



Bioactivity of humic substances and water extracts from compost made by ligno-cellulose wastes from biorefinery

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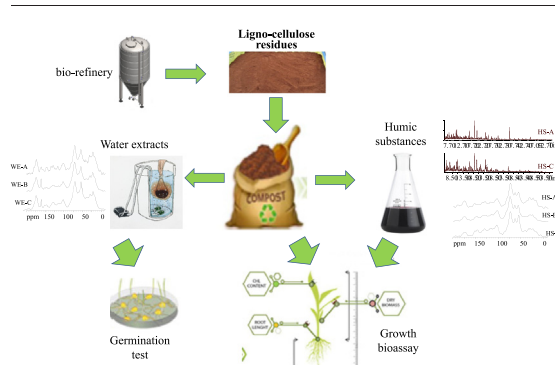
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HIGHLIGHTS

- Ligno-cellulose residues from biorefinery were used in an on-farm composting system.
- Bulk composts and compost extracts were characterized by CPMAS-NMR and TAHM-GC-MS.
- Compost and derived extracts showed a selective incorporation of phenolic derivatives.
- Water extracts and humic substances revealed bioactive properties in cropping tests.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 11 June 2018

Received in revised form 23 July 2018

Accepted 24 July 2018

Available online 25 July 2018

Editor: Shuzhen Zhang

Keywords:

Ligno-cellulose residues
On-farm composting
Molecular characterization
Humic substances
Water extracts
Maize bio-stimulation

ABSTRACT

The ligno-cellulose residues from biorefinery production of bio-ethanol were used as woody structuring material within an on-farm composting system, with the aim to obtain bioactive water soluble and humic fractions from composted materials. The molecular characterization of initial biomasses and final products revealed a transformation towards more stable compounds during composting and showed the selective incorporation of specific phenolic derivatives of ligno-cellulose in both bulk samples and corresponding extracts. While the use of the stable bulk composts as organic fertilizer resulted in a decrease of nitrogen and phosphorous assimilation in maize tissues, a bio-stimulation was shown by water soluble organic compounds and humic substances in germination tests and pot experiments, respectively. The differential responses obtained in maize seedlings and plants were related to the molecular composition and concentration of the applied water extracts and humic substances, thus suggesting a role of phenols and lignin derivatives in the stimulation of maize roots and shoots and the enhancement of P uptake. The results confirm that ligno-cellulose residues may be effectively recycled as composting additives in order to enrich mature compost in aromatic and lignin compounds. A preliminary knowledge on the molecular characteristics and biological properties of composted materials is an essential requirement to select the most suitable derivatives from composted ligno-cellulose wastes in sustainable agricultural practices.

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1. Introduction

The negative environmental impact of fossil fuels consumption has progressively promoted attention on the development of renewable

energy sources. A range of natural materials and biomasses, such as crops, agro-industrial residues and organic wastes, have been used to obtain different bio-energy products (Guo et al., 2014). Bio-ethanol is the current substitute of gasoline that is obtained from the fermentation of plant sugars (Songstad et al., 2009). Earlier feedstuff for bio-ethanol production were food crops, like sugar cane, sugar beet, sorghum, maize, barley, potato and cassava (Haberl et al., 2013). Later, the so-called second-generation bio-ethanol was produced by non-food plants, such as switchgrass, miscanthus, giant cane, energy cane, napier grass, grain sorghum and shrub willow (Cherubini, 2010). However, a large amount of ligno-cellulose residues is left over from the bio-ethanol production. This waste is commonly burnt to obtain thermal energy, thus losing a valuable biologically fixed organic carbon.

An alternative exploitation of the photosynthate carbon in ligno-cellulose residues would be their use as a structuring fraction in the composting process. The recycle of biorefineries wastes in compost is in line with EU provisions, which recommend to employ biomasses waste and by-products, especially woody residues, in the productive chain of a circular economy (European Commission, 2017). Composting is an effective bio-technology that transforms biomasses through microbial activities under controlled, aerobic conditions with an initial thermophilic active stage followed by a mesophilic curing phase associated with the final stabilization of organic components (Spaccini et al., 2016; Pergola et al., 2017). A mature compost is rich of highly humified matter, and, when amended to soil, may profitably contribute to reintegrate the soil organic carbon lost during crop production, and improve the overall soil quality (Cozzolino et al., 2016; Scotti et al., 2016). Recently, it has been pointed out that organic extracts from mature composts may behave as rhizospheric bio-effectors for the stimulation of biochemical and physiological activities of crops (Canellas and Olivares, 2014; Monda et al., 2017). Concomitantly, lignin derivatives directly isolated from ligno-cellulose residues of biorefineries were also shown to stimulate development of both crops aerial and root tissues (Savy et al., 2016).

In this study we composted different amounts of ligno-cellulose residues from biorefinery production of bio-ethanol together with cow manure and maize straw, with the aim to isolate water extracts (WE) and humic substances (HS) from the composted biomasses. The different composts and their WE and HS fractions were first characterized for their molecular composition, and, then, tested for their stimulating capacity towards maize growth

2. Materials and methods

2.1. Materials and composting

The ligno-cellulose residues were provided by Mossi & Ghisolfi (Alessandria, IT). These were obtained by applying on giant reed (harvested in January 2008 from a field near Torino, IT) the PROESA hydrolyzing technology, that releases cellulose through a microbial enzyme treatment (<http://www.biochemtex.com/proesaeng.html>). After the enzyme hydrolysis, the ligno-cellulose residue was separated from the hydrolysate, oven-dried at 333 K for 5 days, and ground with a blender.

Compost based on ligno-cellulose residues was produced in the composting facility at the Experimental Farm of the University of Napoli Federico II, located in Castel-Volturno (CE). Composting was based on a 4 × 6 m static pile with an air insufflation system composed by three perforated rubber tubes of 10 meter length placed at the bottom of the compost pile and fed by a rotative compressor pump. The composting pile consisted in cow manure (70% w/w) added with maize straw and poplar trimming as structuring woody materials (30% w/w). This mixture was uniformly spread by a manure-spreader machine over the insufflation rubber tubes. The same bulk compost mixture was mixed with different amounts of ligno-cellulose residues in litter bags which were placed inside the composting pile. Triplicates of litter bags contained the following composition: A) control bulk

compost without ligno-cellulose residues; B) bulk compost added with 10% (w/w) of ligno-cellulose residues; C) bulk compost added with 20% (w/w) of ligno-cellulose residues. In order to keep a constant composting ratio in the litter bags between manure and the structuring fraction, the original bulk woody material was reduced by 10 and 20% in litter bags B and C, respectively. Each litter bag had a starting fresh weight of 5 kg. The ligno-cellulose residues were crumbled into fragments lower than 2 cm before insertion into the litter bags, which were made of polyester nets with a 1 mm mesh size, currently used in forest litter studies and in the biodegradation tests in composting processes (Spaccini et al., 2016). The litter bags are conceived to isolate the included organic fragments, avoiding both losses and/or unwanted inputs as well as limited or unappropriated fluid exchanges, thereby allowing a suitable moistening and oxygenation rates to support an adequate microbial activity, comparable with the external environmental conditions. The bags were evenly distributed with respect to the insufflation tubes, and finally covered with the bulk composting bulk mixture to obtain a final pile of approximately 1.5 m high.

The composting process lasted 100 days, with a periodic monitoring of temperature (5 min interval) and percent oxygen (insufflations flush every 60 min interval) inside the pile. During the first 50 days the minimum percentage of oxygen insufflated by the air compressor was set at 10%, thereafter at 5%. At completion of the composting process, the litter bags were weighed and representative compost samples were collected from each bag for analyses, extractions, and pot experiments. The properties of the bulk compost and the composts obtained in litter bags with ligno-cellulose residues are shown in Table 1.

2.2. Water extracts (WE) and humic substances (HS) from composts

Water-soluble extracts (WE) were isolated from different composts as it follows: 50 g of air dried compost was suspended in 500 mL of de-ionized water in polypropylene containers and shaken for 24 h in a rotary shaker (Monda et al., 2017). The suspension was centrifuged at 2500 rpm for 10 min and the supernatant filtered through a Wathman 41 filter (Vaccaro et al., 2009). The filtrate was then freeze-dried and stored at 4 °C. The elemental composition, electric conductivity and pH of WE are shown in Table S1 in Supporting materials.

Humic substances (HS) were obtained from each bulk compost as it follows: 200 g of air dried compost samples were suspended in 1 L of 1 M NaOH solution in polypropylene containers and shaken overnight in a rotary shaker. The supernatant containing the humic compounds was separated by centrifugation for 20 min at 7000 rpm, filtered through a Wathman 41 filter, brought to neutral pH using 1 M HCl, dialyzed until Cl-free against distilled water, and freeze dried.

2.3. ¹³C-CPMAS-NMR

The solid-state NMR spectra of compost samples, humic substances and water extracts were recorded on a Bruker AV-300 equipped with a 4 mm wide-bore MAS probe, with the following acquisition parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; ¹H-power for CP 92.16 W; ¹H 90° pulse 2.85 μs; ¹³C power for CP 150.4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross-polarization pulse sequence was applied with a composite shaped “ramp” pulse on the ¹H

Table 1

N and C (%), C/N ratio, and P (mg g⁻¹) content in bulk compost and litter bag composts^a (standard deviation in brackets).

Sample	N	C	C/N	P
A	2.1 (0.13)	30.8 (3.58)	14.9	5.41 (0.01)
B	1.6 (0.12)	33.2 (1.97)	21.3	5.10 (0.02)
C	1.5 (0.23)	35.9 (1.94)	23.7	4.84 (0.04)

^a A = bulk manure/straw mixture, B = as A but with 10% of ligno-cellulose residues, C = as A but with 20% of ligno-cellulose residues.

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