

# Solid $^{13}\text{C}$ -nmr as fundamental tool in the characterization of organic matter during the composting

Martínez-Sabater, E.<sup>a</sup>, Moral, R.<sup>b</sup>, Bustamante, M.A.<sup>b</sup>, Marhuenda-Egea, F.C.<sup>a</sup>, Paredes, C.<sup>b</sup>, Lorenzo, E.<sup>c</sup>, Jordá, J.<sup>a</sup>

<sup>a</sup>Department of Agrochemistry and Biochemistry, Alicante University, Spain.

<sup>b</sup>Department of Agrochemistry and Environment, Miguel Hernandez University, EPS-Orihuela, Ctra Beniel Km 3.2, 03312-Orihuela (Alicante), Spain.

<sup>c</sup>Research Technical Services, Alicante University, Spain.

Phone: 965903525 Fax: 965903880 E-mail: aul.moral@umh.es

## Abstract

Different spectroscopy methods were used to monitor the composting process, evaluate the degradation rate and thus, determine the maturity of the composts obtained. Methods to assess compost maturity are needed in order to assure optimal benefits with the application of composted materials to lands. The aim of the present research is to evaluate the maturity degree reached by different compost elaborated with rabbit and goat manure by means of carbon-13 nuclear magnetic resonance ( $^{13}\text{C}$ -NMR) coupled with cross-polarization magic-angle spinning (CPMAS). The technique of nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning ( $^{13}\text{C}$  CPMAS NMR) is suitable for characterizing directly the main organic composition of complex, insoluble samples as dry powders (Cook, 2004; Deur, 2002).

In this study, changes in the organic matter were produced, as it was shown in an increase in the aliphatic C and carboxyl groups, a decrease in the phenolic groups and total aromaticity, as well as a decline in the polysaccharide levels. From the obtained results, it may be concluded that  $^{13}\text{C}$  CPMAS NMR could be used to improve the characterization of organic matter changes during the composting process (Inbar, 1992).

*Keywords: compost,  $^{13}\text{C}$ -NMR, humification, aromaticity*

## Introduction

Composting is becoming an environmentally friendly and economical alternative method for treating solid organic waste, as winery and distillery residues (Marhuenda-Egea et al., 2007). The composition of the organic matter in soils or waste materials is very complex due to the wide range of chemical compounds and the variety of decomposed and synthesized products. Maturity compost consists of stable organic matter, water, minerals, and ash. Consequently the composting process directly involves the changes of the chemical composition and physico-chemical parameters of the composting mixture, and can be controlled by spectral methods (Smidt et al., 2005).

Solid-state  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS NMR) allows one to obtain information directly and non-destructively on the carbon components of the entire sample without any chemical or physical fractionation, and is well suited to the characterization of natural organic matter (Preston, 1996; Cook, 2004). This technique can be used to detect and even quantify the amounts of carbohydrates, alkyl, aromatic or carboxyl carbons for diverse solid samples (Almendros et al., 1991; Inbar et al., 1992). These studies suggest the accumulation of relatively recalcitrant aliphatic polyesters, such as cutins, suberins and other little known carbohydrate-polyalkyl macromolecules in higher plants (Nip et al., 1987).

The objective of the present research was to carefully examine the organic matter transformation during the composting process of fresh winery and distillery residues (WDR), with the aim of establishing some analytical parameters suitable for estimating

the degree of compost maturity and stability. The progress of the composting process was evaluated by solid-state  $^{13}\text{C}$  NMR spectroscopy.

## Materials and methods

### Composting procedure

Three different piles (P1, P2 and P3) were elaborated using WDR (grape marc (GM) and exhausted grape marc (EGM)), and two organic wastes (cattle manure (CM) and poultry manure (PM)), as nitrogen source. The mixtures were prepared in the following proportions, on a fresh weight basis (dry weight basis in brackets):

- Pile 1: 70 % (76) EGM + 30 % (24) CM
- Pile 2: 70 % (72) GM + 30 % (28) CM
- Pile 3: 70 % (71) EGM + 30 % (29) PM

The mixtures (about 140 kg each) were composted, using natural aeration, in domestic thermo-composter (85 cm high with a 70 x 70 cm base and a 350 L volume). Compost piles were turned over, when the temperature decreased, to improve both homogeneity and the composting process. The bio-oxidative phase of composting was considered finished when the temperature of the pile was stable and near to that of the surrounding atmosphere. Then, the piles were allowed to mature for two extra months. The moisture of the piles was maintained above 40% by adding the appropriate amount of water. Samples were collected every week throughout the composting process, by mixing subsamples coming from seven different zones of the piles.

### Sample preparation and solid-state $^{13}\text{C}$ NMR spectroscopy

Samples were air-dried, ground in an agate mill, then sieved through a 0.125 mm mesh, and milled again with an agate mortar. Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance (CPMAS  $^{13}\text{C}$ -NMR) experiments were performed on a BRUKER AVANCE DRX500 operating at 125.75 MHz for  $^{13}\text{C}$  (Smernik et al., 2002).

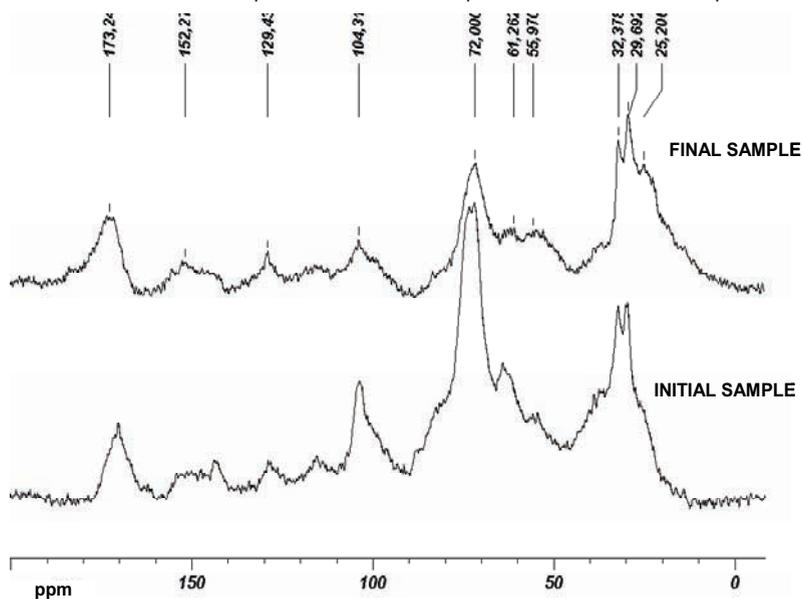
## Results and discussion

The CP-MAS  $^{13}\text{C}$ -NMR spectra show several main peaks in the initial samples: 173, 153, 145, 130, 118, 105, 72, 63, 57, 33 and 30 ppm (Figure 1). A shoulder appears around 25 ppm that could be assigned to acetyl methyl groups in hemicellulose. Peaks at 33 and 30 ppm correspond to chemical shifts that could be assigned to methylene carbons in alkyl chains (from suberin or suberin) and polypeptides and to  $\text{CH}_3$  groups of acetyl groups attached to aliphatic structures (Vane et al., 2006).

The peak that appears around 56 ppm is traditionally assigned to  $\text{O-CH}_3$  groups in lignin (phenolmethoxyl) of coniferyl and sinapyl moieties) and in hemicellulose (glucuronic acid in xylan) (Gómez et al., 2007) and to  $\text{C}_\alpha$  carbons of polypeptides. Signals around 72 to 74 ppm are due to C2, C3, and C5 of cellulose and hemicellulose (secondary carbinol carbons). Peaks at 83 and 88 ppm are due to noncrystalline and crystalline components of C4 and the peak at centred around 105 ppm to anomeric C1 of glucose fragment in a carbohydrate. Signals derived from hemicellulose are contained within the cellulose peaks. This chemical shift has also been assigned to different carbons of lignin-type moieties: The C2 carbons of both guaiacyl and syringyl lignin structures and the C6 carbon of syringyl units. This peak is also attributed to quaternary aromatic carbons in tannins. The region of the spectrum between 110 ppm and 170 ppm can be divided in two sub-regions: the first region between 110-140 ppm is attributed to unsubstituted aromatic C and C-substituted

aromatic carbons (Almendros et al., 1991). The second region between 140-160 ppm is assigned to aromatic carbons linked to O or N. The first region exhibits well-defined peaks at 115 and 130 ppm (Inbar, 1992). The peak at about 115 ppm is assigned to aromatic C that are *ortho* or *para* to oxygen-substituted aromatic C from lignin or lignin-derived products (Inbar, 1992). The peak that appears around 130 ppm is characteristic of unsubstituted aromatic C, including C1 quaternary carbons of syringyl and guaiacyl lignin units and the C6 carbon of guaiacyl. The second sub-region between 140-160 ppm shows peaks that are traditionally assigned to lignin or tannins. The peak centered at 145 ppm is attributed to methoxy-substituted or hydroxy-substituted phenyl C (Inbar, 1992). The peak at 155 ppm is assigned to oxygen-substituted aromatic C, including both C-OCH<sub>3</sub> and C-OH groups. This peak could also be assigned to C4 carbons of guaiacyl units involved in ether linkages to C<sub>β</sub> of adjacent lignin units. The peak centered at 172 ppm in the <sup>13</sup>C CP-MAS spectrum is assigned to carbonyl/carboxyl carbons of ester and amides groups (Inbar, 1992).

Figure 1. Solid-state <sup>13</sup>C NMR spectra of the initial sample and the final of the compost for the Pile 1



<sup>13</sup>C NMR spectroscopy displayed a preferential biodegradation of carbohydrates as well as an accumulation of aliphatic chains (cutin- and protein-like) (Figure 1). On the basis of this study it may be concluded that CPMAS <sup>13</sup>C-NMR could provide information significantly correlated to conventional chemical parameters of compost maturity.

## References

- Almendros, G., R. Fründ, F. J. Gonzalez-Vila, K. M. Haider, H. Knicker and H. - D. Lüdemann (1991). Analysis of <sup>13</sup>C and <sup>15</sup>N CPMAS NMR-spectra of soil organic matter and composts. *FEBS Letters*, 282, 119-121.
- Cook R.L. (2004). Coupling NMR to NOM. *Analytical and Bioanalytical Chemistry*, 378, 1484-1503.
- Gómez, X., Miguel C. Diaz, Mick Cooper, D. Blanco, A. Morán and Colin E. Snape. Study of biological stabilization processes of cattle and poultry manure by thermogravimetric analysis and <sup>13</sup>C NMR (2007). *Chemosphere*, 68, 1889-1897.
- Inbar, Y., Y. Hadar and Y. Chen (1992). Characterization of humic substances formed during the composting of solid wastes from wineries. *The Science of The Total Environment*, 113, 35-48.

- Marhuenda-Egea, F.C., E. Martínez-Sabater, J. Jordá, R. Moral, M.A. Bustamante, C. Paredes and M.D. Pérez-Murcia (2007). Dissolved organic matter fractions formed during composting of winery and distillery residues: Evaluation of the process by fluorescence excitation–emission matrix. *Chemosphere*, 68, 301-309.
- Nip, M., J. W. De Leeuw, P. J. Holloway, J. P. T. Jensen, J. C. M. Sprenkels, M. De Pooter and J. J. M. Sleenckx (1987). Comparison of flash pyrolysis, differential scanning calorimetry, <sup>13</sup>C NMR and IR spectr. Protonation on <sup>13</sup>C CPMAS NMR Quantitation of Charred and Uncharred Wood Solid State Nuclear Magnetic Resonance, 22, 71-82.
- Smidt, E., K. Eckhardt, P. Lechner, H. Schulten and P. Leinweber, Characterization of different decomposition stages of biowaste using FT-IR spectroscopy and pyrolysis-field ionization mass spectrometry (2005). *Biodegradation*, 16, 67-79.
- Vane, C.H., Trevor C. Drage and Colin E. Snape (2005). Bark decay by the white-rot fungus *Lentinula edodes*: Polysaccharide loss, lignin resistance and the unmasking of suberin. *International Biodeterioration & Biodegradation*, 57, 14-23.