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Characterisation of the biodegradability of post-treated digestates via the chemical accessibility and complexity of organic matter

Géraldine Maynaud^a, Céline Druilhe^{b,c}, Mylène Daumoin^{b,c}, Julie Jimenez^a, Dominique Patureau^a, Michel Torrijos^a, Anne-Marie Pourcher^{b,c}, and Nathalie Wéry^{a*}

^aLBE, INRA, 102 Avenue des Etangs, Narbonne, F-11100, France

^bIrstea, UR OPAALE, 17 avenue de Cucillé, CS64427, Rennes, F-35044, France

^cUniversité Bretagne Loire, France

*Corresponding author. Tel: +33 4 68 42 51 86; E-mail address: nathalie.wery@inra.fr

Abstract

The stability of digestate organic matter is a key parameter for its use in agriculture. Here, the organic matter stability was compared between 14 post-treated digestates and the relationship between organic matter complexity and biodegradability was highlighted. Respirometric activity and CH₄ yields in batch tests showed a positive linear correlation between both types of biodegradability ($R^2=0.8$). The accessibility and complexity of organic matter were assessed using chemical extractions combined with fluorescence spectroscopy, and biodegradability was mostly anti-correlated with complexity of organic matter. Post-treatments presented a significant effect on the biodegradability and complexity of organic matter. Biodegradability was low for composted digestates which comprised slowly accessible complex molecules. Inversely, solid fractions obtained after phase separation contained a substantial part of remaining biodegradable organic matter with a significant easily accessible fraction comprising

simpler molecules. Understanding the effect of post-treatment on the biodegradability of digestates should help to optimize their valorization.

Keywords: digestate; biodegradability; complexity; anaerobic digestion; respirometry

Abbreviations

AD: Anaerobic digestion

AT₄: Cumulative oxygen uptake in four days ($\text{gO}_2 \text{ kg}^{-1}\text{VS}$)

BDAero: Aerobic biodegradability (% COD_{tot})

BDana: Anaerobic biodegradability (% COD_{tot})

BMP: Biomethane potential ($\text{NLCH}_4 \text{ kg}^{-1}\text{VS}$)

BOD: Biological oxygen demand ($\text{gO}_2 \text{ kg}^{-1}\text{VS}$)

CO₂: Carbon dioxide

COD_{tot}: Total chemical oxygen demand ($\text{gO}_2 \text{ kg}^{-1}\text{VS}$)

DOM: dissolved organic matter

DRI₂₄: Average O₂ uptake rate in the 24 h of maximum activity ($\text{mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}\text{VS}$)

MB: easily biodegradable fraction (%BDAero)

MH: slowly biodegradable fraction (%BDAero)

MSW: Municipal solid waste

NEOM: Non extractable organic matter (% COD_{tot})

O₂: Oxygen

OFMSW: Organic fraction of municipal solid waste

OM: Organic matter

OUR: Oxygen uptake rate ($\text{mmO}_2 \text{ h}^{-1} \text{ kg}^{-1}\text{VS}$)

PEOM: Poorly extractable organic matter (% COD_{tot})

REOM: Readily extractable organic matter (% COD_{tot})

RT: Retention time

SEOM: Slowly extractable organic matter (% COD_{tot})

SPOM: Soluble extractable fraction from particular extractable organic matter (%

COD_{tot})

TS: Total solid

VS: Volatile solid

1. Introduction

Anaerobic digestion (AD) is a biological treatment that converts organic waste into methane (CH₄). It can thus produce energy, while the digestate is regarded as a potential organic fertilizer. The digestate is a fully fermented nutrient-rich material which can ideally replace inorganic fertilizer (Tambone et al., 2010). Many studies focus on the optimization of CH₄ production from organic waste but rarely on the assessment of the agronomic value of digestate and its valorisation through land application (Teglia et al., 2010). At times, when digestate is not fully stabilized, it can present residual biodegradability and contain complex organic elements such as ligno-cellulosic compounds which the digestion process cannot degrade (Bayard et al., 2015). Consequently, post-treatments such as solid-liquid separation, drying or composting, are generally recommended before land application and the fertilizer produced should also fulfil current standards, *i.e.* efficiency and safety (Teglia et al., 2011).

To be used as an organic fertilizer, stability or biodegradability of a digestate has to be fully assessed prior to land application. The biodegradability of organic residues

can be evaluated using (i) biological methods including aerobic respirometric tests and anaerobic test methods (Binner and Zach, 1999; Wagland et al., 2009) and (ii) non-biological methods including dry matter, organic matter (OM) and total organic carbon content (Godley et al., 2004). The use of the biomethane potential test (BMP) aims at assessing the biogas potential of both organic waste and digestate. It is the most common method applied for measuring the anaerobic biodegradability of organic residues (Schievano et al., 2008). However, the BMP test protocol can vary from one laboratory to another (Raposo et al., 2011) thus leading to large discrepancies. It is also a time-consuming method (>30 days). For these reasons, alternative approaches such as respirometric tests have been considered (Cossu and Raga, 2008; Scaglia et al., 2010). These aerobic methods are based on the oxygen (O₂) measurement uptake or carbon dioxide (CO₂) production which are related to OM biodegradation under controlled conditions. As organic residues can either be directly applied on land or stored prior to their application, they can be exposed to both aerobic and anaerobic environments. An assessment of their biodegradability under aerobic and anaerobic conditions thus provides complementary information on their characteristics. Furthermore, correlations between aerobic and anaerobic tests have been reported by several authors, especially for organic waste before its biological treatment, such as AD processes or composting (Cossu and Raga, 2008; Ponsá et al., 2008; Barrena et al., 2009; Bohm et al., 2010; Liu et al., 2015). However, information is scarce concerning such correlations on digestates.

The objectives of this study are (i) to investigate the biodegradability under both anaerobic and aerobic conditions of 14 post-treated digestates (seven composted or dried solid fractions of digestate and seven solid fractions of digestate obtained after

liquid-solid separation) and (ii) to characterize their OM in order to better assess the impact of post-treatments on their characteristics and stability. In this view, a recent method developed to characterize both the accessibility and complexity of organic wastes (Jimenez et al., 2015) has been applied. This method combines successive chemical fractionations to 3D fluorescence spectroscopy. The final goal was correlating biodegradability and OM characterization and describing the effect of post-treatment on the digestate stability. This could help to define better strategies for agricultural re-use of these organic residues.

2. Material and methods

2.1. Sampling of digestates

Fourteen digestates were sampled from 13 AD sites in France: 7 solid fractions of digestate (SL1S, SL2S, A1S, T1S, T2S, T3S, T4S) obtained following solid-liquid phase separation, 1 dried and composted solid fraction of digestate (A2D) and 6 composted solid fractions of digestate (A3C, A4C, SL1C, O1C, O2C, M1C). The AD sites treated various types of waste, including agricultural waste, sewage sludge from a wastewater treatment plant (WWTP), municipal solid waste (MSW), the organic fraction of MSW (OFMSW), food processing waste (FPW), all according to different digestion processes (wet or dry, mesophilic or thermophilic). The designation of the digestates and their origins are presented in Table 1. All of the samples collected (about 50 kg each) were homogenized for further analyses.

2.2. Physico-chemical characterisation of digestates

Representative aliquots of digestate (about 2 kg) were used to perform all the analytical tests. For each sample, the total solid (TS) content was measured by drying 100 g of sample at 105 °C for 48 h. The volatile solid (VS) content was measured after calcination of this dried sample at 550 °C for 3 h. Prior to total chemical oxygen demand (COD_{tot}) measurements, the dried sample was ground to a particle size of 500 µm. Then, COD_{tot} was determined by titration after digesting 1 g of TS with H₂SO₄ and K₂Cr₂O₇ in accordance with the AFNOR standard NF T90-101. COD_{tot} results were expressed in gO₂ kg⁻¹ VS. All measurements were performed in triplicate.

2.3. Residual biodegradability assays

2.3.1. Aerobic biodegradability assay (BD_{aero})

The aerobic biodegradability was measured on fresh digestates (between 2 and 4 kg according to the bulk density) using a dynamic respirometric method (Berthe et al., 2007). The respirometric device consisted of 10 L hermetically sealed stainless steel cells. These were filled with the studied substrates mixed with plastic Pall rings acting as inert bulking agent. Each cell was maintained at 40 °C by means of a water bath and was supplied with a continuous air flow rate of 70 L h⁻¹. The incoming air was preheated to the water bath temperature while the aeration system also included a rapid recirculation of part of the exhaust air (360 L h⁻¹), thus ensuring homogeneous conditions throughout the substrate. In order to measure the O₂ uptake rate (OUR) during the respirometric experiment, the oxygen concentrations of the inlet and outlet gases were monitored every 2 min using a paramagnetic analyser (Magnos 206, ABB, Zurich, Switzerland). The respirometric test was finally halted when the O₂ uptake rate fell to a low and stable value.

The biological oxygen demand (BOD) of the digestate corresponded to the cumulative O₂ uptake during the test. Two classical aerobic indices were also calculated for each substrate: AT₄ related to the cumulative O₂ uptake in four days and DRI₂₄ related to the average OUR in the 24 h of maximum activity (Ponsa et al., 2010). Moreover, the aerobic biodegradability (BDaero) could be expressed as the ratio between BOD and COD_{tot}, according to Equation 1 (Liu et al., 2015).

$$\text{BDaero}(\% \text{COD}_{\text{tot}}) = (\text{BOD}(\text{gO}_2 \text{ kg}^{-1} \text{VS}) \times 100) / \text{COD}_{\text{tot}}(\text{gO}_2 \text{ kg}^{-1} \text{VS}) \quad (1)$$

A conceptual approach of typical OUR profiles has been described by Trémier et al. (2005): firstly (i) a rise to a peak value, OUR_{max}, associated with the biodegradation of the easily biodegradable OM (MB) and biomass growth, secondly (ii) a break in the exponential growth and OUR decrease assumed to correspond to the biodegradation of the slowly biodegradable OM (MH) that has to be hydrolysed before biological consumption, and thirdly (iii) the stabilization of OUR at a low and constant value associated with the disappearance of all biodegradable OM and the endogenous respiration of the biomass. On the basis of these previous assumptions, a simplified estimation of MB and MH could be carried out: the MB value represented the cumulative O₂ consumption during the rising phase, until OUR_{max}, while the MH value characterised the cumulative O₂ uptake during the decrease phase, from OUR_{max} to endogenous respiration. During this study, different OUR profiles were obtained: typical profiles with a single OUR peak, atypical profiles with two or three O₂ uptake peaks. A simplified estimation of MB and MH was then performed for each separated peak: the MB₁, MB₂ and MB₃ values represented the cumulative O₂ consumption during the rising phase of the first, second and third peak respectively, and the MH_i values (i=1, 2 or 3) represented the cumulative O₂ consumption during the decreasing

phase of the first, second and third peak, respectively. Following the previously described calculations, M_{Bi} and M_{Hi} were expressed in %BDaero.

2.3.2. Anaerobic biodegradability assay (BDana)

An anaerobic batch test was used to quantify the residual CH₄ production in digestate. This was measured on fresh digestate without inoculation for 40 days using the following protocol: 200 g of fresh digestate were suspended in 200 mL of sodium bicarbonate solution (2.5 g L⁻¹) in 500 mL flasks. To ensure anaerobic conditions, the solution in flask was flushed with N₂ for a few seconds. The flasks were sealed with gas-tight rubber stoppers and aluminium seals and incubated at 37 °C for 40 days in the dark and under a constant shaking. Each digestate was tested in duplicate. Biogas production and gas composition were estimated twice a week using a water displacement method and gas chromatography (Perkin Elmer Clarus®580), respectively. The CH₄ yield was calculated using the experimental results and expressed in NL CH₄ kg⁻¹ of VS under standard temperature and pressure conditions (0 °C and 1013 hPa).

Anaerobic biodegradability (BDana) was expressed as the ratio between the mean of CH₄ yield and COD_{tot} of the studied digestate, in accordance with the Equation 2 of Liu et al. (2015).

$$BDana(\%COD_{tot}) = (CH_4 \text{ yield}(\text{NL CH}_4 \text{ kg}^{-1}\text{VS}) \times 100) / (0.35 \times COD_{tot}(\text{gO}_2 \text{ kg}^{-1}\text{VS}))$$

(2)

Due to the low frequency of CH₄ measurements (twice a week), the kinetic curves of the anaerobic batch tests were not analysed. Only the cumulative CH₄ production at the end of the experiment was taken into account.

2.4. Organic matter characterisation

2.4.1. Chemical sequential extractions

Organic matter was characterised according to Jimenez et al. (2015). Firstly, the sample was dried and grinded to 1 mm particle size, and the following sequential extractions were performed on 0.5 g of dried sample. Four fractions of decreasing accessibility were then obtained: (i) soluble extractible OM (SPOM) produced after four sequential extractions (30 mL of CaCl₂ solution 10 mM) of the remaining pellet for 15 min at 30 °C and 300 rpm, (ii) readily extractible OM (REOM) produced after four sequential saline basic extractions (30 mL of NaCl and NaOH 10 mM) of the remaining pellet for 15 min at 30 °C and 300 rpm, (iii) slowly extractible OM (SEOM) produced after four sequential strong basic extractions (30 mL of NaOH 0.1 M) of the remaining pellet for 4 h at 30 °C and 300 rpm, (iv) poorly extractible OM (PEOM) produced after two sequential acid extractions (25 mL of H₂SO₄ 72% (w:w)) of the remaining pellet for 3 h at 30 °C and 300 rpm. Finally, the non-extractible OM (NEOM) was calculated by subtracting the total OM extracted from the sample from the initial OM. At each step, the solubilised OM was recovered by centrifugation (18600 g at 4 °C for 20 min) and filtered at 0.45 µm.

The chemical oxygen demand (COD) of total residues and extracts was measured in accordance with the standard ISO 15705:2002 in order to characterise the OM content of each fraction. Results are expressed as % COD_{tot}.

2.4.2. Fluorescence spectroscopy analysis

Similar to the procedure developed by Jimenez et al. (2014 and 2015), 3D fluorescence spectroscopy was applied to the extracted fractions, from which OM complexity could be quantified. The fluorescence spectrometer used was a Perkin Elmer LS55. Excitation wavelengths varied from 200 to 600 nm with 10 nm increments. According to Jimenez et al. (2014) and Muller et al. (2014), the spectra were decomposed into seven zones corresponding to biochemical fluorescence family-types. The proportion of fluorescence in a given zone “i” $P_f(i)$ was calculated from the fluorescence zone volumes $V_f(i)$ according to Equations 3 and 4. An example of spectra decomposed into seven fluorescence zones is presented in the Supplementary figure 1.

$$V_f(i) (\text{UA mg COD L}^{-1}) = \frac{V_f(i)}{\text{COD}_{\text{sample}}} \times 1 / \frac{S(i)}{\sum_{i=1}^7 S(i)} \quad (3)$$

$$P_f(i) (\%) = \frac{V_f(i)}{\sum_{i=1}^7 V_f(i)} \times 100 \quad (4)$$

Where:

$V_f(i)$ (U.A. mg COD L⁻¹): the raw volume of the zone i,

$\text{COD}_{\text{sample}}$ (mg L⁻¹): the COD concentration of the sample,

$S(i)$ (nm²): the area of a zone i,

$P_f(i)$ (%): the fluorescence proportion of a zone i.

2.5. Statistical analysis

Correlations between the CH₄ yield and each of the parameters obtained from respirometry (BOD, AT₄ and DRI₂₄), and between anaerobic (BD_{ana}) and aerobic (BD_{aero}) biodegradabilities were investigated using linear regression evaluation. The analytical data were processed using principal component analysis (PCA) to identify multiple correlations between variables including BD_{ana}, BD_{aero}, MBI and MHI (i=1,

2 or 3), the complexity related to fluorescence and the %COD_{tot} of extracted OM fractions and NEOM fraction. A Pearson correlation matrix was performed in order to test for correlations between anaerobic and aerobic biodegradabilities and %COD_{tot} of each extracted fraction. Statistical analysis was performed using the Rcmdr package of R-Software (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Characterization of digestates

Table 2 summarizes the physico-chemical and biological properties of digestates. Anaerobic and aerobic biodegradabilities were calculated with the CH₄ yield and BOD, respectively, and with respect to COD_{tot} content of digestate. The TS content ranged between 40.9-74.6 % for dried or composted solid digestate fractions, the highest value being observed for the dried sample A2D. In solid digestate fractions the TS content varied between 22.3-26.4 %. The VS ranged between 39.3-86.5 % TS and the COD_{tot} between 1245-1696 gO₂ kg⁻¹ VS in all the digestates, without any notable differences related to the applied post-treatment.

The calculated aerobic and anaerobic biodegradabilities (BD_{aero} and BD_{Dana}) presented differences amongst digestates depending on post-treatment applied. Indeed, composted or dried solid fractions of digestate were characterized by low CH₄ yields and BOD (0-28 NLCH₄ kg⁻¹ VS and 39.8-254.4 gO₂ kg⁻¹ VS) corresponding to BD_{Dana} of <0.5-5.2 % COD_{tot} and BD_{aero} of 2.8-18.6 % COD_{tot}. The solid fractions of digestate showed higher values (41.1-93.8 NLCH₄ kg⁻¹ VS, BD_{Dana}=7.2-18.6 % COD_{tot} and 237.0-550.0 gO₂ kg⁻¹ VS, BD_{aero}=14.6-38.0 % COD_{tot}). These results demonstrated that the composting step of the solid fraction significantly reduce the biodegradable OM

content. Among the solid fraction composts, A4C was characterized by the lowest biodegradability under both aerobic and anaerobic conditions ($BD_{ana} < 0.5\%$ and $BD_{aero} = 2.8\% \text{ COD}_{tot}$), thus suggesting that the OM of A4C was the most stable of all studied samples. For solid fractions, A1S presented highest biodegradable COD under both conditions ($BD_{ana} = 18.6\%$ and $BD_{aero} = 38\% \text{ COD}_{tot}$). The total retention time of the treatment line for A1S was short (between 14 and 20 days, table 1) when compared to the other digestates (between 15 and 95 days). This could explain the important part of residual biodegradability in this digestate. The values of biodegradability for the solid fractions were in the range of values previously obtained by Bayard et al. in 2005. These authors reported that the BD_{ana} of digestates from AD of residual municipal solid waste or biowaste ranged between 14.6 and 31.4 % COD and the BD_{aero} ranged between 19.8 and 44.1 % COD. Consequently, despite the digestion process, certain digestates still contained a significant portion of residual biodegradable OM, as has been previously reported (Teglia et al., 2011; Bayard et al., 2015). Depending on the post-treatment conditions, this portion can be more or less important. In terms of biodegradability, the differences observed between digestates were probably due to the differences in the structure of OM. The characterization of OM by chemical sequential extractions describing the accessibility of OM (% COD_{tot} for each extract) showed that the remaining OM in all digestates was essentially composed of less accessible fractions ($SEOM + PEOM = 25\text{--}78\% \text{ COD}_{tot}$). The most accessible fractions $SPOM + REOM$ only represented 2.7–10.6 % COD_{tot} . In terms of accessibility, no difference was observed between digestates. Teglia et al. (2011) reported similar results: the remaining OM of digestate and composted digestate obtained after digestion of food processing waste, agricultural wastes, organic fraction of municipal solid waste and sludge of wastewater

treatment plant, mostly comprised slowly biodegradable OM. These authors demonstrated that the easily biodegradable fraction of OM ranged between 2.7 and 6.3 % COD with no observable distinction between raw digestates and composted digestates. The non-extracted fraction (NEOM), calculated according to the extracted fractions, represented a large part of COD_{tot} (between 15.3-70.8 %). For most of the digestates (10 out of 14) the NEOM fraction represented more than 40 % COD_{tot}. Jimenez et al. (2015) reported that the NEOM of sludge digestate ranged from 30.0 to 43.1 % COD_{tot} with more than 40 % COD_{tot} for the digested sludge compost. In the present study, the composted or dried digestates, excepting two composted digestates (A3C and SL1C, <20 % COD_{tot}), also presented a NEOM fraction greater than 40 % COD_{tot}.

3.2. Correlation between both aerobic and anaerobic biodegradabilities

Fig. 1 illustrates linear positive correlations between anaerobic and aerobic biodegradabilities (BD_{Dana}, BD_{Aero}) and between CH₄ yield and parameters obtained from respirometry data (BOD, AT₄ and DRI₂₄) for the 14 post-treated digestates. Concerning the anaerobic biodegradability, the mean value of the CH₄ yield measured in duplicate was chosen for Fig.1 because values indicated a good reproducibility (coefficient of variation <15 %, data not shown).

Significant positive correlations were obtained between BD_{Dana} and BD_{Aero} and between CH₄ yield and BOD ($R^2=0.8$), whereas the correlations between CH₄ yield and AT₄ and between CH₄ yield and DRI₂₄ were less significant (R^2 of 0.7 and 0.6, respectively). This was due to the underestimation of the aerobic biodegradability of one digestate, A1S (point surrounded on Fig. 1b and Fig. 1c), using AT₄ and DRI₂₄.

Indeed, this sample presented the slowest respirometric kinetic, with an oxygen uptake rate of about $40 \text{ mmO}_2 \text{ h}^{-1} \text{ kg}^{-1} \text{ VS}$ after 200 h, and three clear OUR peaks (Fig. 3b).

Short-term aerobic indices such as AT_4 and DRI_{24} appeared to be insufficient to represent the overall biodegradability of this type of substrate still highly biodegradable and with a high activity level for a longer period of time. The biodegradability is, in this case, better estimated with the BOD value.

A significant positive correlation between both aerobic and anaerobic biodegradabilities and biological indices has been observed in previous studies considering raw waste or organic residues during composting treatment (Binner and Zach, 1999; Cossu and Raga, 2008; Barrena et al., 2009; Bohm et al., 2010; Liu et al., 2015). Few studies have reported this correlation for digestates. Bayard et al. (2015) demonstrated a linear regression between both biodegradabilities for four substrates and four raw digestates from residual municipal solid waste and biowaste. Schievano et al. (2008) also reported a positive correlation between anaerobic biogasification potential and O_2 uptake rate on 46 samples including waste mixtures (agricultural, agro-industrial wastes and organic fraction of municipal solid waste) and their raw digestates. Finally, Ponsa et al. (2008) described a positive and linear correlation between aerobic and anaerobic stability indices for organic solid wastes during different stages of biodegradation including raw digestates and digestates post-treated by composting. Hence, the present study appears to be the first that has demonstrated a significant correlation between both aerobic and anaerobic indices for digestate samples post-treated by phase separation and composting.

Interestingly, the slope of the linear correlation between BD_{aero} and BD_{ana} (1.8, Fig. 1d) suggested that the OM contained in all digestates was less biodegradable

under anaerobic conditions than aerobic conditions. This result on digestate samples has already been obtained by Bayard et al. (2015) on raw digestates from AD of residual municipal solid waste and biowaste. According to Monlau et al. (2012), certain biodegradable parts are not accessible to microorganisms during AD, most likely due to the compositional and structural characteristics of ligno-cellulosic materials. Wagland et al. (2009) also reported that microbial growth efficiency under anaerobic conditions was lower than under aerobic conditions.

Consequently the CH₄ yield and BOD constitute good indicators for describing the biodegradability and the stability of digestates. They can thus also represent useful tools for assessing the efficiency of biological treatment process (Cossu and Raga, 2008; Ponsá et al., 2008; Barrena et al., 2009; Bayard et al., 2015).

3.3. Relation between biodegradability, accessibility and complexity of OM

The full dataset was analysed through PCA in order to highlight correlations between the aerobic and anaerobic biodegradabilities (BD_{Dana} and BD_{Daero}), the accessibility of OM and its complexity, obtained by combining chemical extractions and fluorescence spectroscopy. The two major components, PCA1 and PCA2, were sufficient to describe 76.3 % of the data variability (Fig. 2). The PCA1 essentially describes the complexity of each accessible fraction (1-7 zones) and the aerobic and anaerobic biodegradabilities, while the PCA2 mainly describes the chemical accessibility of OM. The PCA correlation circles also illustrated that BD_{Dana} and BD_{Daero} were particularly well correlated with the variables describing the first component, *i. e.* the OM complexity. The low correlation values between digestate biodegradability and OM accessibility was confirmed by a Pearson's correlation

analysis for BDana, BDaero and %CODtot of each extracted fraction of OM (Table 3). Indeed, this analysis demonstrated that the BDana and BDaero biodegradabilities only correlated with the most easily accessible fraction SPOM ($R^2=0.8$ and 0.6 , respectively), and not with the other accessible fractions REOM, SEOM and PEOM. Likewise, Bayard et al. (2015) studied the correlations between the BMP and different OM fractions. These authors pointed to a significant positive correlation between BMP and water soluble COD, and negative correlations between BMP and Van Soest residual fraction and humic substances, respectively. Here, the PCA correlation circle (Fig 2a) illustrated that the two biodegradability indicators were positively correlated with the simplest forms of molecules (zones 1-3 of complexity related to fluorescence) and were negatively correlated with complex molecules (zones 4-7 of complexity related to fluorescence) such as fulvic acid-, melanoidin-, lignocellulose- or humic acid-like molecules. The previous correlations between anaerobic biodegradability and OM complexity have already been reported in studies carried out on wastes intended for AD processes (Gunaseelan, 2007) or treated by AD (Jimenez et al., 2014).

The PCA1 axis governed the classification of digestates into two distinct groups (Fig. 2b). Composted and dried solid fractions of digestate were clearly separated from solid fractions of digestate, revealing that the composting post-treatment step significantly affected the biological and biochemical properties of digestate. This has also been reported by Bayard et al. (2015) on substrates and digestates from biowaste and residual municipal solid waste. Composted and dried solid fractions of digestate were characterized by low aerobic and anaerobic biodegradabilities. They mostly contained the slowly accessible fraction SEOM associated to humic-like substances including complex molecules (zones 4-7 of complexity related to fluorescence) as

reported by Jimenez et al. (2015). Indeed, the complexity of composted solid fractions of digestate could be attributed to humic and fulvic acid-like and lignocellulosic-like compounds found in fluorescence zones 4-7 of the easily and slowly accessible fractions (SPOM+REOM+SEOM), which are known to be recalcitrant and slowly biodegradable. Inversely, solid fractions of digestate without a composting post-treatment were characterized by high amounts of aerobic and anaerobic biodegradability indicators and contained a large part of easily accessible fraction (SPOM) comprising simple forms of molecules. Solid fractions of digestate contained proteins molecules observed in fluorescence zones 1-3 of easily and slowly accessible fractions (SPOM+REOM+SEOM).

These results suggest that OM contained in digestates present different levels of complexity according to the applied post-treatment. Composted or dried solid fractions of digestate characterized by low biodegradability and high complexity were found to be more stable than solid digestate fractions, thus allowing for their agricultural re-use by land spreading (Ponsá et al., 2008; Teglia et al., 2011). On the contrary, the solid digestate fractions, which were characterized by a higher biodegradability and lower complexity, should most likely be subjected to a complete composting step or to a storage period in order to ensure their stabilization before they can be re-used for agricultural purposes. Although the impact of post-treatments on the biodegradability of organic matter in solid-fractions of digestate was here demonstrated, it must be pointed out that other factors such as types of feedstocks or digester operating conditions can also impact the organic matter properties of digestates. Finally, OM characterization using fluorescence spectroscopy is easier and faster to perform than aerobic and

anaerobic tests; it could therefore be applied to evaluate the biodegradability and thus the status of digestate as a relatively stabilized fertilizer.

3.4. Kinetics of respirometric activity

Only the respirometric kinetics have been taken into account in the results. Indeed, the O₂ uptake rate was measured more frequently (every 2 min) than the CH₄ production rate (twice a week). During the respirometric experiments, the OUR kinetics showed various profiles according to the studied digestate (Fig. 3). Nine digestates, including all the dried and composted solid fractions of digestate, were characterized by a profile with only one peak of O₂ consumption, followed by a decrease of activity until endogenous respiration (Fig. 3a). These profiles were classical on solid organic wastes, such as municipal waste (Adhikari et al., 2013) or mixtures of sludge and bulking agent (Mohajer et al., 2009). At the opposite, five digestates showed atypical profiles with a second (SL1S, T1S, T2S, T3S and A1S) and a third OUR peak (T3S and A1S) before reaching the endogenous respiration (Fig. 3b). Atypical OUR kinetics could be explained by combining two phases of OM biodegradation according to (i) the OM accessibility and/or (ii) the released OM components, that both changed with time.

The applied AD process (e.g., temperature and retention time) and the type of treated waste could also impact the OM composition of the obtained digestate as well as its respirometric profile. Atypical kinetics with two OUR peaks have already been reported by Zeng et al. (2016) in a study on digestates obtained after thermophilic AD of municipal solid waste. Atypical kinetics were here observed in digestates from both mesophilic (T1S, T2S, T3S) and thermophilic (A1S and SL1S) AD processes, and with different treated wastes (agriculture waste, territorial waste, urban sludge). These results

demonstrate that the type of digestion process and the nature of treated waste do not seem to explain the observed atypical kinetics of respirometric activity.

In order to understand the appearance of two or three OUR peaks, a PCA was carried out on the different parameters describing the OM accessibility, its complexity and the fractionation of BDaero into MB_i and MH_i (i=1, 2 or 3) according to the number of OUR peaks (calculations described in 2.3.1) (Fig. 4). Two components, PCA1 and PCA2, were sufficient to describe 67.4 % of the data variability. Variables describing the PCA1 axis were MB1 which characterized the easily biodegradable OM linked to the first OUR peak, and the fluorescence-related complexity of each accessible fraction (zones 1-7). As for the previous results, the variable describing the PCA2 axis was the chemical accessibility of OM. MB1 was positively correlated with the simpler molecules of OM fractions (Fig. 4a and R^2 of the correlation between MB1 and fluorescence zones 1-3 of SPOM+REOM+SEOM = 0.5-0.7, *p-values*<0.05, data not shown). Even with the data description using the other PCA axes, the other MB_i and MH_i axes were not significantly correlated with the accessibility and/or the complexity (data not shown). Moreover, axes of PCA led to the separation from other digestates of four out of the five digestates presenting atypical kinetics (Fig. 4b). The parameters then used to describe the OM in this study were not sufficient and/or relevant to explain the observed atypical OUR kinetics. Further work is still required for a better comprehension of the observed multiple peaks. For example, during the respirometric test, enzymatic activities and bacterial and fungal community composition (by high-throughput sequencing analysis) could also contribute to the evolution of the nature of the OM. Moreover, the aqueous compartment of OM could be characterized in terms of apparent molecular weight by size exclusion chromatography.

4. Conclusions

Solid fractions were found to comprise a significant part of remaining biodegradable OM despite the digestion process. Composted solid fractions were characterized by low biodegradable OM containing complex molecules, thus implying the presence of a more stable OM. The relation between complexity and biodegradability that has been demonstrated in this study has allowed for the effect of post-treatments on the stability of digestates to be better understood. Consequently, these data on OM stability should contribute to the improvement of agricultural re-use of digestates as fertilizers.

An example of decomposition of a spectra obtained by fluorescence spectroscopy analysis into seven zones of fluorescence is presented in the Supplementary Figure 1.

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Figure Captions

Fig. 1. Correlations between BOD and CH₄ yield (a), AT₄ and CH₄ yield (b), DRI₂₄ and CH₄ yield (c), and BDaero and BDana (d) of digestates (white diamonds: solid fractions)

of digestate; black circles: composted or dried solid fractions). The mean for duplicates are given for the CH₄ yield and the BD_{ana} is calculated from these mean values.

Fig. 2. Principal Components Analysis on biodegradabilities and organic matter characterization: variables on correlation circle (a) (square symbol for BD_{ana} and BD_{aero}, triangle for %COD_{tot} of extracted OM fractions and NEOM and circle for complexity related to fluorescence of each extractable fraction) and distribution of digestates (b) (solid fractions of digestate in white circles and composted or dried solid fractions in black circles). SP, RE, SE, PE and NE correspond to SPOM, REOM, SEOM, PEOM and NEOM respectively. sp1-7, re1-7, se1-7 and pe1-7 correspond to fluorescence zones (1-7) of extractable fractions SPOM, REOM, SEOM and PEOM, respectively.

Fig. 3. Kinetics of respirometric activity during the first 600h for digestates classified according to the number of OUR peaks: digestates with only one peak (a) and digestates with two or three peaks (b).

Fig. 4. Principle Components Analysis on respirometric kinetics and organic matter characterization: variables on correlation circle (a) (square symbols for MBi and MHi, triangles for %COD_{tot} of extracted OM fractions and NEOM and circles for complexity related to fluorescence of each accessible fraction) and distribution of digestates (b). SP, RE, SE, PE and NE correspond to SPOM, REOM, SEOM, PEOM and NEOM respectively. sp1-7, re1-7, se1-7 and pe1-7 correspond to fluorescence zones (1-7) of each extractable fraction SPOM, REOM, SEOM and PEOM, respectively.

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Table 1. Digestate designation and origin.

Digestate ^a	Nature	Main digester feedstock ^b	Ton of digested waste (T/year)	Digestion process			Phase separation of digestate
				Type of AD	Total RT ^c (days)	Temperature (°C)	
SL1S	Solid fraction	WWTP sludge	4000	Wet	15	55	Centrifugation
SL2S	Solid fraction	WWTP sludge	- ^c	Wet	20-30	37	Thermal centrifugation and filter press
A1S	Solid fraction	Manure	12000	Wet	14-20	53-55	Screw press
T1S	Solid fraction	FPW, slurry	35000	Wet	60	38	Centrifugation
T2S	Solid fraction	FPW	20000	Wet	90-95*	40	Screw press
T3S	Solid fraction	FPW, green waste, fat, WWTP sludge	30000	Wet	47-52*	37	Screw press
T4S	Solid fraction	FPW, WWTP sludge, manure	-	Wet	75-85*	37	Screw press
A2D	Dried and composted solid fraction	Slurry, biowaste, WWTS sludge, fat	12000	Wet	44*	38	Centrifugation
A3C	Solid fraction compost	Manure, green waste	5000	Wet	30-32	40	Centrifugation
A4C	Solid fraction compost	Manure, slurry, green waste	6000	Wet	60	41	Screw press
SL2C	Solid fraction compost	WWTP sludge	-	Wet	20-30	37	Thermal centrifugation and filter press
O1C	Solid fraction compost	OFMSW, FPW	48000	Dry	21	37 or 55°	Screw press
O2C	Solid fraction compost	OFMSW, green waste, fat, FPW	25000	Wet	21	53-57	Screw press and centrifugation
M1C	Solid fraction compost	MSW, biowaste, green waste	100000	Dry	21	38	Screw press and band filter

^aA: agricultural waste, SL: urban sludge, T: territorial waste, O: organic fraction of municipal solid waste (OFMSW), M: municipal solid waste (MSW), S: solid fraction of digestate, D: dried and composted solid fraction of digestate, C: composted solid fraction of digestate, ^bWWTP: wastewater treatment plant, FPW: food

processing waste, ^c:- unknown, ^dRT: retention time including the time of post-digestion when applied (*), ^esample results from the digestate mix originated from two reactors (one at 37°C and one at 55°C) operating in parallel.

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Table 2. Physico-chemical and biological properties of digestates. Analysis of TS, VS, COD_{tot} were performed in triplicate and CH₄ yield in duplicate. The mean and the standard deviation are given for these analyses.

Digestate ^a	TS (%)	VS (%TS)	COD _{tot} (gO ₂ kg ⁻¹ VS)	CH ₄ yield (NL CH ₄ kg ⁻¹ VS)	BOD (gO ₂ kg ⁻¹ VS)	BD _{ana} (%COD _{tot})	BD _{aero} (%COD _{tot})	Chemical sequential extraction (% COD _{tot})				
								SPOM	REOM	SEOM	PEOM	NEOM
SL1S	22.3 ± 0.2	70.4 ± 0.1	1696 ± 6	83.7 ± 3.8	550.0	14.1	32.4	5.3	3.2	18.6	49.5	23.4
SL2S	22.4 ± 0.1	51.7 ± 0.5	1311 ± 44	73.2 ± 2.0	332.1	16.0	25.3	5.6	4.6	10.4	48.4	31.0
A1S	25.6 ± 0.4	86.5 ± 0.4	1439 ± 13	93.8 ± 2.4	546.9	18.6	38.0	6.6	4.1	11.2	32.9	45.2
T1S	23.9 ± 0.1	74.3 ± 0.1	1554 ± 12	66.5 ± 1.3	368.1	12.2	23.7	4.1	3.6	11.1	24.8	56.4
T2S	24.9 ± 0.1	80.1 ± 2.2	1615 ± 14	55.1 ± 3.4	492.6	9.7	30.5	2.7	2.1	4.2	34.3	56.7
T3S	26.4 ± 0.3	82.6 ± 0.6	1508 ± 13	48.0 ± 7.3	446.2	9.1	29.6	3.0	2.2	8.0	40.3	46.5
T4S	22.8 ± 0	61.0 ± 0.5	1627 ± 44	41.1 ± 0.9	237.0	7.2	14.6	1.9	2.4	7.0	17.9	70.8
A2D	74.6 ± 0.8	61.3 ± 0.1	1366 ± 10	19.1 ± 0.3	122.7	4.0	9.0	2.1	1.9	15.8	37.6	42.6
A3C	43.9 ± 0.4	51.7 ± 0.4	1530 ± 35	17.5 ± 0.6	177.8	3.3	11.6	3.1	3.8	30.3	47.5	15.3
A4C	40.9 ± 1.7	47.0 ± 2.4	1425 ± 138	<0.5	39.8	0.0	2.8	1.5	3.8	20.2	19.6	54.9
SL2C	59.1 ± 0.8	50.4 ± 7.3	1440 ± 61	14.6 ± 0.2	93.0	2.9	6.5	5.0	4.8	24.9	46.0	19.3
O1C	60.1 ± 1.1	46.6 ± 1.0	1245 ± 15	22.7 ± 0.2	152.7	5.2	12.3	3.7	1.5	7.5	42.9	44.4
O2C	53.3 ± 1.2	39.3 ± 1.7	1367 ± 133	17.8 ± 0.6	254.4	3.7	18.6	1.6	1.1	4.1	34.0	59.2
M1C	55.8 ± 0.3	42.1 ± 1.1	1541 ± 38	28.0 ± 0.4	176.1	5.2	11.4	2.1	2.2	6.8	29.7	59.2

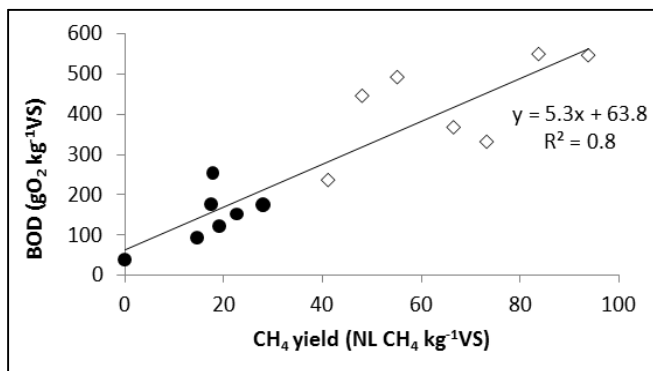
^aA: agricultural waste, SL: urban sludge, T: territorial waste, O: organic fraction of municipal solid waste (OFMSW), M: municipal solid waste (MSW), S: solid fraction of digestate, D: dried and composted solid fraction of digestate, C: composted solid fraction of digestate.

Table 3. Pearson's r correlation analysis between biodegradability under anaerobic (BDana) and aerobic (BDaero) conditions and %COD_{tot} of each extracted fraction of OM (SPOM, REOM, SEOM and PEOM). Significant correlations (** p -values<0.001, ** p -values<0.01 and * p -values<0.05) are in bold.

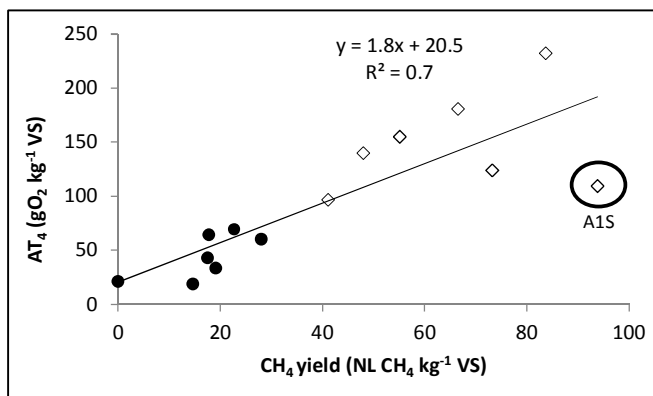
	BDana	BDaero	SPOM	REOM	SEOM	PEOM
BDana	1.0	0.9***	0.8**	0.3	-0.3	0.2
BDaero		1.0	0.6*	0.0	-0.4	0.2
SPOM			1.0	0.6*	0.2	0.5
REOM				1.0	0.6*	0.2
SEOM					1.0	0.4
PEOM						1.0

Fig. 1.

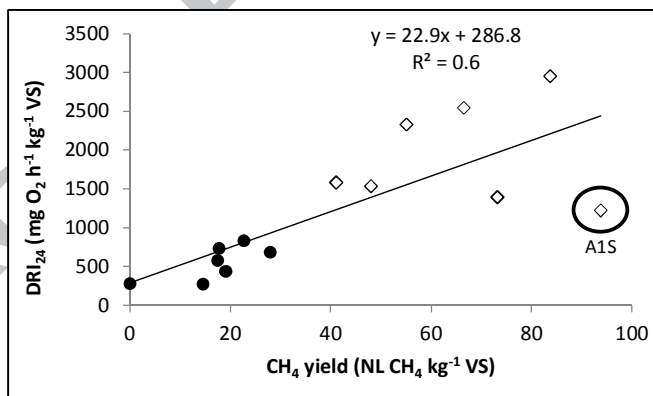
a.



b.



c.



d.

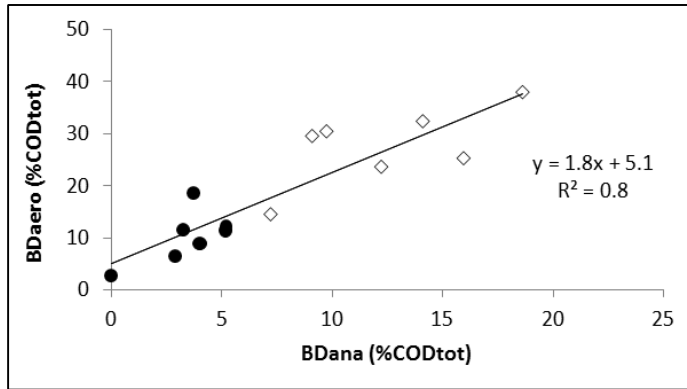
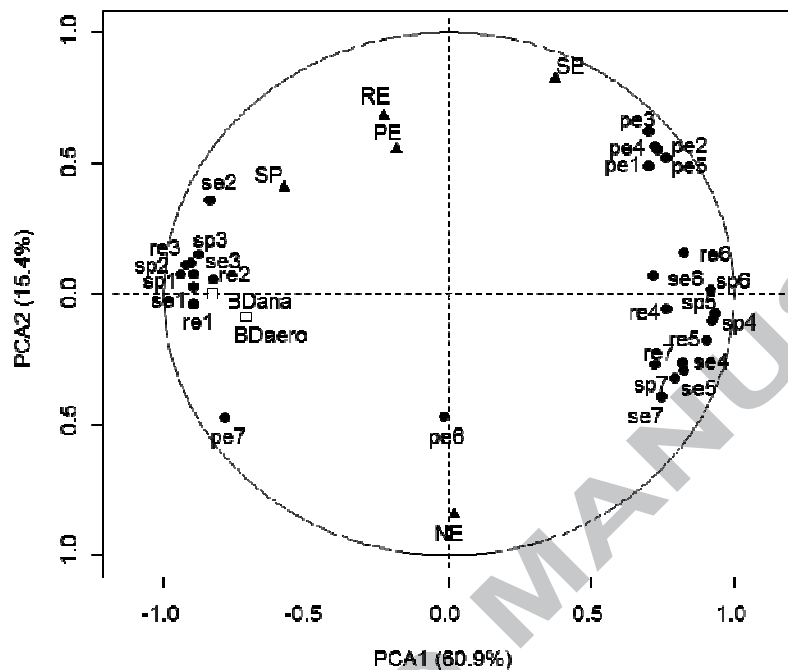


Fig. 2.

(a)



(b)

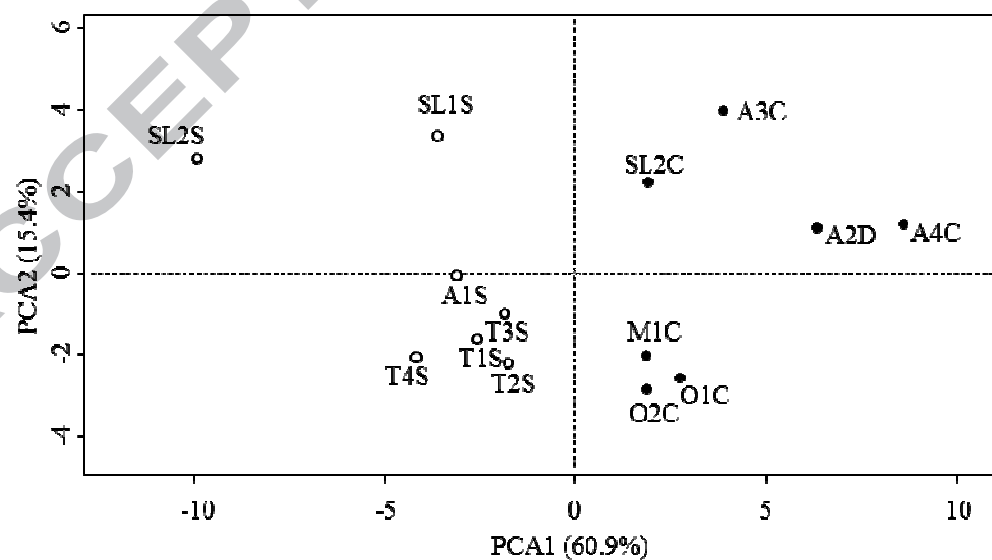
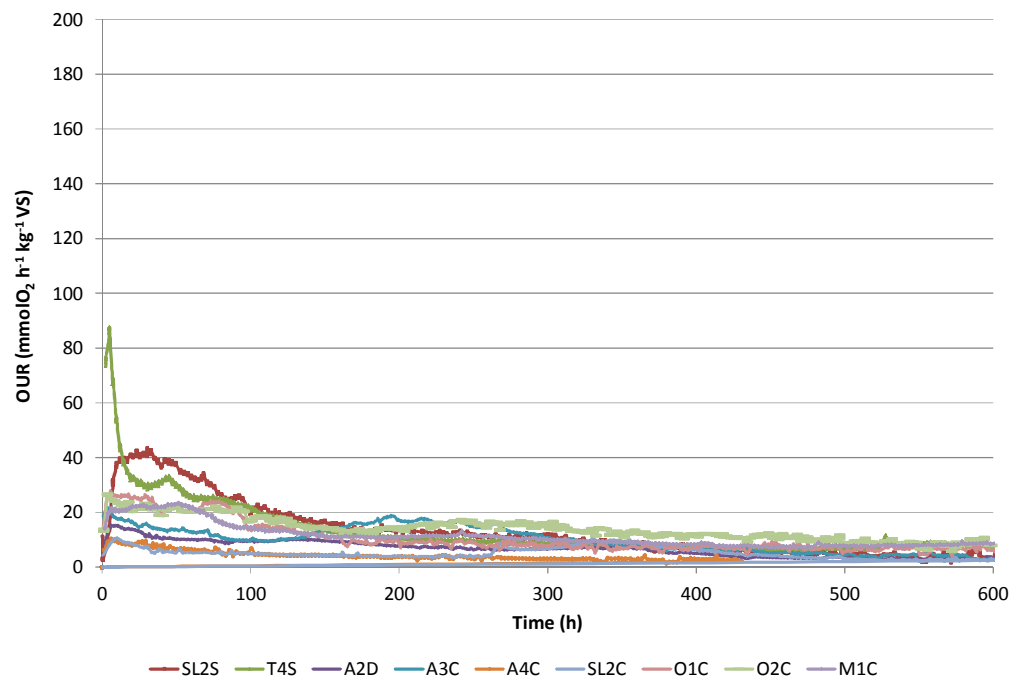


Fig. 3.

(a)



(b)

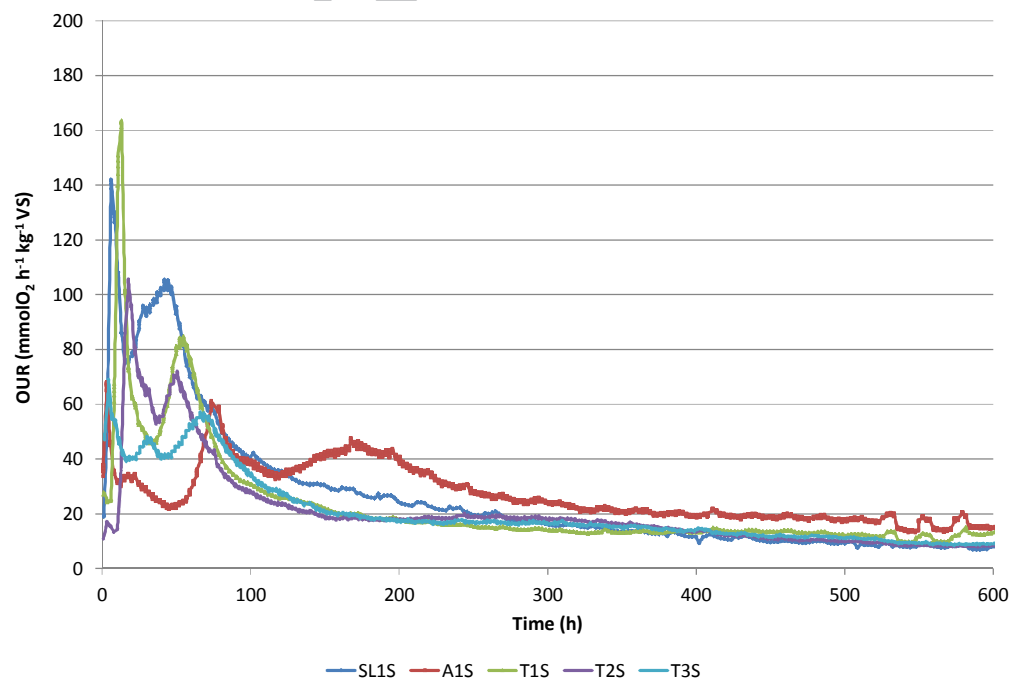
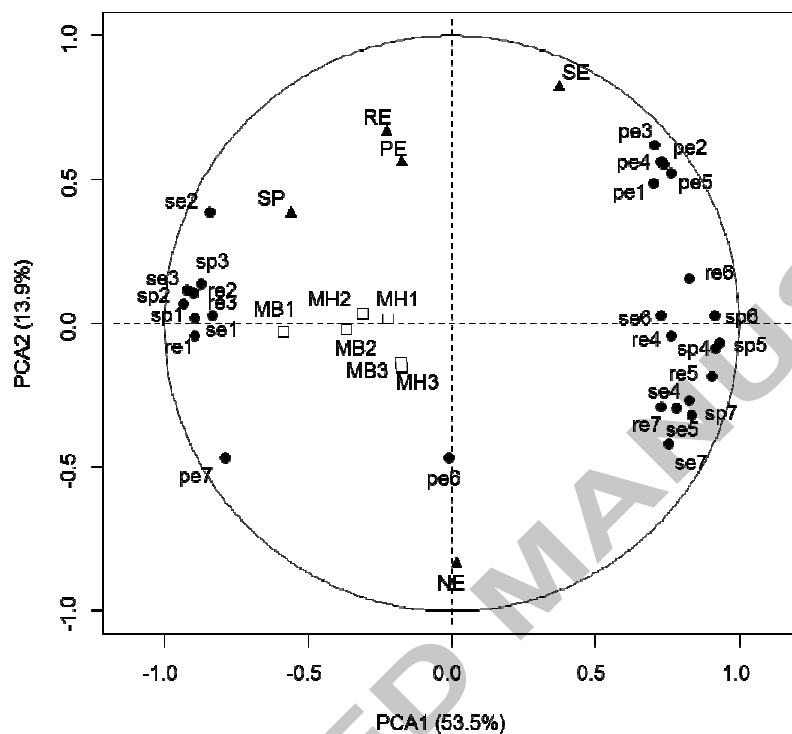
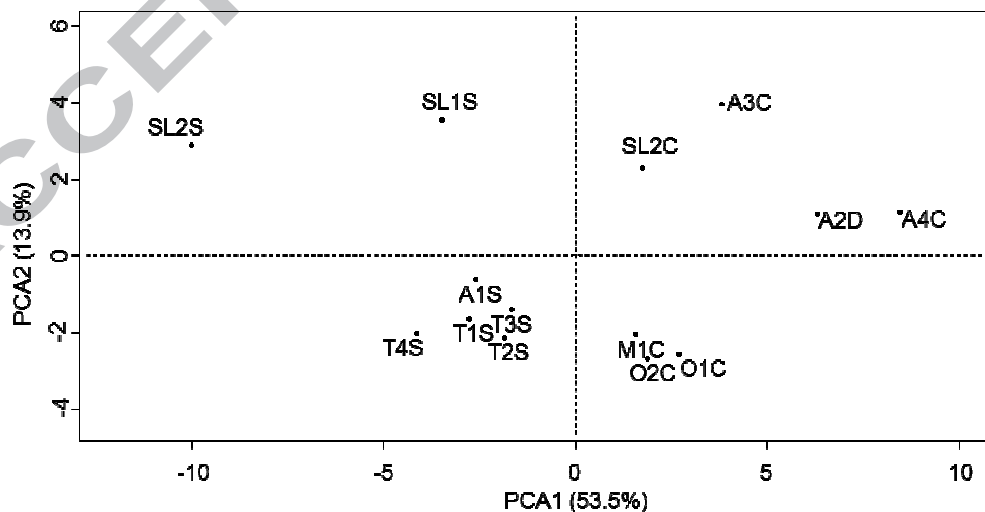


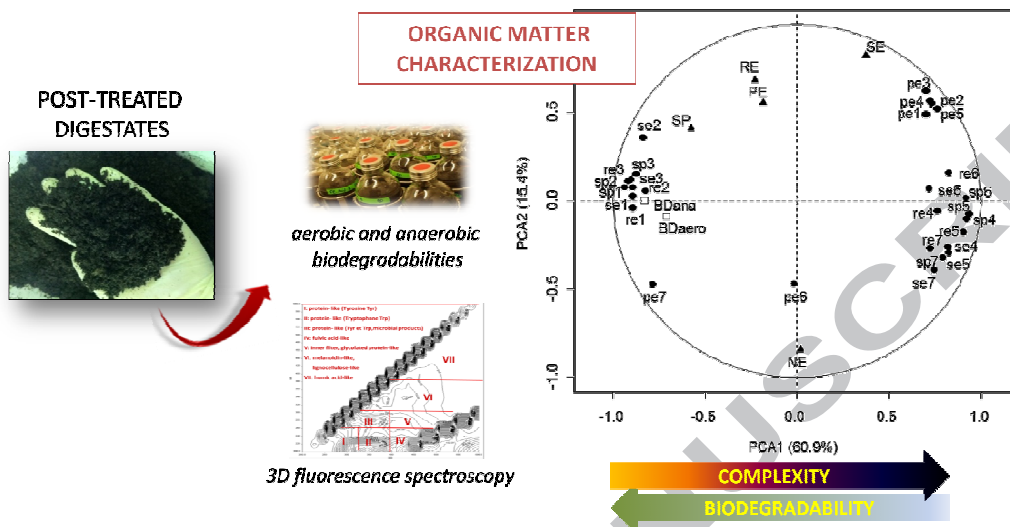
Fig. 4.

(a)



(b)





Graphical abstract

Highlights

- Aerobic and anaerobic biodegradabilities are linearly correlated
- Biodegradability is mostly anti-correlated with the complexity of organic matter
- Post-treatments have a significant effect on biodegradability and complexity
- Kinetics of respirometric activity present multi-peaks for certain digestates

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