Biodegradation (2011) 22:623–635
DOI 10.1007/s10532-010-9436-y

ORIGINAL PAPER

Digestion of cattle manure under mesophilic and thermophilic conditions: characterization of organic matter applying thermal analysis and ^{1}H NMR

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Received: 17 June 2010 / Accepted: 8 November 2010 /

Abstract Digestion of cattle manure collected from a livestock farm together with bedding material (straw) has been studied under mesophilic and thermophilic conditions in batch reactors. The digestion was carried out for a prolonged period with the aim of evaluating the changes undergone by the organic matter. The mesophilic digestion carried out revealed a greater capacity to produce gas and transform organic matter, while a higher conversion rate, but a lower gas yield, was obtained under thermophilic conditions. Degradation of the organic matter was evaluated by means of thermal analysis and ^{1}H NMR. Stabilisation through anaerobic digestion (either mesophilic or thermophilic) resulted in an increase in the quality of the organic matter, as characterised by an enrichment in thermostable compounds, and an accumulation of long chain aliphatic materials. The experiments performed demonstrated the transformation of organic matter into complex materials under anaerobic conditions with an accumulation of aliphatic components under both types of conditions tested. Degradation through mesophilic digestion, in comparison to the thermophilic process, resulted in a greater destruction of straw particles.

Keywords Cattle manure · Stabilisation · Anaerobic digestion · Thermal analysis · ^{1}H NMR

Introduction

Composting and anaerobic digestion are biological treatment processes intended to stabilize organic matter, with the digestion process presenting the additional benefit of obtaining biogas as a valuable end product. However, composting is the technical process available for conversion of waste organic matter into humic substances with a high content in aromatic structures. Humification of biomolecules provides the environmental benefit of carbon sequestration through the reduction in CO_2 release caused by mineralization (Smidt and Tintner 2007). Despite these apparent benefits, the composting process entails a long maturation stage to attain humification of organic matter.

The treatment of biowastes by the anaerobic digestion process is characterised by a long period of permanence inside the reactor, around 20–40 days.

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This time requires to be optimised in order to increase the economic feasibility of the digestion treatment plant, by achieving a reduction in the volume of the reactor needed. In this sense, contrary to the composting process, the lack of a maturation stage is a particularity of the digestion process. Assessment of the quality of the digestate obtained under anaerobic conditions has been studied by different authors (Tambone et al. 2009; Bartoszek et al. 2008; Gómez et al. 2005) as well as the subsequent degradation of digestate through composting (Amir et al. 2006) and sludge aeration (Smidt and Parravicini 2009) as a means of increasing the quality of the organic matter.

The study of the transformations suffered by the organic matter during biological degradation is not an easy task. Different parameters have been proposed for evaluating the quality of organic matter, with special attention given to the thermogravimetric behaviour of the samples when heated up to a desired temperature, as in the case of Thermal Analysis (TA). Owing to the simplicity of sample preparation and the short time needed to obtain results, this technique of analysis has proven simple, fast and yet reliable when dealing with the characterization of organic matter (Melis and Castaldi 2004). On these lines, thermal characterization of organic matter has been studied by several authors (Gómez-Rico et al. 2005; Otero et al. 2002; Font et al. 2001; Dell'Abate et al. 1998). TA and Differential Scanning Calorimetry (DSC) have also been applied to the study of the degradation of organic mater during composting (Klammer et al. 2008; Smidt and Lechner 2005) and to the assessment of compost stability (Dell'Abate et al. 1998).

^{1}H and ^{13}C NMR spectroscopy has been selected as an analytical tool thanks to its capacity for the selective identification of different types of C and H moieties and for comparing differences in concentrations of main functional groups (Adani et al. 2006). The humic substances (HS) two-dimensional (2D) NMR study was introduced by Buddrus et al. (1989). Solid-state ^{13}C NMR spectroscopy in conjunction with thermal analysis has been used for comparing the molecular and structural properties of humic acids isolated from sewage sludges, and for determining changes in amended soils. As an example, humic acids in thermally dried sewage sludge showed an important presence of alkyl and O/N-alkyl compounds (Fernández et al. 2008). In our previous study (Gómez et al. 2010), TA, fluorescence spectroscopy and ^{1}H NMR were proposed as analytical tools for characterisation of the organic matter transformations during the digestion of food wastes under semi-continuous operation at mesophilic and thermophilic conditions. As result, enrichment in aliphatic components was observed under both conditions studied, although the degradation attained at thermophilic temperatures was faster, with a higher content in aromatic structures remaining in the thermophilic digestate, while under mesophilic conditions degradation of these structures was observed.

The aim of this study was to assess the transformations taking place during the digestion process of cattle manure under mesophilic and thermophilic conditions during prolonged stabilisation. TA and ^{1}H NMR were used as analytical tools for studying the degradation of organic matter.

Materials and methods

Substrates and digestion characteristics

The inoculum used for starting up the reactors was obtained from the wastewater treatment plant of the city of León (Spain). The concentrations of total solids (TS) and volatile solids (VS) in the inoculum used were 46.2 and 25.7 g/l respectively. Cattle manure originated from a local livestock farm. Straw was used as bedding material on the farm, gathering liquid and solid dejections together. The cattle manure presented an initial concentration of TS of 172 g/kg, with a percentage of VS of 82.7% (wt%).

The digestion was carried out in triplicate using static reactors (under batch conditions), with a working volume of 1 l and thermostatised at 34 ± 1 and 55 ± 1°C. The reactors were loaded with 300 g of fresh manure, 200 ml of inoculum and 500 ml of tap water. In each case a blank containing only inoculum and tap water was used.

Kinetic analysis

Final cumulative biogas produced was fitted to the modified Gompertz equation (Zwietering et al. 1990) (1) which is a suitable model for describing the process of cumulative biogas production in batch experiments (Sung and Liu 2003):

$$\mathrm{P_{(t)}} = \mathrm{P_{max}} \cdot \exp\left[^{-\exp\left[\frac{\mathrm{R_{max}} \cdot e}{\mathrm{P_{max}}}(\lambda - t) + 1\right]}\right] \tag{1}$$

where $\mathrm{P_{(t)}}$ is the cumulative biogas production (l); $\mathrm{P_{max}}$ is the biogas production potential (l), $\mathrm{R_{max}}$ is the maximum biogas production rate (l/day), λ is lag-phase time (days) and e is 2.718. Data analysis was performed using Origin 6.1 software.

Analytical techniques

Cattle manure used as substrate was characterised by the following chemical analysis: electrical conductivity and pH were determined using a sample diluted in de-ionized water in the proportion 1:25 (w:v as g:ml) shaken for 30 min. Total organic carbon was determined on the basis of Walkey–Black method (1934). N-Kjeldahl was determined in accordance with the APHA et al. (1989). The extract for determination of cations and phosphorus was obtained by wet digestion carried out with HNO_3 65% in a pressurized microwave oven at 170–200°C for 20 min. Total phosphorus and cation content in the extract was quantified by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using Perkin Elmer Optima 2000 DV. Ammonium nitrogen (NH_4^+–N) was determined from the extract after submitting the sample to 1 h of agitation with 20 ml of 2 M KCl solution and quantified by Keeney and Nelson (1982). Cellulose, hemicellulose and lignin content were estimated by analysis of neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude fibre (Van Soest et al. 1991) using ground samples in duplicate with an Ankom 200 Fibre Analyser.

TS, VS, chemical oxygen demand (COD), ammonium and pH were analysed during the digestion process. These parameters were determined in accordance with APHA et al. (1989). Daily gas production was measured using a liquid displacement device. Biogas composition was analysed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A 4-m-long column packed with HayeSep Q 80/100 followed by a 1-m-long molecular sieve column were used to separate CH_4, CO_2, N_2, H_2 and O_2. The carrier gas was Helium and the columns were operated at 331 kPa and a temperature of 50°C.

Thermogravimetry (TG) and Differential thermogravimetry (DTG) are based on a programmed heating of samples in controlled atmospheres, providing qualitative and quantitative information regarding the organic content of the sample (Melis and Castaldi 2004). Differential thermal analysis (DTA) is based on the temperature change in the sample, as compared to that in an inert sample submitted to the same programmed heating. TG curves are usually represented in terms of the percentage of the weight loss experienced by the sample, while DTG curves are obtained from the first derivative of TG profiles, thus representing the rate of weight loss (Gómez et al. 2007). DTA data are represented by the difference between the temperature of the sample and that of the reference material. The higher the temperature at which weight loss occur, the more resistant and ordered structurally is the organic fraction which is burning (Otero et al. 2002).

Sampling on days 17 and 33 were taken from only one of the 3 reactors of the experimental set-up (half of the total volume each time). The other 2 reactors were used for gas measurement and analysis, as well as for sampling on day 77 (at the end of the experiment) using the whole content. The experimental methodology was based on previous work (Gómez et al. 2007) for monitoring organic matter conversion by TA. Samples were divided into two fractions with the aid of a 3 mm mesh for separating straw particles of greater size which do not undergo changes in VS/TS percentage over the course of the digestion process. The two fractions thus obtained were dried at 105°C. Prior to drying, TS, VS, ammonium and COD were analysed (by triplicate) in the slurry fraction not retained by the mesh. The straw particles retained by the mesh were washed with deionised water, with any small particles passing through the mesh being added to the slurry previously obtained. The dried solids from the slurry were subsequently ground with a ball mill Retch 200MM. In the case of the sample taken at the end of the experiments, a composite sample was obtained from the two reactors used for gas measurements. A subsample selected from the ground material was then submitted to TA using a TA Instruments SDT2960 thermobalance. The heating rate applied was 10°C/min up to 700°C with a flow-rate of 100 ml/min of synthetic air.

Ground samples were extracted using dimethyl sulfoxide d_6 (DMSO d_6) as proposed by Francioso et al. (2007) for 24 h and the extract obtained after centrifugation was then analysed by ^{1}H NMR. NMR spectra were recorded with a Bruker Avance 600 spectrometer (5 mm TCi cryoprobe, inverse detection). Nominal frequencies were 600.13 MHz for ^{1}H and 150.90 MHz for ^{13}C. An internal lock on DMSO d_6 was used for all spectra. The chemical shifts (δ) at 298 K were referred to DMSO d_6 (δ for ^{1}H: 2.49 and δ for ^{13}C: 39.5).

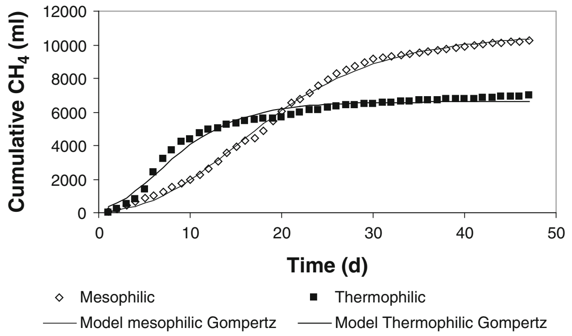

Fig. 1 Mean cumulative CH_4 production for mesophilic and thermophilic systems

Results and discussion

Anaerobic digestion

The chemical characteristics of the substrate used in this study are presented in Table 1 (chemical analyses were expressed in dry basis). CH_4 production for thermophilic and mesophilic systems is presented in Fig. 1. A larger volume of gas was produced at mesophilic temperature although thermophilic reactors presented a higher rate of gas production (see Table 2). Production of gas started on the first day of the experiments, with maximum gas rate obtained between days 6 and 7 for thermophilic reactors and around day 20 for their mesophilic counterpart. Thermophilic systems presented a reduced lag phase (Table 2), although reactors were inoculated with mesophilic microflora. The start up of thermophilic digestion systems has also been studied by Chachkhiani et al. (2004) on the treatment of cattle manure, reporting fast microbial growth and a maximum gas rate on day 10 of experimentation, with the dominant species being the thermophilic microorganisms originally presented on cattle manure (a mesophilic ecosystem) at a subdominant level, which quickly became dominant under thermophilic conditions.

Table 1 Chemical composition of the substrate

Parameter	Substrate
pH	7.5
Conductivity (mS/cm)	7.57
Organic carbon (%)	32.5
Total Kjedahl nitrogen (%)	2.24
Ammonium—N (%)	0.23
Total phosphorus (mg/kg)	9310
K (mg/kg)	33800
Ca (mg/kg)	4010
Mg (mg/kg)	984
Na (mg/kg)	5182
Cellulose (%)	10.6
Hemicellulose (%)	13.1
Lignin (%)	7.4

Mesophilic (35°C) and thermophilic (60°C) digestion of cattle manure have been studied by Sánchez et al. (2000) under batch conditions for a 33 day period, reporting a reduction in the methane yield with the increase of temperature. In the present study, digestion was maintained until day 77. Gas production was measured until total stoppage of gas generation. The methane yield obtained was 234 ± 19 and 159 ± 17 ml/g VS added for mesophilic and thermophilic digesters. Table 2 also presents the characteristics of the substrate based on the separation of particles by the 3 mm sieve, together with those of the digestates obtained under each set of conditions evaluated. Thermophilic digestate was characterized by a lesser removal of particles of a size greater than 3 mm, thus explaining the lower methane yield achieved. In consonance, the total volume of gas produced was also in accordance with this behaviour with the thermophilic reactor presenting a lower cumulative gas production.

Degradation of the particulate substrate was in accordance with the behaviour of gas production which was adjusted to the modified Gompertz model. This equation was developed by dividing gas accumulation into two fractions, one arising from rapidly fermentable feed and the other from slowly

Table 2 Characteristics of batch digestion systems

Substrate characteristics	
Liquid fraction with particles of size less than 3mm	
TS (g/l)	36.9 ± 0.3
VS (g/l)	28.1 ± 0.4
COD (g/l)	42.6 ± 3.5
Organic carbon (%)	28.5 ± 1.5
Solids retained by 3 mm sieve	
Mass (g)	18.1 ± 0.6
%VS	91 ± 2

Batch digestion systems	Mesophilic	Thermophilic
Gompertz kinetic parameters		
P_{max} (ml)	10620 ± 85	6599 ± 64
R_{max} (ml/day)	435 ± 39	487 ± 91
λ (day)	5.7 ± 0.1	1.4 ± 0.2
R^2	0.997	0.966
Digestate at day 77: liquid fraction with particles of size less than 3 mm		
TS (g/l)	25.5 ± 0.2	20.9 ± 0.2
VS (g/l)	16.7 ± 0.4	14.3 ± 0.4
COD (g/l)	35.7 ± 1.6	30.1 ± 1.8
Organic carbon (%)	22.7 ± 1.8	25.4 ± 1.5
Cumulative gas production (l)	17.5 ± 1.1	13.4 ± 0.9
Digestate at day 77: solids retained by 3 mm sieve		
Mass (g)	3.4 ± 0.1	5.6 ± 0.2
%VS	92 ± 3	91 ± 2
% Mass removal	82 ± 5.3	69 ± 5.8

fermentable feed (France et al. 2005). The reduction in the amount of particulate substrate may be seen as relating to the slowly fermentable organic matter, thus presenting thermophilic microflora a limited capacity to assimilate this fraction, which in turn leads to a lower biogas yield.

The systems presented low variability in pH measurements. In the initial state of digestion, the pH value was 7.4, with a reduction to 6.6 by day 3. Thereafter, the pH of the mesophilic reactor returned to its initial value, while the thermophilic reactor reached a higher value (7.8) by the end of the experiments. NH_4^+ content did not reach inhibitory values (Henze and Harremoes 1983; Siegrist et al. 2005). Initial content of NH_4^+–N was 359 mg/l, increasing to 882 mg/l in mesophilic reactors and 707 mg/l in the thermophilic reactors by the end of experimentation.

Thermal analysis

Thermal profiles of the fresh cattle manure are presented in Fig. 2. These profiles are characterised by a three step pattern as reported by Mondini et al. (2003) Thermal profile of fresh cattle manure has been analysed previously (Sánchez et al. 2008). The profile was characterised by two main peaks, once the water released is disregarded. The peak at around 300°C is associated with labile components of organic matter, while the peak at higher temperatures is associated with aromatic structures (Flaig et al. 1975). Although the intensity of the second exothermic peak in DTG profile was lower than that of the first peak (indicating a lower release of mass), a similar intensity for both peaks was observed in the DTA profile, indicating that a higher temperature is reached with the oxidation of complex compounds.

TG, DTG and DTA profiles for digestates curves obtained from mesophilic and thermophilic digestion processes are presented in Fig. 3. The profiles maintained the thermal characteristics of fresh manure. Dehydration reactions take place at low temperatures (Dell'Abate et al. 1998), just as in the previous case (the fresh cattle manure sample). The two exothermic peaks, representing the fractions of different thermal stability, can be distinguished. However, a reduction in the intensity of the first peak was registered for both digestates (mesophilic and thermophilic), indicating no relevant effect of the temperature on stabilisation whenever readily oxidized materials are concerned. The thermophilic sample at day 33 presents a similar thermal pattern at low temperature (300°C) to that of the mesophilic sample at the end of digestion. This behaviour may be indicative of a higher velocity of the thermophilic microflora for degrading labile components.

The continuous weight loss in the temperature range of 350–450°C may be associated with the combustion of char formed at lower temperatures. This process has been reported by Font et al. (2001) and Zhu et al. (2007) in the thermogravimetric study of sewage sludge and landfill sludge. In relation to the second exothermic peak, thermophilic and mesophilic systems are well differentiated. Thermophilic digestates were characterised by lower intensity peaks in the high temperature range. This second exothermic peak was found in the feed sample, and was also registered in digestate samples, with the peak of the

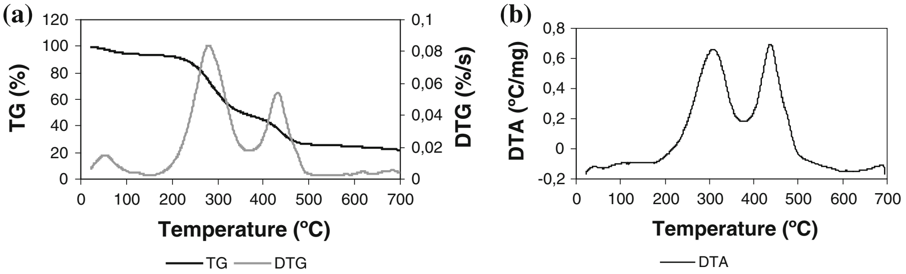

Fig. 2 **a** Weight loss thermograms (TG), differential thermogravimetry (DTG) and **b** DTA of fresh cattle manure used as substrate in the present study

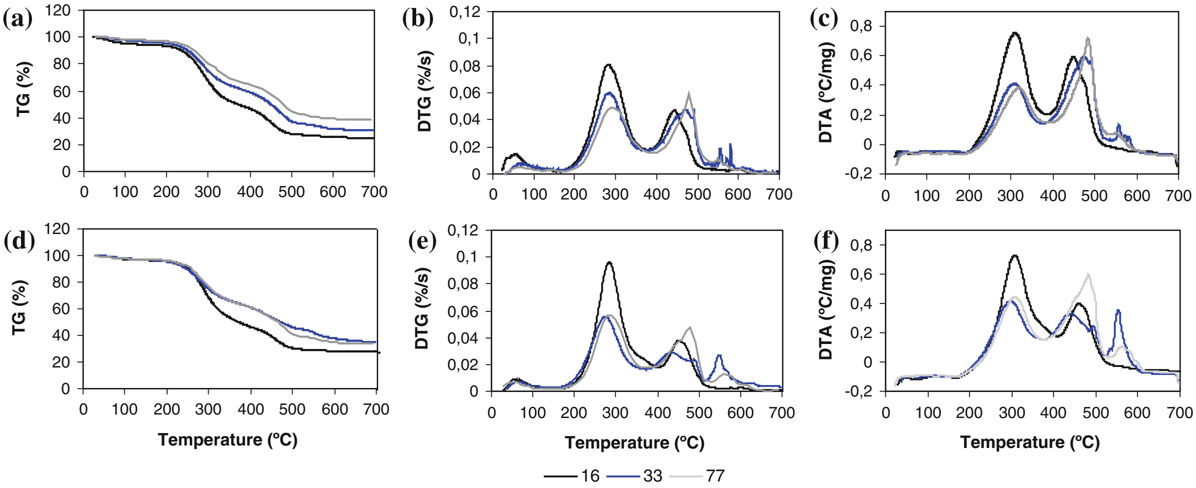

Fig. 3 **a**, **b**, **c** TG, DTG, DTA curves of mesophilic digestates, and **d**, **e**, **f** curves of thermophilic digestates obtained at different time intervals

mesophilic digestate registering a delay as the stabilisation proceeded. This peak has been used for the characterisation of the maturity of compost using DSC profiles (Klammer et al. 2008) and may be associated with lignin components, which are reported to present exothermic peaks around 476°C (Xu et al. 2006). Since it is well-known that lignin is not degraded under anaerobic conditions, the digestion of manure in both cases resulted in a digestate enriched with lignin components which present a complex structure. DTA curve corroborates this fact by the increase in the temperature difference signal which is related to the accumulation of these compounds.

The samples taken from the digestion process at different intervals presented an increment of the mineral content as the substrate was digested. The initial content for the feed was 22.1%, increasing to 24.8% at day 16 and to 38.3% at the end of the mesophilic digestion, while increasing to 27.5 and 33.5% under thermophilic conditions. Although the rate of destruction of the organic matter and posterior transformation into biogas was higher during thermophilic digestion process, this was not linked to a greater degree of mineralisation.

Distortions in DTG profiles have been observed by the presence of a mineral matrix in the sample (Rovira and Vallejo 2000). The content and composition of ashes presented in the organic matter are known to delay the combustion process. Rubiera et al. (2002) and Vamvuka et al. (2006) reported that demineralized samples presented lower burn-out temperatures than those of their parent fuels. In the thermal analysis of concentrated olive mill wastewater, with a high mineral content, an increase in the burn-out temperature was reported by Miranda et al. (2008). In the present study, the higher mineralisation content of digestates samples from mesophilic systems may account for the shift to the right on the temperature scale of the peak registered around 450–500°C.

The increase in the time for stabilisation led to the presence of high temperature peaks in samples evaluated under both temperature conditions. A mass loss registered above 500°C was also reported by Lopez-Capel et al. (2006), this being associated with polycondensation and the formation of increasingly stable C and N forms in the study of the fungal degradation of wheat straw. The formation of complex molecules containing N, has also been reported by Cuetos et al. (2009, 2010) in the study of anaerobic digestion of slaughterhouse wastes, with thermal profiles presenting high intensity peaks centred at around 550°C. The digestate sample obtained on day 33 of stabilisation from the thermophilic reactor presented a peak of particularly high intensity associated with these stable molecules. However, a peak of lesser intensity was registered at the end of the stabilisation, with this fact also being a characteristic of the mesophilic digestate sample. The DTA signal confirmed that the release of mass at this high temperature is an exothermic process. Hence, components being oxidized were able to generate an important increase in the temperature difference signal. In this context stability should not be exclusively related to the presence of C and N complex molecules.

^{1}H NMR spectroscopy

1D ^{1}H NMR spectrum obtained from the fresh cattle manure sample is presented in Fig. 4a. The signal at 2.5 ppm corresponds to DMSO-d_6. The spectrum is dominated by sharp signals in the aliphatic region ascribed to terminal methylic and aliphatic chains. Unsaturated compounds were also identified in two regions, one ascribed to methylenic protons in proximity to the double bond, and the other corresponding to protons in the ethylenic group (see Table 3). Methylic protons in acetic acid present a chemical shift at 2.08 ppm. The region at 3.0–4.0 ppm cannot be perfectly characterised due to overlapping of signals derived from the presence of water in the sample.

Signal intensities in the range of 6.2–8.0 ppm are an indirect method of monitoring the level of substitution on the aromatic ring of lignin (Xu et al. 2006). The intensity of signals in the aromatic region of the spectrum was low, compared to those obtained in the aliphatic region, thus indicating either a low content in aromatic components, or a high level of substitution (Adani et al. 2006). The complexity of the organic matter shown by the ^{1}H NMR spectra was associated with the thermal profile obtained by TG analysis. Aromatic components gave rise to mass loss at high temperature; hence, lignin components identified in ^{1}H NMR were probably responsible for the mass release reported around 470°C in thermal analysis. Protons in the aromatic ring of substituted aromatic amides were identified in the spectrum of Fig. 4a. However C and N stable forms were identified in digestate samples but not in the substrate sample (by the mass loss experienced above 500°C) in DTG profiles. This result may indicate possible transformation of the aromatic compounds containing nitrogen into components of greater complexity, which were subsequently identified by thermal analysis.

Figure 4b shows ^{1}H, ^{13}C HSQC spectra of the fresh cattle manure sample. The unsaturated region which could not be observed in ^{1}H NMR spectra due to overlapping with water signal is now clearly discernible allowing the identification of polysaccharide and protein region being associated with signals between 60 to 110 ppm. Side-chain groups (oxygenated C_α, C_β, and C_γ carbon) of the phenylpropane lignin structural unit provide a minor contribution to this region (Kolodziejski et al. 1982; Haw et al. 1984; Xu et al. 2006). The signals peaking at 56 and 71 ppm suggest that aromatic moieties have a high content of N-alkyl and O-alkyl carbons (Adani et al. 2006). The main contributions giving rise to ^{1}H resonance signals at 3.75–3.92 ppm are likely to arise from CHOH and CH_2OH functional groups which may indicate the presence of methoxyphenylpropyl repeating unit which typically occur in lignin and lignin-like matter (Yasuda et al. 1999) and/or the presence of polysaccharides moieties (Adani et al. 2006).

Signals around 72–74 ppm (3.4 and 3.2 in ^{1}H NMR) are due to C2, C3, and C5 of cellulose as well as carbons from xylans (Sosanwo et al. 1995; Hatcher 1987). C=C and aromatic rings give rise to signals around 120 and 130 ppm in ^{13}C NMR spectra, coinciding with signals at around 7 ppm in ^{1}H NMR

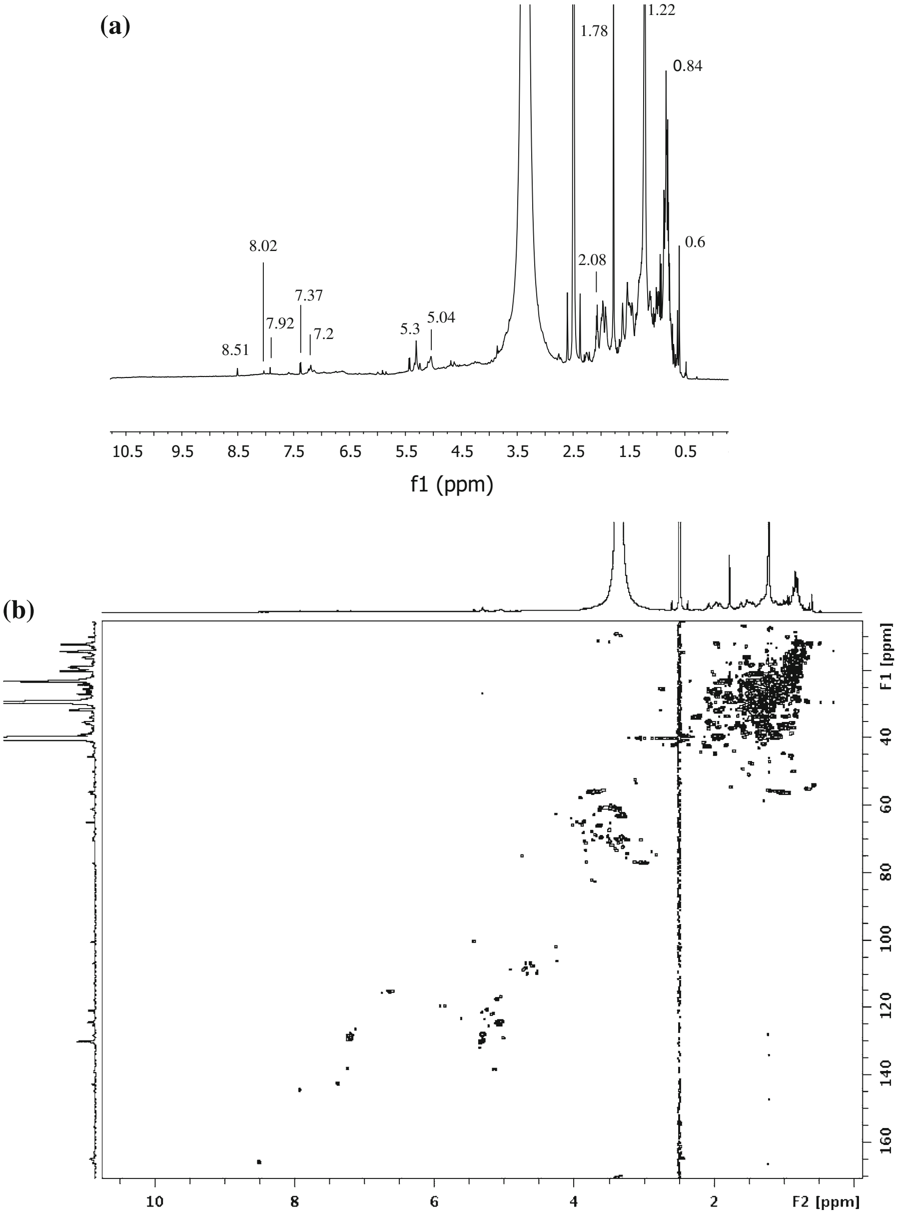

Fig. 4 **a** ^{1}H NMR spectrum of fresh cattle manure sample, **b** ^{1}H, ^{13}C HSQC spectra of fresh cattle manure sample

sprectrum indicative of sysringyl and guaiacyl units (Vivas et al. 2006; Xu et al. 2006) and signals at 5.03 and 5.30 ppm ascribed to unsaturated compounds. The signal at 8.5 in ^{1}H NMR spectrum is associated with 166 signal in ^{1}H, ^{13}C HSQC spectrum, corroborating the presence of nitrogen heteroaromatic compounds.

Spectra obtained from samples at 16 and 77 days for mesophilic and thermophilic digestates are represented in Fig. 5. No major differences were observed in the aliphatic region at an early stage of the mesophilic processes (Fig. 5a). Prolonged stabilisation under mesophilic conditions led to modifications in the aliphatic components of the organic matter, presenting an important relative increase in signals at 0.6 and 0.8 ppm in relation to that at 1.22 ppm, this being indicative of enrichment in terminal methylic groups (Fig. 5b). The great intensity of

Table 3 Signal identification of ^{1}H NMR spectrum of samples taken in the present study

Components	Signal (ppm)	References
Terminal methylic chain	0.6–0.84	Francioso et al. (2007)
Aliphatic chains [$(CH_2)_n$]	1.22–1.42	Guillén and Ruiz (2006)
Methylenic protons in relation to one double bond (unsaturated compounds)	1.94–2.14	Guillén and Ruiz (2006)
Methoxy groups in aromatic rings	5.04	Larghi and Kaufman (2006)
Protons in the ethylenic group	5.3–5.4	Aursand et al. (1998)
Aromatic protons	6.4–7.4	Xia et al. (2003)
Aromatic protons: positions 2 and 6 in structures containing a Cα=O group, and in *p*-coumaric and ferulic acids	7.37	Seca et al. (2000)
Aromatic amides	8.03–8.5	Chang and Liou (2007)

signals registered in the aliphatic region of the ^{1}H NMR spectrum may be explained by components in the extract which are resistant to microbial degradation. The presence of these components was confirmed by results obtained from thermal analysis, in which a continuous mass loss was registered in the DTG profile around 350–400°C. The pyrolysis of components may be attributed to the large aliphatic content of the samples. Results were in accordance to those previously reported by Gómez et al. (2007) in the study of cattle and poultry manure digestion, where an important presence of CH_2 groups was found in digestate samples.

The signal at 1.78 ppm in the fresh sample spectrum was displaced to 1.8 ppm in that of the digestate sample. This modification may be rationalized by changes in substitutions of the main chain due to the degradation of organic matter. There is an important reduction of this signal when its relative intensity is compared with that at 1.22 ppm of final digestate spectrum (Fig. 5b), indicating a decrease in the amount of unsaturated components, this being in accordance with the reduction in signal intensity at 5.3 ppm.

The aromatic region presented low-intensity signals and was characterized by the absences of signals ascribed to coumaric and ferulic acids, and also by the persistence of signals at 7.08 and 7.46 which were ascribed to guaiacyl and syringyl units of lignin. With regard to the spectra obtained form mesophilic digestates, an increase in the intensity of signals at 7.25 and 7.84 ppm relative to that at 5.3 ppm of unsaturated compounds was observed at day 16 of the digestion process. These signals may be derived from those initially registered in the fresh cattle manure spectrum at 7.37 and 7.92 ppm. As previously stated, the signal recorded in the aromatic region was ascribed to lignin fractions, and their higher intensity may be rationalized by a preferential degradation leading to an accumulation of complex components. This statement was in accordance with results obtained from thermal analysis, indicating a reduction in the content of readily oxidized components thus, favoring an increase in the content of complex organic matter. Additionally, the energy release from the thermal degradation of these complex materials was higher as the time of experimentation increased, with results being coincident with those reported by Rovira et al. (2008), suggesting an increase in the quality of organic matter as biological degradation takes place, as it was evident from the enrichment of thermostable fractions (indicated by the mass loss registered at temperatures higher than 450°C in thermal profiles). The increase in the time of stabilisation resulted in the formation of organic components of C and N stable forms, as it was indicated from TG-DTG analysis and ^{1}H NMR spectrum, in which a low intensity signal a 10.2 ppm was registered. This signal was neither initially presented in the fresh cattle manure sample nor in the digestate sample at an early stage of stabilisation and was attributed to amidic protons (In and Kim 2005).

In relation to thermophilic digestates, spectra obtained showed a similar trend to those previously analysed (Fig. 5c, d). However, the thermophilic digestate presented a great degree of conversion at an early stage in stabilisation (day 16) with almost the total volume of biogas being produced during this

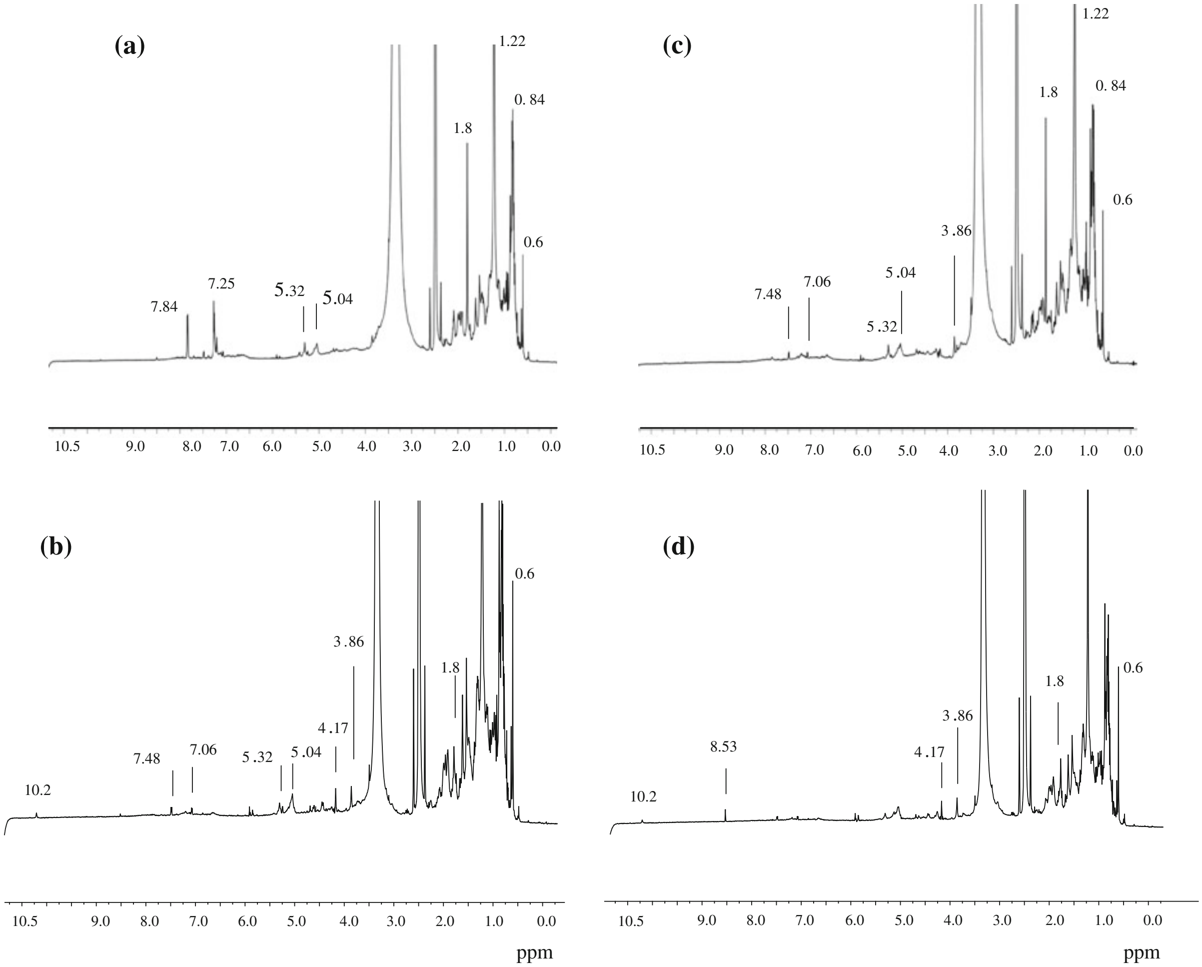

Fig. 5 ^{1}H NMR spectra of mesophilic digestate at **a** 16 days and **b** 77 days, and thermophilic digestates at **c** 16 days and **d** 77 days

period. The degradation of unsaturated compounds needed a longer time, even at thermophilic conditions, as it is shown in Fig. 5d by the persistent presence of signal at 1.86 ppm.

Lignin derived units presented a lower intensity signal in contrast to results obtained from the mesophilic digestate at day 16 (these aromatic doublets placed around 7.48 and 7.06 ppm have a $^3J_{H,H}$ of 7.3 Hz). At the end of the thermophilic digestion it was possible to observe a relative increase of the signal ascribed to N-complex compounds (8.5 ppm), in relation to that of lignin units.

HSQC spectra obtained in the final stage of digestion are presented in Fig. 6 for mesophilic and thermophilic samples. The HSQC fingerprints for both digestates are similar, presenting an accumulation of signals in the aliphatic region at around 20–30 ppm. These aliphatic components present resemblance with suberin and cutin biopolymers (Moire et al. 1999) which are considered to be the protective layers of higher plants. Suberin and cutin are considered to be polyesters, with similar structures (composed of fatty acids), differing in their chain length and their substitution patterns. Suberin contains, in contrast to cutin, lignin-like aromatic components, covalently linked to the aliphatic chain by ester bonds (Kolattukudy 1980).

The main differences were a decrease in signals ascribed to unsaturated compounds at around 5.2 and 5.3 ppm in ^{1}H NMR coupled with 120 and 123 ppm in HSQC in thermophilic digestate. This digestate also presented a major contribution to signals ascribed to the polysaccharide region. Although thermophilic digestion of cattle manure presented a

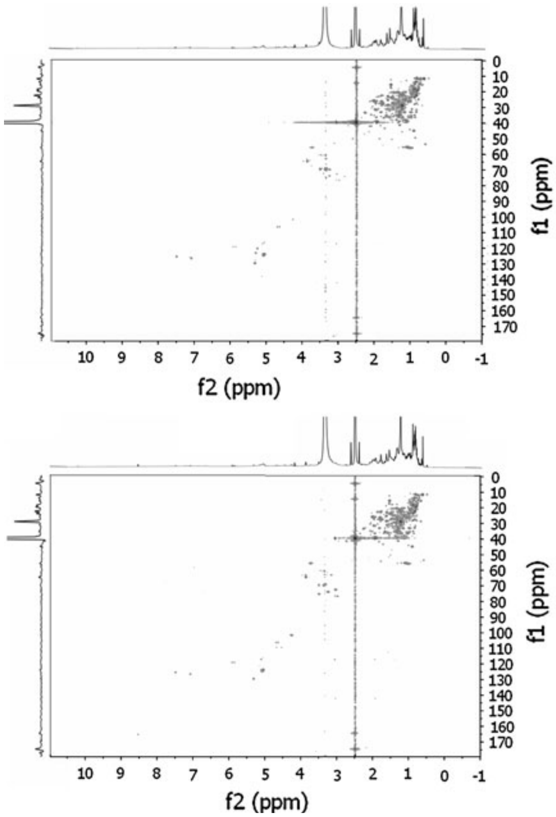

Fig. 6 **a** ^{1}H, ^{13}C HSQC spectra of mesophilic and **b** thermophilic digestate after 77 days of stabilisation

higher rate of stabilisation, the degradation of the organic matter under mesophilic conditions revealed a higher destruction of cellulose (decrease in signal at around 72 and 74 ppm) when digestion time was prolonged.

Conclusions

Results obtained from the digestion process showed a higher degradation of particulate material under mesophilic conditions. Transformation of the organic matter was evaluated by means of thermal analysis and ^{1}H NMR. These analytical techniques demonstrated that prolonged stabilisation at constant temperature resulted in an increase in the quality of the organic matter, as characterised by the accumulation of thermostable compounds, N-complex and long chain aliphatic structures. Results demonstrated the initial degradation of readily oxidised components with the consequent accumulation of the complex fraction, and posterior transformation into stable compounds.

The advantages of the use of thermal analysis are based on the easiness of application, sample preparation and short time needed for the analysis. In this sense, this technique may be useful for control and improvement of process performance by identification of overloading problems associated to the amount of readily oxidised materials. On the other hand ^{1}H NMR spectroscopy allows the identification of functional groups resulting in a better characterisation of the transformation suffered at different stabilisation times by digestate samples. However the wide application of this technique is limited by the higher analytical cost of the equipment needed, thus recommending this analysis for obtaining detailed information related to the transformations undertaken by biological stabilisation processes. Future research in this area should focus in determining relationships between expected gas production and thermal behaviour of substrates, reactor operating conditions and quality of digestate attained, as well as assessing the quality of the organic matter when land application of the stabilised wastes is the disposal option selected.

Acknowledgments The authors wish to thank collaboration of the WWTP of León-SALEAL (mancomunidad municipal para el saneamiento integral de León y su alfoz).They also would like to thank Dr Margarida Gairí (NMR Facility at Parc Cientific de Barcelona) for access to 600 MHz spectrometer.

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