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# Impact of anaerobic digestion on organic matter quality in pig slurry

Claire-Emmanuelle Marcato<sup>a</sup>, Rabi Mohtar<sup>b</sup>, Jean-Claude Revel<sup>a</sup>, Philippe Pouech<sup>c</sup>, Mohamed Hafidi<sup>d</sup>, Maritxu Guïresse<sup>a,\*</sup>

<sup>a</sup> ENSAT (Ecole Nationale Supérieure Agronomique de Toulouse), EcoLab UMR 5245, Auzeville-Tolosane, 31250 Castanet-Tolosan, France

<sup>b</sup> Purdue University, Department of Agricultural & Biological Engineering, 225 South University Street West Lafayette, IN 47907-2093, USA

<sup>c</sup> APESA (Association Pour l'Environnement et la Sécurité en Aquitaine), Hélioparc, 64053 Pau Cedex 09, France

<sup>d</sup> Equipe Ecologie et Environnement, Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390 Marrakech, Morocco

## A B S T R A C T

Changes in pig slurry organic matter (OM) during anaerobic digestion (AD) were studied in a reactor to characterize OM evolution through AD. OM maturity and stability were evaluated using different biological and physico-chemical methods. Germination and growth chamber experiments revealed a higher maturity of digested slurry (DS) than raw slurry (RS). Soil incubations showed that DS was more stable than RS with a C-mineralization of 12.0 g CO<sub>2</sub>-C 100 g<sup>-1</sup> C<sub>Org</sub> after 49 days as compared to 17.6 g CO<sub>2</sub>-C 100 g<sup>-1</sup> C<sub>Org</sub>. Biochemical fractionation showed a relative increase in stable compounds such as hemicellulose-like and lignin-like molecules. Fourier-transform infrared spectroscopy showed some changes in the chemical structures of OM with a reduction in the aliphatic chain, lipid and polysaccharide levels. A comparison between the evolution of OM during AD and the first weeks of a composting process showed almost identical changes. Finally a theoretical method called Fictitious Atomic-group Separation was applied to the elemental compositions of RS and DS. DS was less humified than RS and presented the properties of a fulvic acid, indicating that the observed stability in DS was mainly due to the biodegradation of the most labile compounds.

### Keywords:

Anaerobic digestion  
Humic substances  
Organic matter  
Stability  
Maturity

## 1. Introduction

European energy policies are evolving towards the development of anaerobic digestion for organic residue and waste treatment (European Parliament, 2008). Because of its energy potential, anaerobic digestion (AD) has been studied for decades and the process is well known (Deublein, 2008; Moletta, 2008). That is the reason why this technology is already of particular importance in some European countries for agricultural by-products such as cattle or pig slurries and crop residues. On the other hand, pig slurries are traditionally spread on agricultural lands to recycle fertilizing elements such as N, P or K. When anaerobically digested, they can be managed like raw slurries, by spreading on land, or like substrates for composting. Different studies have been carried out to evaluate the quality of the organic matter from digestates in comparison to composts, in order to better characterize their aerobic post-treatment (Fuchs et al., 2008). However, when anaerobically digested products are directly spread on land, only the nutrients (N and P principally) and the pollutants are taken into consideration (Palm, 2008). Consequently, there is still a lack of

information about the agronomic benefits and disadvantages of the organic matter quality from anaerobically digested pig slurries, although they are widely reused in crop soils. This is of particular interest since soil organic matter is decreasing in crop soils currently in Europe and is receiving increasing attention. At the same time, in a context of climate change and sustainable development, C sequestration in the topsoil is being studied to be accounted as C sinks in national greenhouse gas inventories (Saby et al., 2008).

Organic matter (OM) quality has been widely studied for composts. Bernal et al. (1998) presented the two main criteria to safely use compost in soil: maturity and stability. Maturity implies a stable organic matter content and the absence of phytotoxic compounds and plant or animal pathogens, and is associated with plant growth potential or phytotoxicity. Stability is often related to the compost's microbial activity, but can be associated to physico-chemical parameters (colour, pH, and C/N). In 2005, 12 stability parameters and 7 maturity parameters cited by 49 articles were identified (ADAS Consulting Limited, 2005). Further investigations on composts revealed that stability was best evaluated by combining different characteristics (Bernal et al., 1998; Benito et al., 2003) but there is still no internationally standardized method to evaluate maturity and stability. However, seed-germination tests are widely performed to guarantee maturity and one such procedure has been standardized in France (AFNOR, 2004). Microbial

\* Corresponding author. Tel.: +33 5 62 19 39 37; fax: +33 5 62 19 39 01.  
E-mail address: guïresse@ensat.fr (M. Guïresse).

activity, and more particularly respirometry, is another current method to evaluate compost stability (Barrena Gómez et al., 2006). These two kinds of test are the most widely used, but other methods have been developed to characterize humic substances. For example, Fourier-transform infrared spectroscopy has allowed a much better understanding of humic substances (Stevenson, 1994; Chen, 2003). In consequence, several research works have dealt with humic and fulvic acids in sewage sludge (Gigliotti et al., 2001; Guiesse et al., 2004; Amir et al., 2005; Senesi et al., 2007) or pig slurry (Plaza et al., 2002, 2003; Hernández et al., 2006a) and compared their characteristics to the properties of soil humic substances. Moreover, different authors have established predictive methods to evaluate humic substances or compost behaviour in soil from the chemical characteristics of the organic matter. Linères and Djakovitch (1993) proposed an original approach to predict the OM humic potential using biochemical fractionation to calculate a biological stability index (BSI). In a similar way, an interpretation method of the elemental formula of humic substances was proposed with the goal of understanding their genesis and structure (Tardy et al., 2000; El Hajjouji et al., 2008).

In consequence, the present work aimed to bring new data concerning the changes in the OM quality through a pig slurry anaerobic digestion process and at characterizing the final product from an agronomic point of view. The maturity of both raw and anaerobically digested slurry was first evaluated with a growth chamber experiment. Then, we characterized the organic matter stability of raw and digested slurries by combining four methods inspired from edaphological approaches and compost evaluation: soil incubation and determination of carbon mineralization rate; biochemical fractionation and determination of the biological stability index (BSI); Fourier-transform infrared spectroscopy and identification of the main functional groups; elemental formula and modelling with the "Fictitious Atomic-group Separation" (FAS) method. All these methods were applied considering the organic matter as a whole and not humic and fulvic fractions which are inappropriate to differentiate animal slurries (Plaza et al., 2003; Moral et al., 2005). Large amounts of raw or digested slurry had to be used for biological tests (growth chamber tests and soil incubation) to simulate the mid- and long-term environmental effects of slurry spreading. Since such great quantities can be harmful due to the presence of abundant  $\text{NH}_4^+$  (Mantovi et al., 2005), the research was conducted using solid slurry extracts prepared by removing the aqueous phase containing ammonia after centrifugation.

## 2. Materials and methods

### 2.1. Sample collection, preparation and characterization

Raw slurry (RS) and digested slurry (DS) were collected from the anaerobic digestion plant described by Marcato et al. (2008). This 150 m<sup>3</sup> continuous stirred-tank reactor treated about 11 m<sup>3</sup> d<sup>-1</sup> of pig slurry from a farrow-to-finish herd, with a retention time of 15 days. RS and DS samples were collected using a sampler and were then centrifuged using a Beckam J2-21M/E centrifuge (14 000g, 30 min) in order to concentrate the organic matter, Cu and Zn, in the sample. The resulting extracts were air-dried at 40 °C to obtain a homogeneous powder. Elemental analyses were performed on a Thermo EA1110 (C, H, N and S) and a Thermo Flash EA1112 (O) elemental analyzers. Three replicates were carried out for each sample.

### 2.2. Growth chamber experiment

Preliminary tests were performed to evaluate the maturity of the slurry extracts following the method used for composts, checking the absence of phytotoxic substances for plant growth.

The growth chamber experiment was conducted on a Neoluvisol with cultures of maize (*Zea mays*) and broad bean (*Vicia faba*). Soil was sampled from the surface layer (0–20 cm), air-dried, sieved (<4 mm) and stored at room temperature before use. Soil main characteristics were clay = 35 g 100 g<sup>-1</sup>; silt = 41 g 100 g<sup>-1</sup>; sand = 24 g 100 g<sup>-1</sup>; pH = 6.4; organic C = 1.33 g 100 g<sup>-1</sup>. The methods of characterization followed the work of García-Gómez et al. (2003). Treatments consisted of a single application rate for each slurry and each plant, considering that annual RS and DS spreading is about 60 m<sup>3</sup> ha<sup>-1</sup> (Marcato et al., 2007). Such an application rate corresponds to annual dry matter application rates of 1.62 t RS ha<sup>-1</sup> or 0.96 t DS ha<sup>-1</sup>. The maize assay simulated a long spreading period with high application rates (250 t RS ha<sup>-1</sup> and 160 t DS ha<sup>-1</sup>) and the bean assay represented a shorter period, with application rates of 50 t RS ha<sup>-1</sup> and 32 t DS ha<sup>-1</sup>. Corresponding quantities of slurry extracts were added to a 500 g soil sample to constitute the culture substrates. Three treatments were prepared for each assay: control soil (without any fertilisation), soil amended with raw slurry extract and soil amended with digested slurry extract (Table 1). Each treatment was replicated five times. Pots of 8 cm upper diameter, and a height of 10 cm, with holes in the base for drainage were used, giving a total of 30 pots. The soil moisture was adjusted to 60% of the water-holding capacity (WHC) with tap water.

In the maize assay, slurry amendment led to double the quantity of organic matter (OM) in the culture substrate. Then, preliminary substrate incubation was carried out to allow a mineralization of the organic C. The mixture was placed in the dark at 28 °C for 50 days and the moisture content was maintained at 60% of the WHC.

Dry *Z. mays* and *V. faba* seeds were soaked for 24 h in deionised water. Four seeds of maize and bean were sown in each pot, and then covered with a thin layer of sand to facilitate germination. Pots were placed in a growth chamber (dark, at 25 °C) for a week, and only one germinated seed was kept in each pot. Then, the pots were placed for 7 weeks at 24 °C day, 20 °C night, with a 16-h photoperiod. The relative humidity was kept at 70% day, 75% night. The plants were watered with tap water to maintain the culture substrate moisture at 60% of the WHC. At the end of the growing period, the aerial parts and roots of each plant were collected and the dry weights determined. Statistical analysis of the data was performed by analysis of variance and means comparison between each slurry treatment vs the control treatment. Mean values were tested for statistically significant differences using a *t*-test at *p* < 0.05. Finally, a growth index was calculated as the percentage ratio between the mean dry weight of plants in slurry and control treatments (Barberis and Nappi, 1996).

### 2.3. Soil incubation experiment

Potential C-mineralization of slurry extracts was measured by soil incubation at 28 ± 1 °C for 49 days. The soil used for these incubations was the same as the growth chamber experiment. Slurry input was 0.67 g dry weight (DW) per 25 g soil for raw slurry and 0.44 g DW per 25 g soil for digested slurry. Control soil without any input was also included. Dried slurries were homogeneously mixed with soil samples. In the three treatments (control, RS and DS), equivalent of 25 g dry soil was placed in hermetically closed 800 mL glass jars. Three replicates were performed for each

**Table 1**  
Slurry rates and corresponding theoretical OM amounts in the culture substrates.

	Control soil	Long-term/Maize		Middle term/Bean	
		RS	DS	RS	DS
Slurry extracts (g pot <sup>-1</sup> )		32.8 ± 0.4	21.1 ± 0.1	7.4 ± 0.3	3.9 ± 0.1
OM (g pot <sup>-1</sup> )	12.0	35.1 ± 0.3	25.8 ± 0.1	16.9 ± 0.1	14.2 ± 0.0

treatment and sampling date. Soil moisture content was maintained at 60% of the WHC by weighing and adjusting if necessary with tap water. The CO<sub>2</sub> produced was trapped in 10 mL of 0.5 M NaOH. CO<sub>2</sub> traps were renewed after 1, 3, 7, 14, 28 and 49 days. The residual alkalinity was analyzed by back titration with 0.1 M HCl in an excess of BaCl<sub>2</sub>.

C-mineralization was calculated as the difference between the C-mineralization in control soil and in the soil + slurry extract. It was assumed that mineralization from soil was similar with and without slurry addition. Cumulative C-mineralization was expressed in g CO<sub>2</sub>-C 100 g<sup>-1</sup> slurry organic C.

#### 2.4. Biochemical fractionation and biological stability index (BSI)

Slurries were fractioned following the method proposed by Linères and Djakovitch (1993) that was recently standardized in France (AFNOR, 2005). For this fractionation, 1 g of slurry was mixed with 2 g of calcinated sand in a coarse porosity (40–90 µm) glass crucible. The mixture was boiled in successive extractants using a Velp Scientifica FIWE extractor:

- the soluble and neutral detergent fiber fractions (NDF) were determined by extraction with neutral detergent (100 °C) for 60 min.
- the acid detergent fiber fraction (ADF) was determined by extraction with acid detergent (100 °C) for 60 min.
- the acid detergent lignin fraction (ADL) was determined by cool extraction with sulphuric acid for 3 h.

Dry weight residue between each step was used to calculate the neutral detergent soluble (NDS), hemicellulose-like (HEMI) and lignin-like (LIC) fractions using the following relationships:

$$\text{NDS} = 1 - \text{NDF}; \text{HEMI} = \text{ADF} - \text{NDF}; \text{LIC} = \text{ADL}$$

where the fractions are expressed in g g<sup>-1</sup> slurry.

The Wende cellulose fraction (CEW) was determined by successively boiling the same sand and slurry mixture for 30 min in sulphuric acid (0.26 M) and potassium hydroxide (0.23 M).

The biological stability index (BSI) was calculated according to Gabrielle et al. (2004):

$$\text{BSI} = 2.112 - 2.009 \times \text{NDS} - 2.378 \times \text{HEMI} - 2.216 \times \text{CEW} + 0.840 \times \text{LIC}$$

#### 2.5. FTIR spectra

Fourier-transform infrared (FTIR) spectra were recorded on an FTIR Thermo Nicolet 5700 spectrophotometer on potassium bromide (KBr) pellets, obtained by pressing a mixture of a 2-mg slurry extract sample with 300 mg KBr. To limit moisture interference, both slurries and KBr were separately dried at 105 °C before making the pellets. Spectra were plotted over the range 4000–400 cm<sup>-1</sup>.

#### 2.6. Fictitious Atomic-group Separation (FAS)

This method was proposed by Tardy et al. (2000) and used by El Hajjouji et al. (2008) to evaluate the degree of hydration and oxidation of carbon within a given elemental formula (C<sub>m</sub>H<sub>n</sub>O<sub>p</sub>). Derived from the elemental species composition (CHON) and the stoichiometry of the reactions, the FAS was developed to understand the thermodynamic stability of humic substances in soil-plant-water environments.

For the calculations, elemental composition was first expressed for 1 N, and then one NH<sub>2</sub> was subtracted from the formula since

most of the organic N present in a pig slurry solid fraction is part of proteins (Béline, 2001). Oxygen was then used first to create CH<sub>2</sub>O. If some O remained, CO was created; on the other hand, if all the oxygen was used and hydrogen remained, CH<sub>2</sub> was generated. The remaining carbon was written C; if not enough C was present, free H<sub>2</sub>O, considered as a sign of hyperhydration, was counted as CH<sub>2</sub>O-C. After subtracting NH<sub>2</sub>, the oxidation state of carbon (C<sub>x</sub>) is distributed between -4, such as in CH<sub>4</sub>, and +4, such as in CO<sub>2</sub>. In all cases, the average degree of oxidation of carbon is given by either C<sub>x+</sub> = 2CO/C<sub>t</sub> or C<sub>x-</sub> = 2CH<sub>2</sub>/C<sub>t</sub>, where C<sub>t</sub> is the total number of carbons counted in the elemental formula.

Moreover, total acidity (TA, meq g<sup>-1</sup> C), carboxylic acidity (COOH, meq g<sup>-1</sup> C), alcohol acidity (OH, meq g<sup>-1</sup> C) and molecular weight (MW, g mol<sup>-1</sup> C) can be also calculated from the elemental composition with the following relationships (Tardy et al., 2000), A being the degree of de-polymerisation:

$$A = (\text{CH}_2\text{O} + \text{CO})/C_t$$

$$\text{Log TA} = 0.689 \times A + 0.422$$

$$\text{Log COOH} = 1.319 \times A - 0.217$$

$$\text{Log OH} = 1.366 \times A + 0.385$$

$$\text{MW} = -4.902 \times A + 7.897$$

### 3. Results and discussion

#### 3.1. Growth chamber experiment and maturity

Shoot and root dry weights of bean and maize are shown in Table 2. All the treatments had a maximum germination index since the four seeds of each pot germinated in all cases.

However, after selecting a seed, a difference was noted in bean growth between RS and DS. One RS plant was unable to grow and only four replicates were harvested and the growth index was very low (45%), indicating phytotoxic symptoms (Barberis and Nappi, 1996) while the DS treatment showed only a slight decrease of biomass with a growth index of 89%. This result underlines a positive effect of anaerobic digestion on pig slurry maturity. Three explanations can be formulated for such phytotoxicity: (i) some phytotoxic compounds such as phenols or volatile acids were degraded by AD (Powers et al., 1999); (ii) the C-mineralization of raw slurry extract in soil might have generated large amounts of CO<sub>2</sub> and conditions of asphyxia or anoxia (Guiesse et al., 1995) or might have immobilised the nitrogen (Busby et al., 2007). However, immobilisation of nitrogen cannot explain the drop in growth since the levels of nitrogen in the whole plant did not show deficiency: nodules were present on the roots.

In contrast, when considering the maize assay, the growth indices were good: 260% and 116% for RS and DS, respectively.

**Table 2**

Dry weight yields (g pot<sup>-1</sup>) of shoots and roots of bean and maize.

		Control soil	RS	DS
Bean	Roots	1.11 ± 0.54	0.41 ± 0.45	1.24 ± 0.45
	Shoots	3.31 ± 0.90	1.59 ± 1.30*	2.68 ± 0.59
Maize	Roots	0.42 ± 0.12	1.09 ± 0.59*	0.42 ± 0.39
	Shoots	0.87 ± 0.12	2.27 ± 1.24	1.01 ± 0.91

\*p < 0.05 in the Student t-test.

Moreover, the root biomass of the RS treatment was significantly higher than in the control treatment. In this assay, a previous soil incubation increased the maturity of the culture substrates, and the largest quantity of slurry extract added in the RS treatment (Table 1) to simulate a long spreading period led to an increase in nutrients (organic N in particular) in the culture substrate and to better biomass yield.

The preliminary growth chamber experiment confirmed that both raw and anaerobically digested pig slurry can be safely used as organic fertilizer. Moreover, this assay emphasised the relevance of organic substrate mineralization before sowing. The mineralization reduced the phytotoxic potential which may be due to toxic compounds or competition for O<sub>2</sub> in soil. Finally, the growth chamber experiment showed a higher degree of maturity for DS than RS.

### 3.2. Soil incubation

The amount of CO<sub>2</sub> released after 49 days (Fig. 1) was significantly lower in the control soil (3.6 g CO<sub>2</sub>-C 100 g<sup>-1</sup> soil organic C) than in the soils receiving raw or digested slurry (respectively 17.6 g and 12.0 g CO<sub>2</sub>-C 100 g<sup>-1</sup> organic C supplied). Soil receiving raw slurry showed the highest organic carbon mineralization rate, and both raw and digested slurry-amended soils had mineralization rates statistically different from the control until the end of the assay. However, during the last two weeks, slurry mineralization rates tended towards a similar value. Maximum values were obtained for the three treatments on the first day of the experiment.

These results are consistent with those presented by Plaza et al. (2007) studying the microbial activity in pig slurry-amended soils at rates of 0, 150 and 300 m<sup>3</sup> ha<sup>-1</sup>. Depending on the treatment, these authors obtained a basal respiration from 30 to 65 g CO<sub>2</sub>-C g<sup>-1</sup> d<sup>-1</sup> during the first days, and the mineralization rate of amended soils was statistically significant for about ten weeks, the greatest difference being observed during the first 30 days. However, the initial mineralization rates assessed in our experiment (18–23 g CO<sub>2</sub>-C g<sup>-1</sup> d<sup>-1</sup>) were quite low in comparison to the results of Plaza et al. (2007) and those of Morvan et al. (2006) who screened 47 animal wastes to establish a typological approach between biochemical composition and mineralization kinetics. Busby et al. (2007) found that C from municipal waste was twofold less mineralised in a Troup loamy fine sand when the application rate was eightfold higher. Also, the amount of organic matter added as slurry extracts to simulate several decades of spreading was 4- to 16-fold higher than the quantities used by Plaza et al. (2007). These

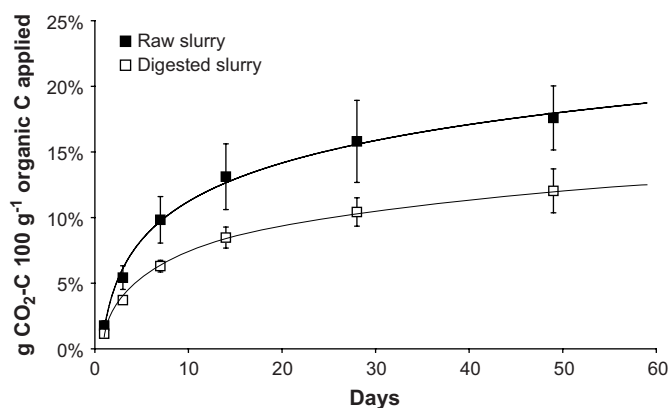


Fig. 1. Cumulative C-mineralization of RS and DS measured during the 49-d soil incubation.

high levels of OM added to the soil might explain the observed C-mineralization difference.

Moreover, the soil incubation assay gave some useful information to identify the effect of AD on the maturity and stability of pig slurry organic matter. The C-mineralization values obtained with raw and digested slurry extracts were similar to those reported by García-Gómez et al. (2003) for compost samples at different degrees of maturity. RS behaved like the organic mixture at the beginning of the treatment (17.6 vs 22.5 g CO<sub>2</sub>-C 100 g<sup>-1</sup> C<sub>org</sub>) while DS (12 vs 12 g CO<sub>2</sub>-C 100 g<sup>-1</sup> C<sub>org</sub>) was similar to the compost after a 4-week treatment, i.e. during the thermophilic phase. Moreover, during the first four weeks of the composting process, about 50% of the initial organic matter was lost. This value is comparable to the conversion rate of 53% presented by Marcato et al. (2008) for the anaerobic digestion plant where raw and digested slurries were sampled. Hernández et al. (2006b) compared C-mineralization during composting of an aerobic and an anaerobic sewage sludge mixed with sawdust. The initial C-mineralization rate of anaerobic sludge was lower and comparable to the values obtained with aerobic sludge after 60 days of composting.

Finally, the comparison of various incubation tests conducted with different substrates shows that whatever the organic waste and the plants considered, the phytotoxicity parallels C-mineralization in the soil. This indicates that the phytotoxicity observed in the growth chamber experiment was more likely due to oxygen depletion than due to toxic compounds. Based on this observation, further information on slurry extract stability is needed to define the effect of anaerobic digestion on the OM quality, and more particularly stability. Several European authors have tried to predict the C-mineralization in soil of various organic substrates from a chemical characterization, and frequently from a biochemical fractionation.

### 3.3. Biochemical fractionation and BSI

Raw and digested slurries showed a similar percentage distribution of organic components with a preponderant NDS fraction and a cellulose fraction that were almost non-existent (Fig. 2). Most of the organic content was recovered in the NDS fraction which represented more than 90% in raw slurry and about 83% in digested slurry. These results are similar to those presented by Francou (2003) studying mixed vegetable and fruit wastes for which 86.7% of the total organic matter was recovered in the NDS fraction. However, calculated biological stability indexes (BSI) were not statistically different between the two slurries: about 0.09 for RS and 0.10 for DS. These values are very low, confirming that pig slurries constitute a poorly humified substrate. These values are

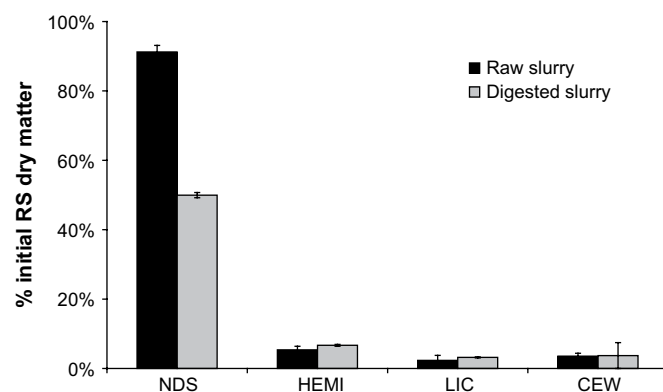


Fig. 2. Biochemical fractionation of RS and DS.

low in comparison to the results presented by Parent (2006) for sludge obtained by a raw slurry separation where the BSI was evaluated at 0.36. On the contrary, these results are consistent with those of Morvan et al. (2006). The lack of significance between RS and DS indexes is consistent with the results of Moral et al. (2005) who used the same method to study different manures but could not differentiate them with this approach. The BSI was developed by Linères and Djakovitch (1993) to classify organic amendments following their degradation ability and does not seem to be well adapted to the characterization of non-humified products such as slurries or manures. Biochemical fractionation appeared to be more efficient than the BSI in distinguishing raw and digested slurries with respect to their organic matter stability. Indeed, stabilisation was observed when considering the fractions independently, due to the bioconversion of the most labile fraction (NDS) into biogas during AD. Thus, the relative amounts of the most stable fractions (HEMI, LIC) increased proportionally. Morvan et al. (2006) preferred to use biochemical fractionation rather than the BSI to characterize several animal wastes (including slurries, litters and manures) and to predict the kinetics of C and N mineralization. Previously, the lignin content had been found to be significantly correlated to the C-mineralization (Bernal et al., 1998; Parnaudeau et al., 2004) or the *Lepidium sativum* L. germination index (Bernal et al., 1998). Studying composts, Francou (2003) proposed the ratio LIC/(HEMI + CEL) to represent the stability and to predict C-mineralization. Considering the raw and digested slurry extracts, the ratios are significantly different (0.24 and 0.44, respectively, for RS and DS). These results better represent the OM stabilisation through anaerobic digestion than the BSI.

### 3.4. FTIR spectra

The FTIR spectra of both raw and digested slurries are illustrated in Fig. 3. The main features of these spectra and their corresponding assignments are: (i) a broad band at about  $3400\text{ cm}^{-1}$  attributed to O–H (phenols, alcohols and carboxylic groups) and N–H (amines and amides A) stretching, (ii) two sharp bands at about  $2930\text{--}2920\text{ cm}^{-1}$  and  $2860\text{--}2850\text{ cm}^{-1}$  corresponding to aliphatic C–H (fatty acids and other long-chain structure) stretching, (iii) a broad band in the region between  $1665$  and  $1635\text{ cm}^{-1}$  due to C=O

stretching in amides (amide I), acids or ketones and C=C stretching in aromatics, (iv) an intense band at about  $1570\text{ cm}^{-1}$  due to N–H deformation and C=N (amide II) stretching, (v) a shoulder at about  $1460\text{ cm}^{-1}$  attributed to C–H (aliphatic structures) stretching, (vi) a medium intensity band at about  $1420\text{ cm}^{-1}$  which is characteristic of C–O stretching (carbonate group), (vii) a weak intensity band at about  $1250\text{ cm}^{-1}$  attributed to C–O stretching, C–N bending and O–H bending of carboxyl, phenols and aromatics, (viii) a band composed of two main peaks between  $1120\text{ cm}^{-1}$  and  $1040\text{ cm}^{-1}$  which might be due to the ring vibration of polysaccharides or polysaccharide-like substances on one hand or, on the other hand, due to symmetric and asymmetric stretching of phosphodiester (contribution of microbial biomass), and (ix) a little sharp band at  $875\text{ cm}^{-1}$  due to C–O out of plane (carbonate group).

Raw and digested slurry FTIR spectra exhibited the same absorbance areas, but they differed in the intensity of some peaks. In digested slurry, the spectra showed a remarkable decrease of: (i) aliphatic structures and lipids (bands at about  $2930\text{--}2920\text{ cm}^{-1}$ ,  $2860\text{--}2850\text{ cm}^{-1}$  and  $1460\text{ cm}^{-1}$ ), (ii) amides (bands at about  $3330\text{ cm}^{-1}$ ,  $1665\text{--}1635\text{ cm}^{-1}$  and  $1570\text{ cm}^{-1}$ ), (iii) polysaccharides ( $1040\text{ cm}^{-1}$ ). These decreases represented the biodegradation of the labile fraction into biogas (Smidt et al., 2002), with a relative increase in more resistant and stable compounds. These observations are coherent with the results of Amir et al. (2005) who showed that sludge decomposition during composting begins by the lipid, protein and carbohydrate components. On the other hand, the digested slurry FTIR spectra revealed an increase in carbonates ( $875\text{ cm}^{-1}$ ) probably due to OM mineralization during anaerobic digestion. Indeed, the bioconversion of organic matter into biogas led to the release of compounds such as Ca which reacts with carbonate ion and precipitates.

Moreover, the FTIR spectra can be compared to those of humic acid (HA) and fulvic acid (FA) extracted from pig slurry (Plaza et al., 2003; Hernández et al., 2006a). RS spectra are quite similar to HA spectra while DS spectra look like FA spectra due to the reduction of aliphatic C–H groups (peaks at about  $2900\text{ cm}^{-1}$  and  $1460\text{ cm}^{-1}$ ). These observations confirm that anaerobic stabilisation of organic matter is mainly due to the build-up of more stable compounds in the dry matter rather than humification processes. This stabilisation was highlighted during the biological treatment of municipal

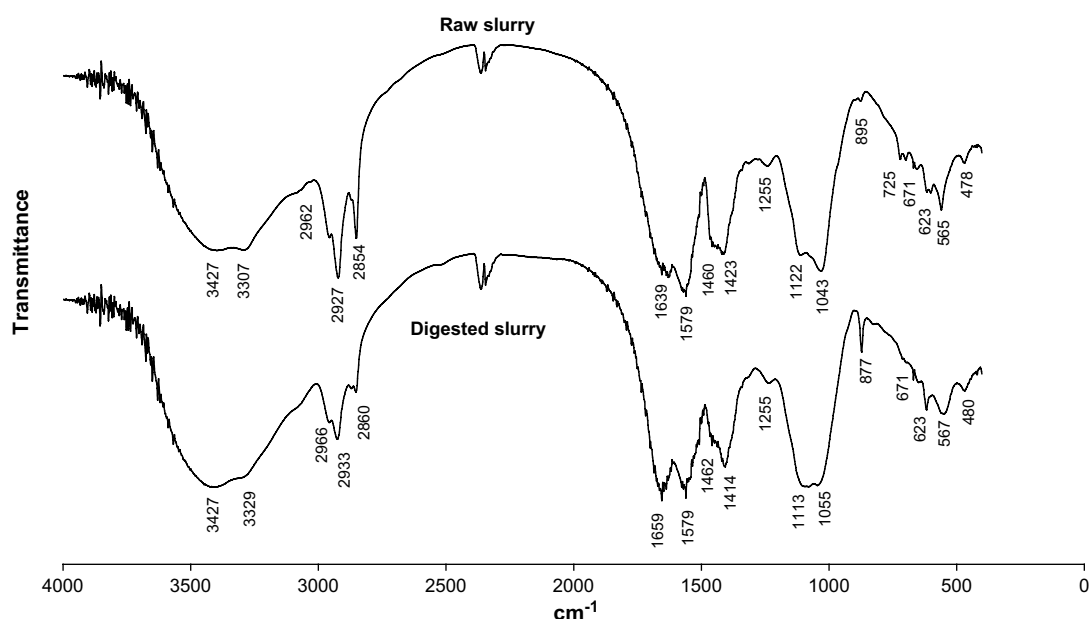


Fig. 3. FTIR spectra of raw and digested slurries.

**Table 3**

Elemental compositions of organic matter in raw and digested solid fractions.

	RS	DS
C	57.96 ± 0.03	48.13 ± 0.38
H	8.32 ± 0.00	6.62 ± 0.08
O	27.48 ± 0.30	39.83 ± 0.20
N	6.15 ± 0.40	5.42 ± 0.09
S	0.09 ± 0.16	0.00 ± 0.00
C/N	9.46 ± 0.61	8.89 ± 0.22

solid waste combining a mechanical, an anaerobic and a composting treatment (Ponsá et al., 2008). The stabilisation was evaluated at the different stages of the process, and AD was found to be the step that stabilised the waste most.

### 3.5. Fictitious Atomic-group Separation (FAS)

The elemental species compositions (Table 3) confirmed that slurry dry matter is richer in organic compounds (mainly formed of C, H, O, N) before anaerobic digestion than after. The elemental composition of RS resembles that of the humic acid-like fractions isolated from composted animal manures and wastes, C representing between 55 and 60% of the OM and O about 30% (Senesi and Brunetti, 1996). These authors presented elemental compositions for the fulvic acid-like fraction from different composted materials which were comparable to the DS composition here, with C and O contents between 45 and 50%.

The carbon oxidation degrees were, respectively,  $-0.829$  and  $-0.216$  for RS and DS, meaning that both RS and DS were from anaerobic ecosystems. DS showed a higher oxidation degree than RS, which is surprising since the DS came from a very reduced environment ( $-300$  mV). This is due to the bioconversion of the OM into a biogas containing about 64% CH<sub>4</sub>. The most reduced OM fraction was then converted into biogas, leading to a relative increase in the C oxidation degree of the remaining OM. These results are consistent with the biochemical fractionation which showed an increase of compounds like lignin or hemicellulose.

The results of the FAS are listed in Table 4. As expected from the FTIR spectroscopy findings, the de-polymerisation degree increased through anaerobic digestion due to the hydrolysis of macromolecules. This phenomenon was also revealed by an increase of FAS hydroxyl-bearing groups in DS (total acidity, carboxylic COOH and alcoholic OH), an increase of the H/C ratio, as well as a decrease in the molecular weight. In comparison to the values given by Tardy et al. (2000), these results confirm that the molecules are less condensed after AD. The different fictitious atomic groups of RS were similar to those of very condensed molecules such as humin, while the values obtained for DS were comprised between those of humic and fulvic acids.

**Table 4**

Imaginary atomic groups of RS and DS calculated from the elemental species compositions in comparison to humin, humic and fulvic acids (Tardy et al., 2000).

	Raw Slurry	Digested Slurry	Humin	Humic Acid	Fulvic Acid
De-polymerisation degree	0.356	0.621	0.387	0.586	0.857
Total acidity (meq g <sup>-1</sup> C)	4.7	7.1	5.8	6.7	10.3
Carboxylic COOH (meq g <sup>-1</sup> C)	1.8	4.0	2.8	3.6	8.2
Alcoholic OH (meq g <sup>-1</sup> C)	1.3	2.9	2.0	2.6	6.1
Molecular weight (g mol <sup>-1</sup> C)	6.2	4.9	6.0	5.0	3.7

## 4. Conclusion

This work aimed at characterizing the modifications occurring in organic matter on anaerobic biological treatment. The model studied was a pig slurry digestion plant. The results showed that during anaerobic digestion, organic matter is stabilised by the degradation of the most labile fraction leading to a relative increase of more stable compounds. The different chemical, biochemical and biological techniques used in this study (elemental analysis, growth chamber test, soil incubation, biochemical fractionation and FTIR) revealed that this anaerobic biodegradation is comparable to the thermophilic phase of the composting process, but not to a humification process. Then, it was proposed that as the most labile fraction governs OM behaviour in soil in the short-term, spreading stabilised anaerobically digested organic matter should be less disturbing for the soil microflora than spreading raw organic matter. Consequently, there will be less risk of competition for nutrient (N in particular) and oxygen between the crops and the soil bacteria. On the other hand, the aerobic post-treatment of anaerobically digested material will be quicker and less odorous than for a raw material. This is of particular interest for the co-composting of sewage sludge and green waste. Indeed, a preliminary anaerobic digestion of sewage sludge would reduce the quantities to be treated as well as the associated harmful effects while conserving the fertilising value and producing renewable energy.

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