Molecular characterization of digestates from Solid-State Anaerobic Digestion of pig slurry and straw using analytical pyrolysis.

Ornella Cavallo¹, José María de la Rosa^{2*}, José Antonio González-Pérez², Heike Knicker², Daniela Pezzolla³, Giovanni Gigliotti³, Maria Rosaria Provenzano¹

¹ *Department of Soil, Plant and Food Sciences, University of Bari “Aldo Moro”, Via G. Amendola 165/A, 70126 Bari, Italy*

² *Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Av. Reina Mercedes, 10, 41012 Seville, Spain*

³ *Department of Civil and Environmental Engineering, University of Perugia, Via G. Duranti 93, 06125 Perugia, Italy*

*Corresponding author: jmrosa@irnase.csic.es

HIGHLIGHTS

- Molecular composition of solid-state anaerobic digestates of pig slurry and straw studied by Py-GC/MS
- Highest OM loss found when using 2 and 4 percolate recirculation per day
- Greater polysaccharides consumption found when increasing recirculation frequency
- Enrichment in Lig-compounds found when increasing recirculation frequency
- Positive effect of percolate recirculation on OM degradation and stabilization

Abstract

Straw and pig slurry Solid-State Anaerobic Digestion (SS-AD) was carried out in a pilot-scale apparatus using percolate recirculation technology. The digestion experiments were performed using 1, 2 and 4 recirculations per day; an additional experiment without percolate recirculation was used as control. The initial mixture and the digestates were analysed by means of chemical analyses and Pyrolysis–Gas Chromatography/Mass Spectrometry (Py-GC/MS), a direct analytical technique that allows investigating the changes in the organic matter (OM) composition of digestates and the effect of percolate recirculation frequency. Chemical analyses suggested a positive effect of percolate recirculation on OM degradation. The highest values of OM loss were found with 2 (26%) and 4 (31%) recirculation cycles per day, that also corresponded with the lowest values of the hydrophilic water extractable organic matter fraction (5.5 and 6.3% respectively). Py-GC/MS showed that the anaerobic digestion proceeded with progressive polysaccharide degradation (from c. 19% in the initial mixture to 10-8% with 2-4 recirculation cycles) and selective enrichment of lignin derived compounds (from c. 58% in the initial mixture to 67-69% with 2-4 recirculation cycles). In addition, a shift in the fatty acids distribution was observed with a decrease in the long/short chain ratio of fatty acid methyl esters. These results indicate that under our experimental conditions, percolate recirculation had a positive effect on the OM degradation. Also OM stabilization is observed with relative increases in recalcitrant lignin at the expense of the more liable polysaccharide fraction. This paper represents the first attempt to apply Py-GC/MS to evaluate the OM quality in digestates obtained by SS-AD of pig slurry and straw optimized by percolate recirculation.

Keywords: solid-state anaerobic digestion; pig slurry; digestate; organic matter stabilization; pyrolysis-GC/MS.

1. INTRODUCTION

Anaerobic digestion (AD) is used in several European countries to treat more than 10% of the organic waste produced [1]. The final products of AD include biogas (*ca.* 65% CH₄, 35% CO₂) and an organic residue called digestate. In the near future, biogas from organic materials (*i.e.* animal manure, food waste, crop silages, etc.) is expected to account for at least 25% of the total bioenergy [2].

Among the different AD technologies, the most widespread operates with materials with dry matter (DM) or total solids (TS) content <10% (wet AD). However, wet AD is not suitable to treat some wastes, such as lignocellulosic and green wastes with a solid bulk structure [3] where solid-state anaerobic digestion (SS-AD) represents a good alternative. In fact, SS-AD is used to treat different materials with TS contents >25% that retain their shape when disposed in an open pile [4]. The SS-AD process is considered advantageous over wet AD for a number of reasons including smaller reactor volume, minimal material handling and lower energy requirements for heating along with biogas production similar to that obtained by wet AD. Moreover, SS-AD produces a final digestate easily manageable due to its low moisture content [5]. This technology currently amounts to *approx.* 54% of the total installed AD capacity in Europe, and the number of these systems has been increasing since 2005 [6].

Although SS-AD can be seen as an efficient alternative to wet AD, some drawbacks have been also found associated to the technique. For example, the use of high organic loadings, as in most of the SS-AD processes, may cause inhibition phenomena due to the accumulation of short-chain volatile fatty acids (VFAs), which results in a decrease of pH and biogas production. It is well known that, during the first step of AD, proteins, lipids and carbohydrates are transformed into amino acids, long-chain fatty acids, and sugars, respectively. All these compounds are then converted by

fermentative bacteria into a mixture of short chain VFAs that are successively degraded by acetogenic bacteria to acetate, carbon dioxide, and/or hydrogen with the final step being methanogenesis. Inhibition by VFAs can be overcome by spreading the liquid fraction of the digestate, *i.e.* the percolate, on the material to be treated (approximately 10% by weight of treated waste) [4 and ref. therein].

In a preliminary study, with the aim to optimize SS-AD of straw and pig slurry by percolate recirculation, Pezzolla et al. [7] investigated the effect of recirculation frequency on biogas production, quality of the liquid fraction (*i.e.* percolate) and final digestate. They observed an increase in biogas production when using frequencies of 2 and 4 recirculations per day, especially during the first 10 days of the AD process. In fact, the increase in the frequency of percolate recirculation promoted hydrolysis in the solid fraction with the subsequent release of readily available organic compounds into the liquid fraction. These compounds were successively degraded in the percolate causing an increase in biogas production especially when the recirculation was performed 4 times per day. In addition, this accelerated degradation led to a faster mineralization of proteins in the solid fraction producing a buffer system in the percolate. The authors concluded that percolate recirculation avoids the inhibitory effects during the SS-AD, although they also indicated the need of further investigations in order to evaluate the chemical and spectroscopic characteristics mainly from the solid fraction of digestates [7].

Therefore, the aim of the present study was to investigate the effect of percolate recirculation at different frequency on the organic matter (OM) composition in the above-mentioned digestates by means of chemical analyses and analytical pyrolysis (Py–GC/MS).

The Py–GC/MS technique is widely used for the direct study of materials, which are difficult to analyze by conventional methods, one of the main advantages of this technique is the use of a small

amount of sample with no prior treatment or extraction needed. Furthermore, analytical pyrolysis (Py-GC/MS) can be used as a semi-quantitative technique by calculating the relative abundance of the different compounds as percentage of total chromatographic areas.

Consequently, Py-GC/MS represents a powerful tool for analyzing organic wastes of different nature and origin. In fact, it has been applied to the characterization of compost [8,9], digested sewage sludge [10], mulched soil [11], agricultural soil and its humic fractions [12,13] and more recently to the study of fermentation processes of lignocellulosic materials [14,15]. Only a few papers have reported previously the application of Py-GC/MS to study AD processes [16, 17]. Nevertheless, to the best of our knowledge, it has never been applied to study digestates obtained from straw and pig slurry, thus this study represents the first attempt to apply Py-GC/MS to evaluate the OM quality in digestates obtained by SS-AD.

2. MATERIALS AND METHODS

2.1. Experimental set-up

The initial mixture was composed of pig slurry and straw at a ratio of 3:1 (w/w). The three main constituents of straw are cellulose, hemicelluloses (carbohydrates) and lignin [14, 17]. The animal manure is a complex solid/liquid system that contains a mixture of raw fibre, cellulose, woody parts, plants residues in various degree of decomposition, mineral materials, excretions from the digestive system such as bacteria and products of their metabolism, nitrogen compounds from the metabolism of protein and non-protein substances as well as vitamins and hormones. In general, swine manures contain about 40% of fiber (including hemicellulose, cellulose, and lignin) whereas proteins amount at about ¼ of dry matter [18].

Inoculum, produced from a previous AD of the same substrates was added at the same amount of pig slurry to the initial mixture along with 2 L of demineralized water. Dry matter (%) of the initial

mixture was 20.7 ± 1.3 , volatile solids (VS; g/kg) were 877 ± 5.4 , total organic carbon (TOC; g/kg) value was 491.0 ± 5.7 and C/N 41.3 [7]. The experimental pilot-scale apparatus was described in detail in [7]. In short, this consists of 3 polyethylene reactors of 15 L capacity equipped with a percolate recycling system and a hydraulic gasometer for biogas production measurement (Fig. 1). Two different AD technologies were compared with and without percolate recirculation, the latter as a control. The effect of different frequency of recirculation was also investigated: once (every 24 h), twice (every 12 h) and four (every 6 h) times per day. The experiment was performed under mesophilic conditions (35 ± 2 °C) during 50 days and each recirculation lasted 45 min.

At the end of each digestion process, about 100 g of samples were retrieved from each polyethylene reactor. Aliquots of these samples were used to carry out the chemical analyses. The remaining samples were thoroughly mixed in order to obtain a composite sample that was successively dried and carefully grounded in a planetary ball mill (500 rpm) to have a representative sample for each experiment. The analyzed samples were the following: the initial mixture, the digestate samples 1SS-AD, 2SS-AD, 4SS-AD obtained with 1, 2 and 4 recirculations per day, respectively, and control from the AD without percolate recirculation.

2.2. General chemical analyses

Moisture content and total VS were determined by weight loss upon drying at 105 °C for 24 h and subsequently heated at 550 °C in a muffle oven for 24 h, respectively. The TOC content was measured by the Springer-Klee wet dichromate oxidation method [19]. The loss of OM in the final digestates was calculated according to the equation used by Gigliotti et al. [20] to describe OM loss during composting. To determine the water-extractable organic matter (WEOM), dried samples were extracted with deionized water (1:10 w/v) and filtered with 0.45 µm membrane filter. The hydrophilic (Hi) and hydrophobic (Ho) fractions of WEOM were obtained as described in Said-

Pullicino et al. [21] and the C content measured by using Pt-catalysed, high temperature combustion (800 °C) followed by infrared detection of CO₂ produced (MULTI N/C 2100/2100S, Analytikjena AG, Jena, Germany). The C content of the Ho fraction was obtained by difference between water extractable organic C (WEOC) and C concentration in the Hi fraction. Humic-like substances were obtained using the alkaline extraction as described in Ciavatta et al. [22] and recently used by Provenzano et al. [23] for digestates from sewage sludge and fruit and vegetable wastes. The alkaline extract was acidified to separate humic (HA) from fulvic acids (FA). The supernatant containing FA was purified on an insoluble polyvinylpyrrolidone column (Aldrich, Germany) and the eluate, containing the not-humified fraction (NH), was discarded. Total extractable carbon (TEC), concentration of the filtered alkaline extract, as well as that of NH fraction were measured as previously described for WEOC. The HA + FA C was obtained by difference between TEC and NH C. The degree of humification (DH) and the humification ratio (HR) were also calculated as described in Gigliotti et al. [24]. All chemical analyses were carried out in triplicate ($n=3$) and standard errors (SE) were calculated and reported.

2.3. Pyrolysis-Gas Chromatography/Mass Spectrometry

Py-GC/MS was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i) attached to a gas chromatograph (Agilent, model 6890N, Agilent, Santa Clara, CA). 1–2 mg from each composite sample were placed in small crucible capsules and introduced into a pre-heated furnace at 400 °C for 1 min. The evolved gases were then directly injected into the GC/MS for analysis. The GC was equipped with a low-polarity DB-5 (J&W Scientific) fused silica capillary column (30 m length, 0.25 µm inner diameter and 250 µm film thickness). The oven temperature was held at 50 °C for one minute and then increased up to 100 °C at 30 °C min⁻¹, from 100 to 300 °C at 10 °C min⁻¹ and was stabilized at 300 °C for 10 min. The carrier gas was helium at a controlled flow of 1 mL min⁻¹. The detector was a mass selective detector (Agilent, model

5973MSD, Agilent, Santa Clara, CA) and mass spectra were acquired with a 70 eV ionizing energy. The identification of individual compounds was achieved by single ion monitoring (SIM) for different homologous series, via low-resolution mass spectrometry and by comparison with stored data (NIST and Wiley libraries) and with previously published mass spectra [25, 26]. The pyrograms were then integrated and the relative abundance of each pyrolysis product calculated as a percentage (%) of the total chromatogram area. The initial mixture and 1SS-AD samples were pyrolyzed in triplicate and the total ion chromatograms compared (Supplementary Material. S2) to verify: i) the homogeneity of the samples and ii) the reproducibility of analytical pyrolysis. The peaks of each chromatogram were identified and integrated following the same routine described above. The deviation found between the integrated areas for each specific compound was always less than 0.2%.

3. RESULTS AND DISCUSSION

3.1. Chemical evaluation of OM during the SS-AD

The co-digestion of pig slurry and straw through the SS-AD led to a decrease in TOC in all tests (Table 1), particularly when percolate recirculation was performed 2 or 4 times per day, even resulting in the highest biogas production [7]. These findings were supported by the highest OM loss in 2 and 4SS-AD, indicating a positive effect of percolate recirculation on OM degradation. The analysis of WEOC revealed a depletion of the easily available fraction of OM in all final digestates, except for the control (4SS-AD < 2SS-AD $\approx$ 1SS-AD < initial mixture < control). The highest WEOC concentration in the control digestate was probably due to the increase of WEOM in the first stages of the AD, followed by an incomplete degradation of this fraction. The decrease of WEOM is commonly associated to the OM stabilization [21, 27, 28], suggesting in this case that the control digestate was least stabilized. The fractionation of WEOM may be useful to know the

composition of the soluble fraction extracted from the digestate. The Hi fraction, mostly characterized by carbohydrates, amino sugars and low-molecular-weight organic acids [29, 30], decreased during the SS-AD, in particular in 2 and 4SS-AD. The Ho fraction, including mainly polymeric structures [29, 30], showed a decrease in all digestates except in the control.

These results suggested that the observed WEOC trend could depend both on the mineralization of most labile soluble compounds and on the release of new complex organic substances due to the polymerization and condensation reactions that occur in the solution [21]. On the opposite, the highest concentration of WEOC in the control digestate might probably be attributable to the presence of polymeric materials derived from fresh straw, which account for the high Ho fraction.

The analysis of humic-like substances showed that the HA+FA content increased in all final digestates, suggesting that the recirculation did not affect the humification process in the final digestate altogether. However, the DH and HR parameters showed the highest values in 2 and 4 SS-AD samples (Table 1), probably due to the intensive mineralization of OM.

3.2. Pyrolysis–GC/MS

The total ion current (TIC) chromatograms of the pyrolysis products (Fig. 2) showed about one hundred compounds. The identified compounds were grouped into 7 families according to their chemical nature or known biogenic origin: aromatics (Ar-), lignin-derived compounds (Lig-), polysaccharide-derived compounds (Ps-), N-compounds (N-), *n*-alkane/*n*-alk-1-ene doublets (AKA) and alkanols (AKA), *n*-alkanoic acids (FA-) and sterols and terpenoids (ST-). Table 2 lists the identified compounds and their relative abundances (%).

For simplification, each TIC chromatogram can be analyzed by dividing it in three main domains [8]. Under our chromatographic conditions, the first domain (2-6 min elution time) was characterized by the presence of Ps-derived compounds and N-compounds; the second one (6-13

min) mostly by Lig-derived products, whereas in the third one (13-30 min) FA, sterols and terpenoids and other compounds derived from lipids were eluted.

In general, the pyrograms of all samples were in accordance with the expected composition of the straw-pig slurry mixture and dominated by Ps- and Lig- derived compounds that mainly eluted between min 2 and 13 (Fig. 2). The Ps-derived molecules comprised furanic compounds commonly ascribed to the Ps fraction of microorganisms [9] and cyclo-ketones and pyran molecules generated by the degradation of cellulosic materials [31]. The most abundant structures were the lactone 2-hydroxy-3-methyl-2-cyclopenten-1-one, furfural (2-furancarboxaldehyde), furfuryl alcohol (2-furanmethanol) cyclopentanedione (1,2-cyclopentanedione) (Table 2). The Lig-derived compounds were very abundant and diverse and are listed separately in Table 3 with an indication of their specific phenylpropanoid unit type: G for guaiacyl, S for syringyl and H for hydroxyphenyl. Lignin is a biopolymer constituted mainly by phenolic compounds of the G and S types, with different distribution in gymnosperm and angiosperm plants. Gymnosperms mainly contain lignin units of the G-type, while angiosperms contain G- and S-type units in similar proportions [32]. The most representative were vinylguaiacol (2-methoxy-4-vinylphenol), syringol (2,6-dimethoxy phenol), vinylphenol (4-vinylphenol) and guaiacol (2-methoxy phenol). The analysis of different lignin-type units associated to changes in their relative abundance can provide information about lignin degradation during the anaerobic fermentation process. Lignin of the G-type has a higher degree of cross-linking compared to that of the S-type, thus it is more resistant to biodegradation. For this reason, a progressive increase in the guaiacyl-to-syringyl (G/S) ratio has been reported during humification. This ratio is used as an indicator of the vegetation type and the degree of microbial reworking of lignins [33]. In this line, Dignac et al. [31] reported a progressive increase of the G/S ratio during composting of green waste. The G/S ratio calculated for the starting mixture and the different digestates studied in this work was found to be almost constant (c. 1.4). This result

591 together with the increase in the relative abundance of lignin compounds with successive percolate
592 recirculations (from 58% in the initial mixture to 64% in the control and 69% in 4SS-AD; Fig. 3;
593 Table 3) suggested that the AD process seemed not to degrade the lignin structures. This finding is
594 consistent with the differences between AD and the humification process, which is mainly aerobic.
595 Few other aromatic compounds like phenol were also identified in the pyrolysates but these are
596 ubiquitous pyrolysis products of unspecific origin [31]. In our samples, they mounted from 1 to 3%
597 (Table 2).
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600 When compared with the starting mixture, all digestate samples showed an evident decrease in Ps-
601 derived compounds in accordance with the first step of AD. Increasing percolate recirculation
602 frequency resulted in a major consumption of Ps-derived compounds following the order: 4SS-AD
603 (8%) < 2SS-AD (10%) < 1SS-AD (12%) < control (15%) < initial mixture (19%) (Fig. 3; Table 2).
604 These findings are consistent with the highest OM loss (%) in 2SS-AD and 4SS-AD, confirming the
605 positive effect of percolate recirculation on OM mineralization. Moreover, these samples showed
606 the lowest values of WEOC, particularly of the Hi fraction, pointing to that during the recirculation
607 process the easily available molecules from the solid fraction may had been solubilized in the
608 percolate and ultimately converted into biogas. These results may be also explained in part as a
609 consequence of the preferential polysaccharide degradation, confirmed by the increase in biogas
610 production, and the subsequent release into the liquid fraction of degradation products of higher
611 solubility when percolate recirculation was performed. In fact, Pezzolla et al. [7] reported a similar
612 result, in all tests with percolate recirculation the total reducing sugars (TRS) yields found in the
613 percolates were higher than those in the control until the end of the experiment. Furthermore, they
614 found increasing contents of TRS when increasing the recirculation frequency.
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Several N-compounds were detected both in the initial mixture and in digestates, the most abundant were pyridine derived from alanine, indole and methylindole mainly derived from tryptophan-containing peptides and proteins [34-37]. The relative abundance of N-compounds showed no significant variations among samples, ranging from 5% in the initial mixture and control, to 6-7% in SS-AD samples (Table 2).

Lipids-derived compounds represented c. 16% of the initial mixture TIC chromatogram area and included *n*-alkanoic acids, sterols and terpenoids and AKA (Table 2). Cholestan-3-ol, 24-ethyl-colestan-3- β -ol, γ - and β -sitosterol and Friedelan-3-one are the main identified sterols and terpenoids although the last triterpene was present only in the initial mixture. The relative abundance of sterols and terpenoids was homogeneous in all samples ranging from 5% (2SS-AD) to 3% for the rest of digestate samples.

Regarding the *n*-alkanoic acids, the short chain propanoic (C₃) was the most abundant. This is a VFA and it is not included in the total fatty acids account. Propanoic acid may derive from the starting material (swine manure), present as the end product of the fermentation of dietary fibers by anaerobic intestinal microbiota or formed in the course of the AD [38]. A clear reduction trend is observed with the increase of recirculation frequency for this VFA. This result may be related with the concomitant biogas increase as seen in [7], which may be caused by a combination of i) a mitigation of the known inhibition effect due to VFAs accumulation and ii) to a progressive VFAs degradation by acetogenic bacteria that precede methanogenesis.

Among the natural lipids, the ubiquitous tetradecanoic (C₁₄) and hexadecanoic (C₁₆) acids were found in abundance. No clear trend is observed in the total fatty acid content (excluding VFA C₃) ranging from 2.8% in the initial mixture to 2.0% in 2SS-AD. The distribution of the *n*-alkanoic acids and the methyl esters series were analyzed in detail by single ion monitoring (SIM), extracting

the diagnostic ions of m/z 73 for fatty acids (FAs) and m/z 74 for *n*-fatty acid methyl esters (FAMES). Extracted single ion chromatograms (SIC) of m/z 73 did not show a good resolution. However, it was possible to identify a medium-long chain FA series (C_{12} - C_{18}). SIC of m/z 74 revealed a FAMES pattern ranging from C_{13} to C_{30} with maximum at C_{14} and C_{16} for all samples (Figs. 4 and 5) and included saturated, unsaturated/polyunsaturated and also branched structures. FAMES may be produced by transesterification of fats with methanol, but are also produced by microorganisms [39]. The FAME complex series found here, mainly composed of the typical C_{14} - C_{16} long-chain fatty acids, are unlikely to be synthesized *de novo* through methylation and other ways points to a microbial origin and to pre-existing biogenic membrane esters.

The notable differences were evident between the FAME series of the initial mixture and the digestates (Fig. 5 and Supplementary Material S1). The ratio L/S for FAMES, calculated as long chain (C_{21} - C_{30}) / short-chain (C_{13} - C_{20}), shows a marked decrease in L-FAMES with fermentation following the order: initial mixture > 2SS-AD $\geq$ control $\geq$ 1SS-AD > 4SS-AD. Consequently, all digestate samples exhibited a lower L-/S- FAMES ratio with respect to the initial mixture, reaching the lowest value for 4SS-AD. This indicates that during our experiment AD promoted a chain shortening with L- to S-FAMES conversion and percolate recirculation enhanced this phenomenon. Branched-FAMES (from C_{13} to C_{16}) and one unsaturated ($C_{18:1}$) FAME were also found (Table 2 and Figs. 5 and Supplementary Material S1). Monounsaturated C_{12} - C_{20} and branched FAMES are typical bacterial FA [39], therefore they can be considered as microbial biomarkers. The relative abundance of FAMES consistently increased with the anaerobic digestion reaching a maximum content for 4SS-AD. This points to an accumulation of microbial biomass in the digestates during AD also indicating that the higher the percolate recirculation frequency the greater the microbial response.

Unlike *n*-alkanoic acids, AKA family did not exhibit considerable differences due to AD. In fact, their percentage in digestates was comparable to the initial mixture (ca. 4-5%) with a slight increase only in 2SS-AD peaking at 6.3% (Table 2).

4. CONCLUSIONS

Our results indicate that percolate recirculation favoured the AD of pig slurry and straw. The highest degree of OM degradation and stabilization was achieved when SS-AD was performed at the highest frequency of recirculation (4 times per day). Direct analytical pyrolysis confirmed that AD proceeds with polysaccharides degradation resulting in a selective accumulation of more recalcitrant lignin-derived compounds in the digestates. Percolate recirculation was found to play a key role in favouring digestion. In fact, an increase in recirculation frequency resulted in a greater consumption of polysaccharide-derived compounds following the order: 4SS-AD < 2SS-AD < 1SS-AD < control with a concomitant relative increase in lignin compounds. The positive effect of percolate recirculation on OM degradation and stabilization, was also underlined by the highest values of OM loss in 2 and 4SS-AD as well as by the analysis of WEOC, which revealed a depletion of the easily available fraction of WEOM in all digestates, except for the control (4SS-AD < 2SS-AD $\approx$ 1SS-AD < initial mixture < control). Also signs for the mitigation of methanogenesis inhibition by VFAs favoured by recirculation were found. Analytical pyrolysis was also useful in detecting a number of well-known microbial biomarkers and a conspicuous decrease in FAME chain length, indicating that percolate recirculation favoured microbial growth.

Acknowledgements

The Spanish Ministry of Economy, Industry and Competitiveness (MINEICO) the *Agencia estatal de Investigación* and FEDER programme are thanked for funding the projects CGL2016-76498-R and CGL2016-78937-R (AEI/FEDER, UE). J.M. De la Rosa thanks MINEICO for funding his

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829 “*Ramon y Cajal*” contract (RYC2014-16338). Alba Carmona Navarro and Desire Monis Carrere are
830
831 recognized for their technical assistance with analytical pyrolysis.
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Figure Captions:

Figure 1. Schematic representation of the experimental apparatus [7].

Figure 2. Total ion current (TIC) chromatograms from Py–GC/MS of the initial mixture and control, 1SS-AD, 2SS-AD and 4SS-AD digestate samples. All pyrograms are normalized for 2-methoxy-4-vinylphenol (Lig-15, min 8).

Figure 3. Changes in the relative abundance (%) of the chemical families identified by pyrolysis–GC/MS: aromatics (Ar-), lignin-derived compounds (Lig-), polysaccharide-derived compounds (Ps), N-compounds (N-), ketones, *n*-alkane/*n*-alk-1-ene doublets and alkanols (AKA-), *n*-alkanoic acids (FA-) and sterols and terpenoids (ST-).

Figure 4. Extracted Single ion chromatograms (m/z 73) of the initial mixture and control, 1SS-AD, 2SS-AD and 4SS-AD digestate samples.

(*) Fatty acid methyl ester (FAME); (M) Fatty acid, α -methyl. C_x refers to the total number of carbon atoms. All chromatograms are normalized for the first peak (min. 2.2)

Figure 5. Relative abundance of *n*-fatty acid methyl esters (FAMES) calculated from the extracted single ion chromatograms (m/z 74) in the initial mixture and digestate samples. On the top right Long (L) to short (S) FAMES ratio calculated for the initial mixture and digestate samples.

(*) docosanedioic acid, dimethyl ester; (M) FAME, α -methyl-

SUPPLEMENTARY MATERIAL

Supplementary 1 (S1). Extracted single ion chromatograms (m/z 74) of the initial mixture, control, 1SS-AD, 2SS-AD and 4SS-AD digestate samples. (*) Fatty acid methyl ester (FAME); (**) FAME, α -methyl-; (u) unsaturated FA. C_x refers to the total number of carbon atoms. All chromatograms are normalized for C₁₆* (min 14.8)

Supplementary 2 (S2). Total ion current (TIC) chromatograms from Py–GC/MS (1.5 mg) of the three replicates of initial mixture and 1SS-AD samples. All chromatograms are normalized for 2-methoxy-4-vinylphenol (Lig-15, min 8).

Table 1. Chemical characteristics of organic matter (OM) in the initial mixture and digestates.

<i>Parameters</i>	<i>initial mixture</i>	<i>control</i>	<i>1 SS-AD</i>	<i>2 SS-AD</i>	<i>4 SS-AD</i>
<i>pH*</i>	8.22 ± 0.18	8.90 ± 0.10	8.22 ± 0.06	8.69 ± 0.06	8.82 ± 0.08
<i>TOC (g kg⁻¹)*</i>	491.0 ± 5.7	411.8 ± 2.8	417.7 ± 6.1	376.9 ± 6.7	230.9 ± 9.0
<i>OM-loss (%)</i>	0.0	18.0	14.6	25.8	31.0
<i>WEOC (g kg⁻¹)</i>	34.2 ± 2.2	47.6 ± 3.6	30.0 ± 0.1	29.3 ± 1.1	27.1 ± 2.0
<i>Hi (g kg⁻¹)</i>	10.8 ± 0.3	9.1 ± 0.0	7.7 ± 0.1	5.6 ± 0.1	6.3 ± 0.3
<i>Ho (g kg⁻¹)</i>	23.4 ± 1.6	38.5 ± 3.6	22.2 ± 0.8	23.7 ± 1.2	20.8 ± 1.7
<i>HA+FA (g kg⁻¹)</i>	107.2 ± 6.4	148.3 ± 2.2	131.1 ± 4.1	143.3 ± 9.4	146.2 ± 17.8
<i>DH (%)</i>	65.8	72.0	73.0	75.2	75.8
<i>HR (%)</i>	21.8	36.0	31.4	38.0	63.3

WEOC: water-extractable organic carbon; Hi: hydrophilic fraction; Ho: hydrophobic fraction; HA+FA: humic and fulvic-like acids; DH: degree of humification; HR: humification ratio
* as determined by Pezzolla et al. (2017)

Table 2. Compounds identified by analytical pyrolysis* and their relative abundances (%) in the initial mixture and digestate samples.

<i>Family</i>	<i>Pyrolysis products</i>	<i>Peak label</i>	<i>initial mixture</i>	<i>control</i>	<i>1SS-AD</i>	<i>2SS-AD</i>	<i>4SS-AD</i>
Ps-derived compounds	1-hydroxy-2-butanone	Ps-1	1.3	1.6			0.4
	furfural	Ps-2	2.4	2.0	2.1	1.6	1.0
	2-furanmethanol	Ps-3	1.7	1.4	1.5	0.9	0.6
	2-cyclopenten-1-one, x-methyl-	Ps-4	0.5	0.4		0.1	
	3,4-dihydropyran	Ps-5	1.4	0.6	1.4	0.6	0.4
	1,2-cyclopentanedione	Ps-6	1.6	1.1	1.1	0.5	0.4
	2-butanone, 3,3-dimethyl-	Ps-7	0.5	0.3	0.3	0.2	0.1
	2-furancarboxaldehyde, 5-methyl-	Ps-8	0.4	0.4	0.4	0.3	0.2
	2-cyclopenten-1-one, x-methyl-	Ps-9	0.4	0.4	0.4	0.3	0.1
	cyclohexanone, 3-methyl-	Ps-10	0.9	0.8	0.3	0.4	0.3
	1-hydroxy-2-pentanone	Ps-11	0.5	0.6	0.5	0.5	0.4
	2-cyclopenten-1-one, 2-hydroxy-3-methyl	Ps-12	2.8	2.6	2.5	2.6	2.8
	2-cyclopenten-1-one, 2,3,-dimethyl-	Ps-13	0.2	0.4	0.2	0.1	
	3,5-dimethylcyclopentenolone	Ps-14	0.5	0.5	0.4	0.3	0.2
	cyclohexanol, 3,5-dimethyl-	Ps-15	0.8	0.5	0.3	0.2	
	maltol	Ps-16	0.2	0.3	0.2	0.4	0.4
	2-cyclopenten-1-one, 3-ethyl-2-hydroxy-	Ps-17	1.3	0.8	0.7	0.7	0.6
	1-methoxy-4-(1'-methylethyl)ciclohexa-1,4-diene	Ps-18	0.8				
	3,4-dihydro-6-methyl-2H-1-benzopyran-2-one	Ps-19	0.7	0.4	0.2	0.2	0.5
	Total		18.9	15.1	12.4	10.0	8.4
N-compounds	pyridine	N-1	0.9	1.4	2.6	2.3	1.1
	methanedianiline, N,N,N',N'-tetramethyl-	N-2	1.3		0.7		0.6
	pyridine, x-methyl-	N-3		0.4	0.5	0.1	0.2
	benzyl nitrile	N-4	0.3	0.3	0.3	0.5	0.4
	benzenepropanenitrile	N-5		0.3	0.7	0.6	0.6
	1H-inden-1-one, 2,3-dihydro-	N-6	0.5	0.5	0.4	0.3	0.4
	indole	N-7	1.1	1.2	0.8	1.1	1.5
	1H-indol, 6-methyl	N-8	0.8	0.5	0.7	0.4	0.8
	Total		5.0	4.6	6.7	5.3	5.7
Aromatic compounds	phenol	Ar-1	1.5	1.7	1.5	1.2	1.3
	benzoic acid, methyl ester	Ar-2	0.6	0.9	0.6	0.4	0.3
	3-methylthiobenzofuran	Ar-3	0.2	0.4			0.3
	Total		2.3	2.9	2.1	1.6	2.0
n-alkane/ene, ketones and alkanols	1-octen-3-ol	AKA-1	1.8	1.3	1.5	1.2	0.7
	dodecene	AKA-2		0.1	0.1	0.2	0.2
	Alkyl	AKA-3	0.3	0.6	0.7	0.8	0.8
	eicosane	AKA-4					0.2
	heptacosane	AKA-5	0.3	0.3	0.4	0.3	0.3

	nonacosane	AKA-6	0.9	0.9	1.1	1.4	1.2
	hentriacontane	AKA-7	0.4	0.6	0.8	1.0	0.7
	1-heptacosanol	AKA-8	0.3	1.0	0.4	1.0	0.5
	2-pentadecanone, 6,10,14-trimethyl-	AKA-9	0.2	0.3	0.3	0.3	0.3
	2-heptadecanone	AKA-10		0.1	0.2	0.1	0.1
	Total		4.3	5.1	5.4	6.3	5.0
<i>n</i>-alkanoic acids	propanoic acid*	FA-1	2.8	2.2	2.0	1.2	1.4
	tetradecanoic acid	FA-2	0.8	0.8	0.7	0.8	1.2
	tetradecanoic acid, 12-methyl-, methyl ester	FA-3		0.1	0.1	0.2	0.2
	pentadecanoic acid, methyl ester	FA-4	0.1	0.2	0.2	0.2	0.3
	tetradecanoic acid, 12-methyl-	FA-5	0.2				
	pentadecanoic acid	FA-6		0.2	0.3	0.2	0.4
	pentadecanoic acid, 13-methyl-, methyl ester	FA-7		0.1	0.1	0.2	0.3
	hexadecanoic acid, methyl ester	FA-8	0.2	0.5	0.4	0.4	0.5
	n-hexadecanoic acid	FA-9	1.1	0.8	0.9	0.8	0.8
	hexadecanoic acid, 3-hydroxy-, methyl ester	FA-10		0.2	0.2	0.3	0.3
	9-octadecenoic acid (oleic acid)	FA-11	0.4	0.2	0.1	0.1	
	9-12-octadecadienoic acid (linoleic acid)	FA-12	0.3	0.1	0.1	0.1	0.2
	18-octadec-9-enolide	FA-13	0.2	0.1		0.2	0.3
	docosanedioic acid, dimethyl esters	FA-14	0.2				
	Total fatty acid methyl esters		0.5	1.1	1.0	1.3	1.6
	Total fatty acids**		2.8	2.1	2.1	2.0	2.6
Sterols and terpenoids	cholest-x-ene	ST-1	0.8		0.3	0.3	0.3
	squalene	ST-2	0.1	0.2	0.2	0.3	0.4
	cholesta-8,24-dien- 3 β -ol 4 β -methyl-	ST-3	0.4	0.1	0.1	0.3	0.2
	cholestan-3-ol	ST-4	0.3	0.8	1.0	1.9	1.2
	ergostanol	ST-5		0.3	0.2	0.5	0.3
	stigmasta-5,22-dien-3-ol	ST-6	0.2	0.3	0.3	0.6	0.3
	24-ethylcholestan-3- β -ol	ST-7	0.4	0.7	0.6	1.0	0.6
	γ -sitosterol, β -sitosterol	ST-8	0.5	0.6	0.4	0.6	0.5
	Friedelan-3-one	ST-9	2.7				
	Total		5.4	3.0	3.2	5.3	3.1

*) Lignin-derived compounds (Lig-) are shown in Table 3.

**) Propanoic acid was not considered.

Table 3 Lignin-derived compounds (Lig-) and their relative abundance (%) in the initial mixture and digestate samples.

<i>Pyrolysis products</i>		<i>Peak label</i>	<i>initial mixture</i>	<i>control</i>	<i>1SS-AD</i>	<i>2SS-AD</i>	<i>4SS-AD</i>
<i>phenol, 2-methyl-</i>	H	Lig-1	0.5	0.6	0.4	0.3	0.3
<i>phenol, 2-methyl- isomer</i>	H	Lig-2	0.7	0.8	0.7	0.7	0.8
<i>phenol, 2-methoxy</i>	G	Lig-3	4.3	5.7	6.3	6.7	6.6
<i>4-ethylphenol</i>	H	Lig-4	0.9	1.0	0.8	0.8	0.7
<i>phenol, 2-methoxy-x-methyl-</i>	G	Lig-5	0.3	0.7	0.2	0.1	0.2
<i>ethanone, 1-(2-methylphenyl)- (acetoguaiacone)</i>	G	Lig-6	0.2	0.3	0.2	0.2	0.2
<i>creosol (methylguaiacol)</i>	G	Lig-7	1.2	1.2	1.2	1.2	1.2
<i>catechol</i>	H	Lig-8	1.8			0.4	
<i>phenol, 4-(methylthio)-</i>	?	Lig-9	0.8	0.4	0.8	0.4	
<i>4-vinylphenol</i>	H	Lig-10	4.3	6.0	5.8	5.6	5.8
<i>3,4-dimethoxytoluene</i>	S	Lig-11	0.6	0.5	0.5	0.4	0.5
<i>1,2-benzenediol, 3-methoxy</i>	G	Lig-12	2.2	2.0	1.3	1.3	2.1
<i>phenol, 4-ethyl-2-methoxy (ethylguaiacol)</i>	G	Lig-13	1.6	2.0	1.9	1.6	1.9
<i>phenol, 4-methoxy-3-methyl- (methylguaiacol)</i>	G	Lig-14	0.6				
<i>2-methoxy-4-vinylphenol</i>	G	Lig-15	6.9	8.7	10.9	10.9	8.8
<i>phenol, 4-(2-propenyl)-</i>	H	Lig-16	0.7	0.6	0.8	0.3	0.6
<i>phenol, 2,6-dimethoxy</i>	S	Lig-17	5.3	7.6	7.3	7.5	6.4
<i>phenol, 2-methoxy-4-(2-propenyl)-</i>	G	Lig-18	0.8	0.8	0.7	0.5	0.7
<i>phenol, 2-methoxy-4-propyl-</i>	G	Lig-19	1.0	0.9	0.9	0.4	1.1
<i>benzaldehyde, 4-hydroxy-3-methoxy-</i>	G	Lig-20					0.3
<i>benzaldehyde, 4-hydroxy-3-methoxy- isomer</i>	G	Lig-21	1.4				
<i>phenol, 2-methoxy-4-(2-propenyl)-</i>	G	Lig-22	2.6	4.4	5.4	4.6	5.3
<i>1-(4-hydro-3-methoxyphenyl)propyne</i>	G	Lig-23	0.5	0.3	0.3	0.3	0.4
<i>acetovanillone</i>	G	Lig-24	0.9	1.2	1.7	1.4	2.2
<i>4-ethyl-2,6-dimethoxyphenol</i>	S	Lig-25	0.9	1.6	1.3	1.4	1.9
<i>3-(4-hydroxyphenyl)-3-oxopropanal</i>	H	Lig-26	0.3				
<i>2-propanone, 1-(4-hydroxy-3-methoxyphenyl)-</i>	G	Lig-27	1.1	1.6	1.6	1.7	1.6
<i>trans 2,6-dimethoxy-4-propenylphenol</i>	S	Lig-28	0.4	0.4	0.5	0.3	0.7
<i>2,6-dimethoxy-4-vinylphenol</i>	S	Lig-29	3.7	4.5	4.3	5.2	4.8
<i>propiovanillone</i>	G	Lig-30	0.4	0.5	0.5	0.7	0.9
<i>phenol, 2,6-dimethoxy-4-(2-propenyl)-</i>	S	Lig-31	0.6	0.6	0.6	0.8	0.9
<i>homosyringaldehyde</i>	S	Lig-32	0.3	0.3	0.1	0.2	0.3
<i>2,6-dimethoxy-4-vinylphenol</i>	S	Lig-33	0.5	0.2	0.0	0.2	0.1
<i>phenol, 2,6-dimethoxy-4-(2-propenyl)-</i>	S	Lig-34	0.8	0.9	0.7	0.9	1.2
<i>syringaldehyde</i>	S	Lig-35	0.9	0.6	0.9	1.0	0.6
<i>ethyl 4-hydroxy-3-methoxyphenylacetate</i>	G	Lig-36	0.8	0.8	0.7	1.0	1.3
<i>(E)-2,6-dimethoxy-4-(prop-1-en-1-yl)phenol</i>	S	Lig-37	2.5	2.7	2.5	3.0	3.8
<i>acetosyringone</i>	S	Lig-38	1.6	1.3	1.2	1.7	1.7
<i>3-(4-Hydroxy-3-methoxyphenyl)-2-propen-1-ol, 4-Hydroxy-3-methoxycinnamyl alcohol</i>	G	Lig-39	0.7	0.4	0.3	0.8	0.8
<i>syringylacetone</i>	S	Lig-40	1.2	1.0	0.9	1.3	1.4
<i>propiosyringone</i>	S	Lig-41	0.3	0.7	0.6	0.5	0.7
<i>2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester</i>	G	Lig-42	0.2	0.2	0.2	0.2	0.3
Total			57.7	63.9	65.0	66.5	69.2
<i>G-type units</i>			27.8	31.8	34.3	33.8	35.9
<i>S-type units</i>			19.7	22.8	21.3	24.2	25.0
<i>H-type units</i>			9.3	8.9	8.5	8.1	8.3
G/S ratio			1.4	1.4	1.6	1.4	1.4

Molecular characterization of digestates from Solid-State Anaerobic Digestion of pig slurry and straw using analytical pyrolysis.

Ornella Cavallo¹, José María de la Rosa^{2*}, José Antonio González-Pérez², Heike Knicker², Daniela Pezzolla³, Giovanni Gigliotti³, Maria Rosaria Provenzano¹

¹ *Department of Soil, Plant and Food Sciences, University of Bari “Aldo Moro”, Via G. Amendola 165/A, 70126 Bari, Italy*

² *Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Av. Reina Mercedes, 10, 41012 Seville, Spain*

³ *Department of Civil and Environmental Engineering, University of Perugia, Via G. Duranti 93, 06125 Perugia, Italy*

*Corresponding author: jmrosa@irnase.csic.es

S1. Extracted single ion chromatograms (m/z 74) of the initial mixture, control, 1SS-AD, 2SS-AD and 4SS-AD digestate samples. (*) Fatty acid methyl ester (FAME); (**) FAME, x-methyl-; (u) unsaturated FA. Cx refers to the total number of carbon atoms. All chromatograms are normalized for C16* (min 14.8)

S2. Total ion current (TIC) chromatograms from Py-GC/MS (1.5 mg) of the three replicates of initial mixture and 1SS-AD samples. All chromatograms are normalized for 2-methoxy-4-vinylphenol (Lig-15, min 8).