 February 2009

Accepted 24 February 2009

Available online 16 March 2009

Keywords:

Compost

Humic acids

DRIFT

CPMAS-¹³CNMR

Thermochemolysis

ABSTRACT

The molecular composition of humic acids (HA) extracted from compost at increasing maturity stages was determined by off-line TMAH-thermochemolysis-GC-MS, in combination with solid-state nuclear magnetic resonance (NMR) and infrared (IR) spectroscopies. While spectroscopy measurements followed the bulk changes, thermochemolysis provided a detailed molecular variation of HA composition. Both thermochemolysis and spectroscopy indicated that polysaccharides, alkyl, cyclic, and aromatic compounds were the predominant components of HA, the stable fraction of compost. NMR dipolar dephasing (DD) experiments confirmed that HA extracts contained lignin in lower amount than its oxidized degradation products. The progressive compost maturity was reflected in HA extracts by a decrease of carbohydrate content and a selective preservation of hydrophobic alkyl molecules, such as medium- and long-chain fatty acids, aliphatic alcohols, linear hydrocarbons, and plant polyester derivatives, like long-chain alkyl dicarboxylic acids, and ω -hydroxyacids. Spectroscopy results showed a concomitant entrapment in HA of biolabile compounds, such as peptidic moieties. The wide range of identified lipid components and plant biomarkers may represent useful tools to trace origin, quality, and transformation of amended compost in soil ecosystems.

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1. Introduction

Organic biomasses recycled in compost are useful in improving both environmental quality and sustainable agriculture. The application of composted organic matter to soil produces beneficial effects on the chemical, biochemical and physical quality of soil and its plant nutrition capacity (Kowaljow and Mazzarino, 2007; Weber et al., 2007; Hargreaves et al., 2008). A particular advantage of compost amendment to soil is the increase in colloidal humified organic matter that affects the quantitative and qualitative long-term status of soil organic matter (Adani et al., 2006, 2007). Hence, research has been focused on the compost stable fraction, often referred to as humic acid-like components (Veeken et al., 2000; Amir et al., 2006; Campitelli et al., 2006). The incorporation of humified compost in soil was shown to strongly affect the quality of endogenous humic substances (Rivero et al., 2004; Adani et al., 2007) and to reduce mineralization of biolabile compounds, thereby enhancing the role of soil organic matter (SOM) as a sink of organic carbon (Spaccini et al., 2002; Fortuna et al., 2003; Piccolo et al., 2004). Moreover, the humified material in compost may also

be an important resource in processes of soil remediation from contaminants (Conte et al., 2005).

A detailed molecular characterization of humified constituents formed during composting process appears, thus, as an essential requirement for evaluating the stability of organic material (Larré-Larrouy and Thuriès, 2006) and understanding the role of compost in agricultural and environmental processes. Non-destructive spectroscopic methods such as diffuse reflectance infrared Fourier transform (DRIFT) and ¹³C cross-polarization magic-angle-spinning nuclear magnetic resonance (¹³C-CPMAS-NMR) have already been used to identify the content and distribution of organic molecules in a wide range of solid organic matrices (Martin et al., 2001; Lguirati et al., 2005; Adani et al., 2006, 2007; Spaccini and Piccolo, 2007). However, detailed molecular information is obtained by off-line pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) and followed by gas chromatography-mass spectrometry (Pyr-TMAH-GC-MS). This technique was able to characterize the molecular composition of soil organic matter and bulk compost (Deport et al., 2006; Spaccini and Piccolo, 2007). It involves the solvolysis and methylation of ester and ether bonds present in natural organic materials that enhance both thermal stability and chromatographic detection of resulting methylated acidic, alcoholic, and phenolic groups. Moreover, the off-line technique allows

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the analysis of a large quantity of solid material, and, thus, a more effective qualitative and quantitative measurement of pyrolytic products.

The objective of this work was to apply a combination of spectroscopic techniques and pyrolysis (Pyr-TMAH-GC-MS) to evaluate the molecular changes occurring in humic acids extracted from compost at different degrees of biological maturity.

2. Materials and methods

2.1. Compost and humic acids

The biomass used in compost (GeSeNu Srl, Perugia, Italy) was as follows: 50% domestic organic wastes, 30% refuse from plant trimming, 20% vegetal residues from tobacco. These materials were mixed, ground, and sieved at 12 mm. Compost products were obtained aerobically after 30 days of a common active phase, followed by three different curing phases of an additional 30, 60 or 120 days, that yielded compost samples of different maturities. Compost samples were oven dried at 40 °C until constant weight, powdered by an electrical agate ball mill (Retsch PM 200), and finally sieved at 500 μm .

Free-lipid components were first removed from the compost samples with two consecutive extractions (2 + 24 h) by a 1:10 w/v of dichloromethane–methanol (2:1 v/v) solution. The humic acids (HA) were then extracted by shaking compost (20 g) overnight with 100 ml of 0.1 M NaOH– $\text{Na}_4\text{P}_2\text{O}_7$ (1:1 v/v) solution under N_2 atmosphere. After having centrifuged away the supernatant, the solid residue was washed with distilled water until pH 7. The supernatants and washings were combined, filtered on a quartz filter (Whatman GF/C), and acidified to pH 1 with concentrated HCl. The precipitated HA were purified by a 48 h shaking with 0.1 M HCl/0.3 M HF solution (1:50 w/v). The final residue was dialyzed against deionized water, and freeze dried. The extractions were conducted in triplicate for each compost sample that produced the following humic matter: HA-60 HA-90; HA-150, (humic acid extracted, respectively, after 60, 90 and 150 days of the composting process).

2.2. Infrared spectroscopy

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) spectra of humic acids, were recorded with a PE Spectrum-One spectrometer, equipped with a diffuse reflectance accessory, and by accumulating up to 100 scans with a resolution of 4 cm^{-1} . Before DRIFT analysis, dry samples were finely ground with an agate mortar while diluting with oven-dried KBr powder (5/100, w/w).

2.3. Solid-state ^{13}C NMR spectroscopy

Solid-state NMR spectroscopy (^{13}C -CPMAS-NMR) was conducted on a Bruker AV-300, equipped with a 4 mm wide-bore MAS probe. NMR spectra were obtained by applying the following parameters: 13000 Hz of rotor spin rate; 1 s of recycle time; 1 ms of contact time; 20 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconia rotors with Kel-F caps. The pulse sequence was applied with a ^1H ramp to account for non-homogeneity of the Hartmann–Hahn condition at high spin rotor rates. For the interpretation of the ^{13}C -CPMAS-NMR spectra, the overall chemical shift range was divided into the following main resonance regions: alkyl-C (0–45 ppm); methoxyl-C and N-C (45–60 ppm); O-alkyl-C (60–110 ppm); aromatic-C (110–160 ppm); carboxyl- and carbonyl-C (160–200 ppm). For the dipolar dephasing experiment a suitable

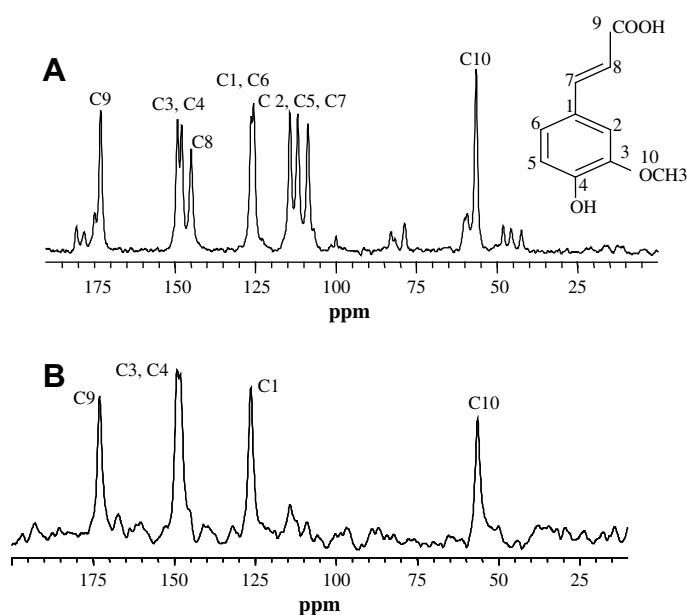


Fig. 1. CPMAS- (A) and dipolar- (B) dephasing- ^{13}C -NMR spectra of a ferulic acid standard.

delay time of 45 μs was based on a earlier experiment performed on ferulic acid standard (Fig. 1a,b)

2.4. Off-line pyrolysis TMAH-GC-MS

About 200 mg of HA were placed in a quartz boat and moistened with 1 ml of tetra-methyl ammonium hydroxide (TMAH 25% in methanol) solution. After drying the mixture under a gentle stream of nitrogen, the quartz boat was introduced into a Pyrex tubular reactor (50 \times 3.5 cm i.d.) and heated at 400 °C for 30 min (Barnstead Thermolyne 21100 furnace). The released gaseous products were continuously transferred by a helium flow (100 ml min^{-1}) into a series of two chloroform (50 ml) traps kept in ice/salt baths. The chloroform solutions were combined and concentrated by rotoevaporation. The residue was redissolved in 1 ml of chloroform and transferred in a glass vial for GC-MS analysis. The thermochemolysis was conducted in triplicate for each HA. The GC-MS analyses were conducted with a Perkin Elmer Autosystem XL by using a RTX-5MS WCOT capillary column, (Restek, 30 m \times 0.25 mm; film thickness, 0.25 μm) that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min isothermal), rate 7 °C min^{-1} to 320 °C (10 min isothermal). Helium was used as carrier gas at 1.90 ml min^{-1} , the injector temperature was at 250 °C, the split-injection mode had a 30 ml min^{-1} of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z , with a cycle time of 1 s. Compound identification was based on comparison of mass spectra with the NIST-library database, published spectra, and real standards.

For quantitative analysis, external calibration curves were built by mixing methyl-esters and/or methyl-ethers of the following standards: heptadecane, tridecanoic acid, cinnamic acid, octadecanol, 16-hydroxy hexadecanoic acid, docosandioic acid, and beta-stosterol. Increasing amount of standards mixture were placed in the quartz boat and moistened with 0.5 ml of TMAH (25% in methanol) solution. The same thermochemolysis conditions as for

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