



Biotransformation of organic matter during composting of solid wastes from traditional tanneries by thermochemolysis coupled with gas chromatography and mass spectrometry



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ABSTRACT

In the present paper, Thermochemolysis (or Thermally Assisted Hydrolysis/Methylation) is used as a way of following the biotransformation of organic matter during composting of solid wastes from traditional tanneries. The qualitative analysis of the pyrograms allowed us to discriminate between four main families of compounds: aromatic compounds, lipids, polysaccharides and nitrogen-containing substances. The quantitative data revealed some variations in the chemical composition of the macromolecular content.

Lipids are the most transformed fraction during the composting process. Animal and plant fatty acids showed a sensible decrease. Fungal fatty acids mainly increased during the thermophilic phase whereas bacterial fatty acids increased when the growth of fungi is repressed. During composting of solid wastes from traditional tanneries an adapted microbial community has been developed. Various lignin-derived units during composting showed a strong increase in *p*-hydroxyphenyl type-compounds in comparison with the other aromatic structures due to high decomposition of grass cuttings. Tannin derived compounds also increased throughout the composting. Lignin and condensed tannins polymers are partially degraded and stabilized by covalent linkage to more complex organic molecules such as humic substances.

Nitrogen containing compounds increased during composting as a consequence of the high rate of degradation of the other fractions and/or their combination with polyphenolic structure leading to the formation of humic substances. In our study a correlation was found between NTK and nitrogen containing substances.

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1. Introduction

Composting consists of two narrowly linked processes, degradation and humification: a part of compost organic matter is aerobically mineralized by microorganisms, whereas the partially decomposed or undegraded part leads to the formation of humic substances. The final product, compost, is a soil amendment (Ros et al., 2006; López-González et al., 2014), an efficient tool for bioremediation processes (d'Errico et al., 2013; López-González et al., 2014) or soil restoration (Tejada et al., 2009; López-González et al., 2014) and has capability for improving soil's physical, chemical and

biological properties in particular by increasing available nutrients mainly in the organic soil fractions (Larchevêque et al., 2005; Cellier et al., 2012).

Composting is a dynamic process where a wide variety of microbial populations, including bacteria, *Actinobacteria* and fungi play key roles in the biotransformation of organic substrates (Moreno et al., 2013; López-González et al., 2015).

Stabilization of organic matter during composting reflects a good progress of the process and an increasing stability of organic matter has been often used to define compost maturity while stabilized organic matter characterizes mature composts (Becker and Kotter, 1995; Francou et al., 2005; Francou et al., 2008).

Furthermore, the quality of compost for agronomical use depends on the degree of organic matter stabilization (Francou et al., 2008).

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Advanced physico-chemical techniques such as Fourier transform infrared (FTIR), Fluorescence, and Nuclear Magnetic Resonance (^{13}C NMR) spectroscopies and thermal analyses, such as Differential Scanning Calorimetry (DSC), have been used to characterize organic matter of composts (Fuentes et al., 2007; Droussi et al., 2009). Thermochemolysis has proved to be a good method to monitor organic matter evolution (Som et al., 2009). It provides high sensitivity using minimal amounts of sample and has been successfully applied to the characterization of different materials (Peris-Vicent et al., 2009; Kaal and Janssen, 2008; Gomes Reisa et al., 2011).

Thermochemolysis is a thermally assisted derivatization of polar groups (Kaal and Janssen, 2008; Gomes Reisa et al., 2011; Challinor, 2001) using a methyl donor reactant such as Tetramethylammonium hydroxide (TMAH) (Robb and Westbrook, 1963).

This technique allows the breakdown of organic compounds which are subsequently isolated and identified (Bracewell et al., 1980), possibly quantified, and related to the original moieties present in the structure of the organic macromolecular material (Jarde et al., 2003). Thus, thermochemolysis–GC/MS gives structural information and an organic fingerprint of the organic macromolecular material, enabling us to compare the samples to other ones (Jarde et al., 2003).

Thermochemolysis avoids decarboxylation since it produces methyl esters from carboxylic acids and methyl ethers from hydroxyl groups, thus rendering many of the polar products volatile enough for GC analysis (Chefetz et al., 2002).

The aim of the present study is to provide a detailed characterization of the organic matter evolution using thermochemolysis–GC/MS during composting of solid wastes from traditional tanneries.

2. Material and methods

2.1. Composting

Solid wastes were collected from a traditional unit located in the old town of Marrakech while grass cutting wastes were collected from the urban commune of Marrakech. A windrow constituted with 50% tannery wastes and 50% grass cuttings was composted during six months.

The mixture was turned every 15 days to aerate and homogenize the substrate. The biological activity was monitored by measuring daily the temperature during composting. A composite sample was collected at different spots of the windrow at the beginning of composting and after 1, 3, 6, months. The composite samples were homogenized and a representative samples was taken of the composting substrate by the quartage method (AFNOR, 1999).

2.2. Pyrolysis–gas chromatography–mass spectrometry

About 2 mg of freeze-dried composite samples were dissolved in chloroform and mixed with 10 μl of a methanol solution containing 50% (w/w) of tetramethyl ammonium hydroxide (TMAH). The samples were then heated for 5 min to 40 $^{\circ}\text{C}$ to evaporate the methanol. The remaining solid was transferred to a 25 \times 1.9 mm (i.d.) quartz tube filled with quartz wool. The tube was then heated from 350 to 650 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C ms}^{-1}$ and maintained at 650 $^{\circ}\text{C}$ for 10 s. The thermochemolysis products were carried into the GC or the GC–MS by a flow of He gas. GC separations were done on a Varian 3900 gas chromatograph using a BPX (SGE) capillary column (30 m long, 0.25 mm i.d., 0.25 μm phase thickness). Column temperature was programmed from 60 to 300 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C min}^{-1}$ and held at 300 $^{\circ}\text{C}$ for 30 min. GC–MS analysis was performed in similar conditions on a Thermo Fisher Focus GC chromatograph coupled with a DSQ II

mass spectrometer. The compounds were identified on the basis of their GC retention times and by comparison of their mass spectra with standards and data from the literature.

3. Results and discussion

The chromatograms of Thermochemolysis coupled with gas chromatography and mass spectrometry (TMAH–GC–MS) of raw solid wastes from traditional tanneries and at different stages of composting (0, 1, 3 and 6 months) are illustrated in Fig. 1. Qualitative analysis revealed numerous individual compounds belonging to the compound classes of aromatic compounds, lipids, polysaccharides and nitrogen-containing substances and each class of the main compounds identified has been studied in detail.

The relative abundance P_i of each pyrolysis product from raw solid wastes from traditional tanneries and at different stages of composting (0, 1, 3 and 6 months) is calculated as the ratio between the area of the corresponding peak to the sum of areas of all the peaks considered in the programs (Ayuso et al., 1996). $P_i = \frac{a_i}{\sum_i a_i}$

a_i is the area of each peak in the pyrogram (Table 1).

3.1. Aromatic compounds: (Table 2)

Lignin, the second most abundant renewable resource on the earth, is difficult to degrade (Huang et al., 2006; Pérez et al., 2002). It limits enzyme activity or reduces the number of enzymes able to act on these polymers (Hall et al., 2010; Ruiz-Dueñas and Martínez, 2009). Noteworthy, the *p*-hydroxyphenyl constituents of lignin were found to be dominant compounds (Table 3) with a progressive increase during composting. A great part of the *p*-hydroxyphenyl would be subjected to grass cutting lignin. The guaiacyl type compounds increased during the first month of composting decreased in the intermediate step of composting and then increased at the end of the process. Nevertheless, the syringyl units significantly decreased during the process. Their decrease may be due to decomposition by microorganisms. The lignin derived compounds identified in this study showed a highest content of alkyl and methoxy phenol. Saiz-Jimenez et al. (1989) attributed the greater part of poly-methoxy-phenol and hence lignin residues in the pyrolysis product of humic acids to the incomplete degradation or selective preservation of grass lignin during earthworm composting. Temperature of thermochimolysis is also problematic: high temperature results in excessive thermal fragmentation and favors excessive decomposition and unwanted reactions rather than a selective base-catalyzed hydrolysis and methylation (Shadkani et al., 2009). Depolymerisation of lignin by microorganisms is occurring during composting. Veeken et al. (2000) showed that during composting, there was an increase of aromatic compounds mainly methoxy-phenols with respect to other compounds suggesting an increased solubility of lignin.

Actinomycetes and bacteria attacked the lignin molecule mainly by removal of methoxyl groups and breakdown of C_{α} – C_{β} linkage, whereas some of fungi could completely degrade lignin to carbon dioxide and water (Tuomela et al., 2000; Huang et al., 2010). López-González et al. (2015) reported that ligninolysis bacteria were poorly represented among the bacteria studied at the bio-oxidative stage and were more numerous at the end of the process during composting of ligninocellulose wastes. At the biooxidative phase of composting, fungi activities were associated to lignin degradation (Malherbe and Cloete, 2002; Mohammad et al., 2012). During composting, organic molecules, including lignin are degraded, mineralized or used as building blocks for humic substance synthesis (Smidt et al., 2008). As previously described by Ziechmann (1994), lignin moieties are known to be a suitable provider of phenolic compounds needed as starting material for humification processes.

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