Statistical analysis to correlate bio-physical and chemical characteristics of organic wastes and digestates to their anaerobic biodegradability

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Abstract:

Solid waste biodegradability is a key parameter to control and optimize dry solid anaerobic digestion of biowaste (BW) and residual municipal solid waste (RMSW), and allow an assessment of biomethane potential and biostability achieved by the process.

The objective of the study was to compare biochemical methane potential (BMP) data to selected analytical parameters commonly used to predict bioreactivity of solid organic samples, such as global analyses (VS, TOC, COD TKN on solid samples), biochemical analyses (humic substance index, carbohydrates and lignin contents, total lipid, and total protein), leaching behaviour (VFA, COD, N-NTK and $N\text{-}NH_3$), and respirometric activity (*ie*. BOD_{28} on solid samples suspended in liquid medium, for this study). The data set was obtained by analysing for the parameters listed above two Residual Municipal Solid Wastes (RMSW) and two Biowaste (BW), collected from three full-scale dry AD plants, and their respective digestates produced by the selected plants (ie altogether 4 wastes and 4 digestates).

The Principal Component Analysis (PCA) and Partial Least Square Regression (PLS-R) methods were used to investigate the correlations between parameters. Significant effect of the origin of waste samples and AD process was shown with principal component analysis (PCA). Therefore, it was concluded that the parameters used were partly redundant and that the set of parameters could be significantly reduced. Predicting anaerobic bioreactivity from single parameters such as VS, COD, TOC and carbohydrate content was found not relevant. On the contrary, other global parameters such as total protein and total lipid contents and respirometric activity were significantly correlated with biomethane potential.

PLS regressions were tested from all the global and biochemical variables. Respirometric activity measured by BOD_{28} was strictly correlated to BMP. A good prediction of the biodegradability was also obtained from total lipid and total protein contents. Van Soest parameters did not provide useful information here to predict biodegradability. Several chemical and biological parameters should therefore be used to predict BMP_{60}.

Keyword: high solid anaerobic digestion, biomethane potential, biochemical analyses, municipal solid waste, biological oxygen demand, stability, organic matter.

List of abbreviations:

BD_{Aero}: Bioconversion yield in aerobic condition ($\%_{COD}$)

BD_{Anae}: Bioconversion yield in anaerobic condition ($\%_{COD}$)

BOD_{28}: Biological Oxygen Demand on suspended solid in liquid phase. 28 days of incubation ($mg_{O2}.g^{-1}_{TS\ or\ VS}$)

BMP_{60}: BioMethane Potential on suspended material. 60 days of incubation ($NmL.g^{-1}_{TS\ or\ VS}$)

CELL: Cellulose ($\%_{VS}$)

COD_{Tot}: Chemical Oxygen Demand on Solid material ($mg_{O2}.g^{-1}_{TS}$)

COD_{Sol}: Chemical Oxygen Demand on leachate collected from leaching test (L/S ratio = 10) on solid material en filtration at 0.45 µm ($mg_{O2}.g^{-1}_{TS}$)

D: Digestate

DRI: Dynamic Respiration Index

HSAD: High solid anaerobic digestion

HEM: Hemicellulose ($\%_{VS}$)

HSI: Humic Substance Index

PCA: Principal Component Analysis

PLS: Partial Least Square

RMSW: Residual Municipal Solid Waste

S; Substrate

SOL: Soluble fraction ($\%_{VS}$)

TOC: Total Organic Carbon

TS: Total solid (%$_{wS}$

VS: volatile solid (%$_{TS}$)

wS: wet solid

1 Introduction

High solid anaerobic digestion (HSAD) of heterogeneous waste such as biowaste, organic fraction from MSW (OFMSW), and residual municipal waste (RMSW) is under development in Western Europe as a promising industrial option of bio-energy production from organic waste (EurObserv'ER, 2013).

For complex and heterogeneous wastes such as RMSW and biowaste, no simple method is available to predict and optimize AD performances. Consequently, several authors have developed analytical procedures to assess the intrinsic biomethane potential of waste (Chandler *et al*., 1980; Buffière *et al*., 2006; Schievano *et al*., 2008, 2009; Mottet *et al*., 2010; Monlau *et al*., 2012). Moreover, estimation of the residual digestate stability is also needed to define adequate post-treatments (de Araujo Morais *et al*., 2008; Cossu & Raga, 2008; Barrena *et al*., 2009; Bayard *et al*., 2010; van Praagh *et al*., 2009; Scaglia *et al*., 2010).

The biochemical methane potential (BMP) in batch mode is the most common way for assessing the anaerobic biodegradability of solid waste and methane production yields and rates. However this procedure is time consuming (from 30 to 100 days), and the precise protocol can vary significantly from one author to another (Raposo *et al*., 2011). Consequently, many authors have suggested alternative approaches, such as organic matter quantification, biochemical characterisation, and aerobic respirometric tests, and tried to establish correlation models with BMP.

Organic matter (OM) quantification can be considered as the first level, and the easiest

characterization. OM content in solid waste is commonly quantified by the loss on ignition method expressed as volatile solid content (VS), total organic carbon with combustion method (TOC_{solid}), or chemical oxygen demand with chemical oxidation (COD_{solid}).

As shown by numerous authors, biodegradability of organic solid waste is strongly influenced by the biochemical composition of organic solid waste, including proteins, lipids, carbohydrates such as soluble sugars and fibers (hemicellulose, cellulose and lignin) (Chandler *et al*., 1980; Noike *et al*., 1985; Eleazer *et al*., 1997). Cellulose + hemicellulose content is often used as a parameter to estimate the biodegradability, assuming that these carbohydrate polymers are the most significant sources of carbon in waste, and may contribute to up 90% of the total biomethane potential (Rodriguez e*t al*., 2005). Besides, cellulose is considered to be more difficult to biodegrade than soluble sugars and hemicellulose (Noike *et al*, 1985). Finally, lignin is generally considered as non-biodegradable under anaerobic conditions (Stinson & Ham, 1995; Young & Frazer, 1987) and to decrease the accessibility of cellulose (Tong *et al*., 1990). Consequently, the biodegradation of lignocellulosic complexes is a strong limitation of organic substrate conversion to methane in AD.

Several authors have reported experimental works to study correlations between BMP and physico-chemical characteristics of fresh feedstocks (Buffière *et al*., 2006; Gunaseelan, 2004), sludges (Mottet *et al*., 2010; Tambone *et al*., 2010), digestates (Tambone *et al*., 2009; Schievano *et al*., 2008, 2009), refuse from landfills of various

ages (Eleazer *et al*., 1997; Kelly *et al*., 2006), and wastes collected from mechanical biological treatment plants (Binner *et al*., 1999; Cossu & Raga, 2008; de Araujo Morais *et al*., 2008; Barrena *et al*., 2009; Valencia *et al*., 2009; Bayard *et al*., 2010; Scaglia *et al*., 2010). Acceptable correlations have been reported in the literature between BMP and cellulose + hemicellulose content in residual organic fraction from MSW (Eleazer *et al*., 1997), or cellulose + lignin content in biowaste (Chandler *et al*., 1980; Buffière *et al*., 2006; Gunaseelan, 2007). , Aerobic respirometric tests have also been considered as a more rapid alternative to the BMP test (Cossu & Raga, 2008; Ponsa *et al*., 2008 Scaglia & Adani, 2008; Barrena *et al*., 2009; Schievano *et al*., 2008, 2009; Scaglia *et al*., 2010). Good simple linear correlations were reported with the respiration index RI_4 (Cossu & Raga, 2008), specific oxygen uptake rate SOUR (Schievano *et al*., 2008), and dynamic respiration index DRI (Scaglia *et al*., 2010).

The purpose of this study was to compare various methods to characterize chemical, biochemical and biological properties of both heterogeneous substrates and digestates collected from three HSAD plants. The objective was to evaluate the correlations between BMP and other parameters.

The data set was obtained by analysing two Residual Municipal Solid Wastes (RMSW) and two Biowaste (BW), collected from full-scale dry AD plants, and their respective digestates produced by the selected plants (ie altogether 4 wastes and 4 digestates).

The Principal Component Analysis (PCA) and the Partial Least Square regression method (PLS) were used to describe and correlate the various parameters.

2 Materials and Methods

2.1 Solid substrates and digestates collection and preparation

Residual MSW (RMSW) and biowaste (BW), called here “substrates” (S), and their respective digestates (D) were sampled from three dry AD treatment plants located in France and Spain. Designation and information on samples are summarized in Table 1. The substrates were collected after biological–mechanical pre-treatment operations, before the injection into the digestion unit. The digestates were collected at the output of the digesters. The digestions were carried out in “high solids” anaerobic digesters HSAD, with total dry matter content between 20 and 30%$_{wM}$. Pneumatic mixing was implemented by injection of pressurized biogas from the bottom of the vertical digesters. For each HSAD plant, digesters were being operated under steady-state conditions before and during the sampling period.

Two feed-in Residual MSW and two digestates were collected from two distinct AD plants: S1 & D1, from HSAD plant #1 designed to treat RSMW < 12 mm, and S2 & D2, from AD plant #2 designed to treat residual RMSW < 60 mm. Two feed-in biowaste and two digestates were collected from AD plant #3, once in winter: S3 and D3, and once in summer: S4 and D4.

All the samples collected, about 100 kg each, were homogenized and stored at 2°C prior to further sample preparation. Samples were shredded with a low-speed shredder Blik® monorotor M420 and then sieved down to 10 mm, to obtain homogenous matter. For

each solid sample, a homogenous sub-sample of 5 kg was sampled to determine dry matter (DM) by drying at 80°C in a large-scale oven until a constant weight and volatile solids (VS) by calcination at 550°C for 4h. Afterwards, two shredding in a cutting mill Retsch® SM 200 were carried out on dry aliquotes of 1 kg to pass successively through a 4 mm and then a 1 mm sieve. All the analyses were performed in triplicates, using the same dried and crushed samples.

2.2. Organic matter quantification and elemental analysis

Total solid (TS); volatile solid (VS), chemical oxygen demand on solids (COD_{Tot}), and total organic carbon (TOC) were analysed with standard methods (APHA, 1998). In addition, elemental contents in C, H, and N were determined by dry combustion (LECO® CHN analyser, model 600).

2.2. Organic matter analyses

2.2.1. Content in humic-like substances

Humic substances are commonly analysed in environmental samples such as soils and sediments as an indicator of OM stabilisation in natural systems. Humic-like substances were also analysed to study the state of degradation of organic matter in compost from MSW (Castaldi *et al*., 2005) or mechanically-biologically pretreated MSW (van Praagh *et al*., 2009). Humic-like substances content was determined according to the acido-alkaline extraction method (Francou *et al.*, 2008). The samples were first treated for an alkaline extraction of the humic substances (humic acids HA + fulvic acids FA)

with 100 mL of 0.1 M NaOH for 2h. After centrifugation (20 min, 8 000 g), the supernatant (20 mL) was acidified to pH 1.5 with 1M H_2SO_4 in order to precipitate the humic acids overnight. Afterwards, the acido-soluble fulvic fraction was separated by centrifugation. Total organic carbon in the two supernatants was analyzed by combustion followed by infrared detection of CO_2 with a TOC analyzer Shimadzu® model 5050A. Finally, a humic substance index (HSI) was calculated from Eq. 4.

$$HSI = TOC_{HA}/TOC_{FA} \text{ where } TOC_{HA} = TOC_{HA+FA} - TOC_{FA}. \quad (1)$$

2.2.2. Proteins and lipids

Proteins content was estimated from the quantification of organic nitrogen content calculated from the difference between NTK and $N\text{-}NH_3$. Organic nitrogen was multiplied by 6.25 to determine proteins content (APHA, 1998). Lipids extraction was based on the method developed by Achour (2008), in which organic solvents were selected to avoid the solubilisation of plastic materials. Aliquotes of 10 g of samples were extracted for 4 h with 200 mL of 50-50 (V/V) heptane-ethanol mixture at room temperature. The solvent was then extracted in a rotary evaporator and the solid residue dried at 60°C. Lipid content was estimated from the weight loss.

2.2.3. Carbohydrates content

Carbohydrates content was determined according to the standard procedure AFNOR XPU 44-162 (2005) based on van Soest's procedure which was initially developed to analyse crude fibres in agricultural feedstocks (van Soest *et al.*, 1991). The sequential extraction was based on a first step of neutral detergent extraction (soluble fraction,

SOL), a second step of acid detergent extraction (hemicelluloses, HEM) followed by the last extraction in strong acid (H_2SO_4, 72%), which was supposed to correspond to cellulose (CELL). The non extractable organic matter (residue, RES) was considered to correspond to a mixture of lignin and plastic polymers. These measurements were performed with the FiberCap® 2023 Manual System FOSS®. After each extraction, the residual organic matter was estimated by ignition loss at 550°C for 4h. The results were calculated in percentage of volatile solids.

2.4. Biodegradation measurements

Biodegradation measurements under aerobic and anaerobic conditions were both performed using manometric methods under batch conditions on dried, crushed and sieved solid samples (< 1 mm). Results are expressed under normal temperature and pressure conditions (NTP: 273 K, 101325 Pa). Blanks containing only liquid medium and seed inoculum solution were incubated and monitored in parallel with the tests. Aerobic and anaerobic biodegradability were determined on the basis of COD content in solid samples.

2.4.1. Aerobic respiration

The experimental protocol used to measure the biological oxygen demand ($BODS_{28}$) was adapted from the standard procedure ISO 14851 (1999). Tests were carried out in a 510 mL BOD flasks OXITOP-Control® closed by a manometric head which allows to monitor the pressure inside the bottle. Carbon dioxide produced from biodegradation was trapped by sodium hydroxide pellets in the headspace of the test flasks.

Consequently, the recorded pressure decreased proportionally to the oxygen consumed. Aliquotes of 100 mg of dried sample were mixed with 10 mL of inoculum (20 $g_{VS}.L^{-1}$) for a sample to inoculum VS ratio of 1:2, and 50 mL of mineral medium. The inoculum was collected from a municipal wastewater treatment plant and cultivated in the laboratory at 35± 1°C until no oxygen consumption was detected prior to be used. The mineral medium was prepared by mixing 40 mL of solution A (KH_2PO_4: 28.25 g, K_2HPO_4: 146.08 g in 1000 mL distilled water*)*; 30 mL solution B ($CaCl_2$,$2H_2O$: 3.66 g, NH_4Cl: 28.64 g in 1000 mL distilled water*)*; 30 mL solution C ($MgSO_4$,$7H_2O$: 3.06 g, $FeSO_4$,$7H_2O$: 0.7 g, $ZnSO_4$: 0.4 g in 1000 mL distilled water*) ;* distilled water to complete to 1000 mL. The samples were incubated for 28 days at least in the darkness under permanent magnetic stirring. All the assays were performed in triplicates. A positive blank containing mineral medium, seed inoculum solution and glucose was incubated and monitored in parallel with the tests.

The theoretical oxygen consumption can be calculated from the chemical formula describing the composition of the organic matter contained in the samples. Aerobic biodegradability was defined as the ratio between $BODS_{28}$ and CODS (*see* Eq. 2).

$$BD_{Aero} = \frac{BODS_{28}\ (g_{O_2}.kg_{TS}^{-1}) \times 100}{CODS\ (g_{O_2}.kg_{TS}^{-1})} \quad (2)$$

2.4.1. Biomethane potential

The protocol used was adapted from the standard procedure ISO 14853 (1999). Serum flasks of 600 mL were used where samples of 2 g dry matter (DM) were suspended in 200 mL of aqueous solution composed of 20 mL of nutrient medium, 100 mL of seed

inoculum (around 10 $g_{VS}.L^{-1}$) for a sample to inoculum VS ratio of 1:2, and 80 mL distilled water. The inoculum was collected from a sewage sludge digester and cultivated in the laboratory at 35± 1°C until no biogas production was observed prior to be used. Nutrient medium consisted of: 10 mL NH_4Cl aqueous solution (27 $g.L^{-1}$); 10 mL of aqueous solution with KH_2PO_4 100 $mg.L^{-1}$; Na_2HPO_4 400 $mg.L^{-1}$; $MgCl_2,6H_2O$ 100 $mg.L^{-1}$; $CaCl_2,2H_2O$ 50 $mg.L^{-1}$. To ensure anaerobic conditions, the solutions in the flasks were flushed with a flow of N_2 for 5 minutes after introducing inoculum. The bottles were then sealed with air-tight rubber caps and plastic seals, and incubated at 35 ± 2°C in the dark until no further biogas production was detected (90 days at least), with a daily manual shaking. A positive blank containing liquid medium and seed inoculum solution and glucose was incubated and monitored in parallel with the tests. All assays were performed in triplicates.

Biogas production was monitored using a pressure transducer Digitron® Model 2085P. The biogas was discharged regularly to prevent pressure from exceeding 2000 mbar. Gas composition was periodically analyzed with an Agilent® gas micro-chromatograph with thermal conductivity detectors and equipped with a Poraplot U® column for CO_2 and H_2S separation and a Molsieve® one for O_2, N_2, and CH_4. Biogas and methane productions of the assays were determined by subtracting the blank average productions of biogas and methane. Biogas Potentials (BP) and Methane Potentials (usually called Biochemical Methane Potentials, BMP) were calculated from the experimental results. Anaerobic biodegradability was finally expressed as the ratio between BMP and CODS

(Eq. 3):

$$BD_{Anae} = \frac{BMP(Nl.kg_{DM}^{-1}) \times 100}{0.35 \times CODS(g_{O_2}.kg_{DM}^{-1})} \quad (3)$$

2.4. Statistical approach

Principal components analysis (PCA) was used to investigate the relationships between the parameters followed: TOC, VS, COD_{Tot}, COD_{Sol}, HSI, van't Soest fractions (SOL, HEM, CELL, and RES), BOD_{28}, and BMP_{90}. PCA is a data reduction technique where the new variables (principal components or factors) are calculated from linear combinations of the original variables. The principal components are orthogonal to each other, so there is no redundant information. The first principal component, or factor, accounts for the greatest variability in the data. Matlab® software was used to perform the PCA.

3. Results

3.1. Chemical characterisation

Results are shown in Table 2. It can be seen that VS content ranged between 56.8 and 70.1 % in the substrates and between 47.1 and 56.6 % in the digestates . As displayed on Figure 1, a good correlation (coefficient of correlation r^2 = 0.81 and 0.88 respectively) was obtained between VS and TOC and between VS and COD, indicating that all three methods could be used indifferently to determine the total organic matter contents of substrates or digestates selected for this study. Digestates were characterised by lower carbon/nitrogen (COT/N-NTK) ratios than substrates due to nitrogen conservation and

carbon release (as CO_2 and CH_4) during the AD process (Tambone *et al*., 2009), confirming that C/N may be considered as an indicator of the progression of biodegradation (Gunasselan, 2004). Schievano *et al.* (2008) observed a positive correlation between PBM and TOC/N-TKN.

Leaching tests results are reported in Table 2. Substrates leachates were characterized by low pH values < 6.5, due to the presence of volatile fatty acids (VFA). The pH of the digestates leachates were more alkaline, as a consequence of VFA biodegradation and ammonia production. A significant amount of the total COD (COD_{Tot} in solid samples) , from 9 to 21.5% for substrates and from 3 to 16% for digestates, was hydrosoluble. The major trend observed is the decrease of COD_{Sol} with AD treatment, except for winter sample from AD plant #3. It is likely that the organic matter in this winter sample contained a higher fraction of recalcitrant compounds. Moreover, it can be seen in Table 2 that VFA concentration (expressed in equivalent g of COD. kg-1_{TS}) was not well correlated with COD_{Sol}, suggesting that part of the recalcitrant organics were water soluble Data on soluble nitrogen compounds (N-NTK and N-NH_4^+) underlined the increase of soluble proteins and ammonium during AD. The alkaline pH > 7.5 suggested the possibility of NH3 losses into the gas phase during the AD process. Consequently, results from leaching tests may be of some interest to evaluate biological stability of organic waste samples, such as for example before and after AD.

Table 2 + Figure 1

3.2. Biochemical characterization

The biochemical characteristics of the selected substrates and digestates are reported in Table 3, including humic'like substance index (HSI), total proteins content (PROT), total lipids content (LIP), and the four van Soest fractions: SOL is the amount of organic matter extracted with the neutral detergent NDS, HEM is the difference the neutral detergent and the acid detergent residue, CELL is the fraction extracted by 72% sulphuric acid, and RES is the VS residue after 72% sulphuric acid treatment, supposed to correspond to lignin compounds and other non-extractable fractions like plastic materials which are supposed to be present in RMSW. Significant differences can be observed depending on the origin and nature (substrates vs. digestates). Globally, substrates were characterized by lower humic substance indices (HIS) and protein contents, higher lipid, hemicellulose and cellulose contents. The "winter biowaste" S3 discussed above was the substrate with the highest residual content (21%$_{VS}$), twice more than in the other three substrates (10%$_{VS}$). High residual contents were also observed in digestates, ranging from 16%$_{VS}$ (D2 – RMSW, and D4 "summer biowaste") to around 26%$_{VS}$ (D1 – RMSW, and D3 "winter biowaste").

Surprisingly, digestates were also characterized by higher amounts of NDF extractible matter (SOL fraction), reaching 68%$_{VS}$ for D2, and never less than 40%$_{VS}$ for the three others. A decrease in extractible organic fraction was expected, as a consequence of preferential anaerobic biodegradation of soluble forms. On the contrary, it was observed that AD induced probably a significant hydrolysis of the particulate OM initially

contained in the substrates, resulting in the decrease of hemicellulose and cellulose contents and in the relative accumulation of non-extractible organic compounds (RES) such as plastics and lignin.

Table 3

3.2. Biological characterization

Biological oxygen demands (BOD_{28}) and biomethane potentials (BMP_{90}) of substrates and digestate are presented in Table 4. Results are expressed both on a dry matter (TS) and an organic matter basis VS, and biodegradability was calculated with respect to COD_{Tot} content of samples, following eqn. (2) for aerobic biodegradability (BD_{Aero}), and eqn. (5) for anaerobic biodegradability (BD_{Ana}). Differences can be observed between the biodegradability of the wastes depending on the type of waste, its nature (substrates vs. digestates), the collection spot and the season. BOD_{28} ranged from 580 to around 1000 $gO_2.kg^{-1}_{VS}$ for the substrates, and from around 300 to 480 $gO_2.kg^{-1}_{VS}$ for the digestates. Substrate “Winter biowaste” (S3) exhibited the lowest BOD_{28}, corresponding to an aerobic biodegradability BD_{Aero} of 42%$_{COD}$, significantly lower than S1, S2 and S4 (82, 71 and 86, respectively). Moreover, digestates were all characterised by lower BD_{Aero} ranging from 20 to 44%$_{COD}$ (D4, “summer biowaste”). These results were well correlated with the lignin content, as already discussed in the previous section. In addition, BMP_{90} ranged from 200 to 410 $Nml_{CH4}.kg^{-1}_{VS}$ for substrates, corresponding to the anaerobic biodegradability BD_{Ana} from 54 to 80%$_{COD}$. Digestates were characterised by a lower BMP_{90}, from 75 to 125 $Nml_{CH4}.kg^{-1}_{VS,}$ with BD_{Ana} from 15 to 30%$_{COD}$.

From these results, it can be deduced that all digestates contained a significant amount of biodegradable matter, yet with a lower anaerobic biodegradability as compared to aerobic biodegradability. Biodegradability data (BD_{Ana} / BD_{Aero}) and PBM_{90} to BOD_{28} values are compared in Fig. 2. In accordance with previous observations (Cossu & Raga, 2007; Ponsa *et al.*, 2008; Schievano *et al*., 2008), significant correlations were found on the set of data, with regression coefficient r^2 of 0.76 and 0.79 for BD_{Ana} / BD_{Aero} (Fig. 2A) and BMP_{90}/BOD_{28} (Fig. 2B), respectively. Moreover, the positive slope of 0,66 between BD_{Ana} and BD_{Aero} clearly indicated that OM contained in all samples was less biodegradable under anaerobic conditions as compared to aerobic conditions. However, Scaglia *et al.* (2010) underlined the impossibility to directly compare biomethane potential and aerobic respirometry because of the different experimental conditions adopted, including sample preparation and conditions to perform biological tests. The biodegradable part of the organic matter might be less accessible to micro-organism under the experimental conditions of anaerobic assays, likely due to structural characteristics of lignocellulosic materials contained in samples, as previously suggested by Triolo *et al*., (2011) and Monlau *et al*. (2012).

Table 4

Figure 2

4. Descriptive approach with PCA

To identify the quantitative and qualitative features affecting the biochemical methane potential from the selected substrates and digestates, the set of data was analysed

through PCA. The main components defined by the analysis are presented in Figure 3 and the linear matrix of correlations is reported in Table 5.

Three main components PC1, PC2 and PC3 were selected by the Kaiser criterion (>1), which explained 87% of the variability of the data, among which only 10% for the third component. Consequently, only the first two components were selected for the description of the data, corresponding to 77% of their variability. The typology of variables and samples represented on the factorial plans established by components PC1 and PC2, is illustrated in Figure 4. The Figure 4B clearly separated the substrates from the digestates into 2 distinct categories, revealing that the AD treatment affected significantly the bio-physical and chemical characteristics of the wastes. Axis PC1 expressed the opposition between the nature of the two categories of samples. Axis PC2 revealed the effects of the origin of the samples. Indeed, samples from AD plant #1 and 3 (S1, D1 and S3, D3) were represented opposite to those from AD plant #2 and 4 (S2, D2 and S4, D4). The typology of variables on Figure 4A enables to understand this clear separation. The global and biologic variables were represented as two distinct groups with good correlations within each group. In particular, the correlation between BMP and BOD was very strong with $r^2 = 0.81$ (see Fig. 4A, and Table 5).

Some biochemical variables were revealed as particularly relevant to estimate the biological stability of the digestates. The opposition of RES and HSI variables to BMP and BOD indicated that the formers were good indicators of biostability. A strong negative correlation was found between RES and the two bioreactivity parameters,

which confirmed the major effect of non-extractive fraction. Our results showed negative correlation (r^2 = -0.76) between RES and BMP, which is in agreement with previous studies. Triolo *et al.* (2011) reported a good negative correlation (r^2 = -0.76) between lignin and BMP on a set of 28 samples. Nevertheless, the interpretation and the exploitation of van Soest fractions other than the residual fraction RES remained difficult. No correlation was found between NDS soluble fraction (SOL), hemicellulose and cellulose, whereas humic substance index (HSI, r^2 = -0.77) total lipid content (LIP, r^2 = 0.77), and total protein content (PROT, r^2 = 0.85) were found as the parameters most strongly correlated with BMP.

5. Conclusions

The analysis of samples from HSAD plants, before and after digestion, showed a strong influence of waste origin and anaerobic treatment on their characteristics. In particular:

- The descriptive analysis PCA highlighted the very distinctive characteristics of the substrates and digestates from the treatment. This opposition was revealed by the grouping of the biological variables, opposed to variables RES and ISH. These two variables constitute good indicators of the bioreactivity of samples;
- The van Soest fractions SOL HEM and CELL did not appear relevant for the estimation of biostability. On the contrary, results showed that the residual fraction RES associated with the other available variables was a good indicator for the description of the bioreactivity of the samples. Finally, the other van

Soest fractions were found poorly informative with respect to th biodegradability of the samples

- The predictive analysis showed that the prediction of the biodegradability from the global and biochemical variables was good. In particular, non-extractible van Soest's fraction with 72% sulfuric acid (RES), humic substance indice (HIS), lipid (LIP) and Protein (Orit) content were shown particularly relevant indicators.

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Tables

Table 1. Substrates and digestates collected: designation and origin.

HSAD #	1	2	3	3
Type of waste	RMSW	RMSW	Biowaste “winter”	Biowaste “summer”
Substrates designation	S1	S2	S3	S4
Digestate designation	D1	D2	D3	D4

Table 2. Global analyses and leaching behaviour of substrates and digestates.

Global analyse	Substrates				Digestates			
	S1 (RMSW)	S2 (RMSW)	S3 (BW)	S4 (BW)	D1 (RMSW)	D2 (RMSW)	D3 (BW)	D4 (BW)
DM ($\%_{wM}$)	45.1 ± 0.5	33.9 ± 1.1	50.4 ± 0.4	44.3 ± 0.7	17.9 ± 0.2	20.1 ± 0.5	18.9 ± 0.3	20.4 ± 0.2
VS ($\%_{TS}$)	69.2 ± 0.6	70.1 ± 1.7	66.9 ± 1.6	56.8 ± 5.1	47.1 ± 0.5	52.5 ± 0.9	56.6 ± 1.4	47.3 ± 1.5
TOC ($\%_{TS}$)	38.8 ± 1.9	35.4 ± 1.8	38.1 ± 2.0	32.1 ± 1.8	25.3 ± 1.2	25.2 ± 1.3	31.9 ± 1.6	21.5 ± 1.0
COD_{Tot} ($g_{O2}.kg^{-1}_{TS}$)	885 ± 19	1026 ± 10	933 ± 14	772 ± 10	685 ± 3	831 ± 32	828 ± 26	510 ± 53
N-TKN ($g.kg^{-1}_{TS}$)	11 ± 0.5	22 ± 2	11 ± 0.5	20 ± 1	14 ± 1	26 ± 05	13 ± 0.5	15 ± 0.5
COT/N-TK	35.2	16.1	34.6	16.0	18.1	9.7	24.5	14.3
Leaching behaviour	Substrates				Digestates			
	S1 (RMSW)	S2 (RMSW)	S3 (biowaste)	S4 (biowaste)	D1 (RMSW)	D2 (RMSW)	D3 (biowaste)	D4 (biowaste)
pH	6.4	4.8	5.6	5.2	7.5	7.9	8.7	8.1
Total VFA ($g_{O2}.kg^{-1}_{TS}$)	51.7 ± 0.1	23.2 ± 0.2	45.5 ± 0.6	35.1 ± 1.0	3.4 ± 0.1	15.6 ± 0.1	7.4 ± 0.1	14.9 ± 2.5
COD_{Sol} (g_{O2}.20,6))	156.0 ± 4.5	221.0 ± 1.7	87.9 ± 0.2	157.2 ± 0.4	19.5 ± 2.0	30.2 ± 0.2	135.7 ± 0.6	67.0 ± 0.1
N-NTK ($g.kg^{-1}_{TS}$)	4.2 ± 0.05	5.4 ± 0.3	1.9 ± 0.05	5.1 ± 0.1	17.1 ± 0.6	25.0 ± 0.3	15.3 ± 0.7	15.6 ± 0.1
$N\text{-}NH_4^+$ ($g.kg^{-1}_{TS}$)	2.26 ± 0.01	1.61 ± 0.01	1.01 ± 0.01	2.48 ± 0.01	14.08 ± 0.07	19.68 ± 0.07	10.47 ± 0.11	11.35 ± 0.04

Table 3. Biochemical analyses of substrates and digestates.

	Substrates				Digestates			
	S1 (RMSW)	S2 (RMSW)	S3 (biowaste)	S4 (biowaste)	D1 (RMSW)	D2 (RMSW)	D3 (biowaste)	D4 (biowaste)
HSI (TOC_{HA}/TOC_{FA})	0.03 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.20 ± 0.04	0.56 ± 0.03	0.74 ± 0.02	0.45 ± 0.04	0.61 ± 0.02
Proteins (PROT $\%_{VM}$)	8.3 ± 0.1	7.7 ± 1.0	8.5 ± 0.1	18.6 ± 0.9	16.2 ± 0.8	12.7 ± 1.0	13.3 ± 0.2	16.1 ± 0.4
Lipids (LIP $\%_{VM}$)	12.7	13.8	11.4	14.6	11.3	11.8	9.2	10.9
Soluble Fraction (SOL $\%_{VM}$)	24.2 ± 0.21	55.5 ± 3.0	22.1 ± 0.6	49.4 ± 1.0	40.2 ± 1.5	67.7 ± 1.1	41.6 ± 0.7	64.5 ± 1.3
Hemicellulose (HEM $\%_{VM}$)	15.5 ± 0.7	19.2 ± 3.1	20.7 ± 0.8	14.5 ± 1.0	25.9 ± 2.6	8.0 ± 1.7	8.8 ± 1.1	7.3 ± 2.2
Cellulose (CELL $\%_{VM}$)	50.8 ± 0.7	15.3 ± 0.68	36.6 ± 2.5	26.8 ± 1.0	6.6 ± 1.0	8.4 ± 1.0	24.0 ± 1.1	12.3 ± 3.1
Residual fraction (RES $\%_{VM}$)	9.5 ± 0.12	10.0 ± 1.0	20.6 ± 2.3	9.3 ± 0.3	27.2 ± 1.0	16.0 ± 1.1	25.6 ± 1.9	15.9 ± 2.3

Table 4. Bioreactivity analyses of wastes and digestates.

	Substrates				Digestates			
	S1 (RMSW)	S2 (RMSW)	S3 (biowaste)	S4 (biowaste)	D1 (RMSW)	D2 (RMSW)	D3 (biowaste)	D4 (biowaste)
$BODS_{28}$ ($gO_2.kg^{-1}_{TS}$)	727 ± 53	724 ± 10	389 ± 10	664 ± 17	210 ± 40	167 ± 15	164 ± 11	225 ± 25
$BODS_{28}$ ($gO_2.kg^{-1}_{VS}$)	1051 ± 77	978 ± 13	581 ± 15	1054 ± 27	446 ± 85	316 ± 28	290 ± 19	476 ± 53
BD_{Aero} ($\%_{COD}$)	*82.1*	*70.6*	*41.7*	*86.0*	*30.7*	*20.1*	*19.8*	*44.1*
BMP_{90} ($Nml_{CH4}.kg^{-1}_{TS}$)	*157 ± 1*	*287 ± 4*	*132 ± 6*	*146 ± 2*	*35 ± 1*	*65 ± 1*	*64 ± 1*	*56 ± 0.5*
BMP_{90} ($Nml_{CH4}.kg^{-1}_{VS}$)	228 ± 1	410 ± 10	197 ± 8	257 ± 4	75 ± 3	124 ± 2	112 ± 0.5	118 ± 0.5
BD_{Ana} ($\%_{COD}$)	*50.7*	*79.9*	*40.4*	*54.0*	*14.6*	*22.3*	*22.1*	*31.4*

Table 5. Correlation matrix among all parameters to compare BMP to bio-physico and chemical data.

	VS	COD	TOC	CODS	PROT	LIP	HSI	SOL	HEM	CELL	RES	BOD	BMP
VS	1	0.91	0.94	0.75	-0.84	0.41	-0.86	-0.53	0.27	0.71	-0.50	0.66	0.78
COD		1	0.81	0.64	-0.84	0.33	-0.61	-0.33	0.26	0.41	-0.34	0.44	0.74
TOC			1	0.68	0.69	0.33	-0.90	-0.74	0.36	0.82	-0.33	0.61	0.62
CODS				1	-0.42	0.50	0.75	-0.17	0.02	0.46	-0.61	0.76	0.85
PROT					1	-0.04	0.51	0.41	-0.20	-0.46	-0.61	0.77	0.85
LIP						1	-0.57	-0.08	0.28	0.17	-0.82	0.86	0.77
HSI							1	0.64	-0.35	-0.84	0.59	-0.86	0.77
SOL								1	-0.55	0.78	-0.18	-0.24	0.03
HEM									1	0.08	0.20	0.31	0.22
CELL										1	-0.35	0.51	0.29
RES											1	-0.81	-0.76
BOD												1	0.84
BMP													1

Figures

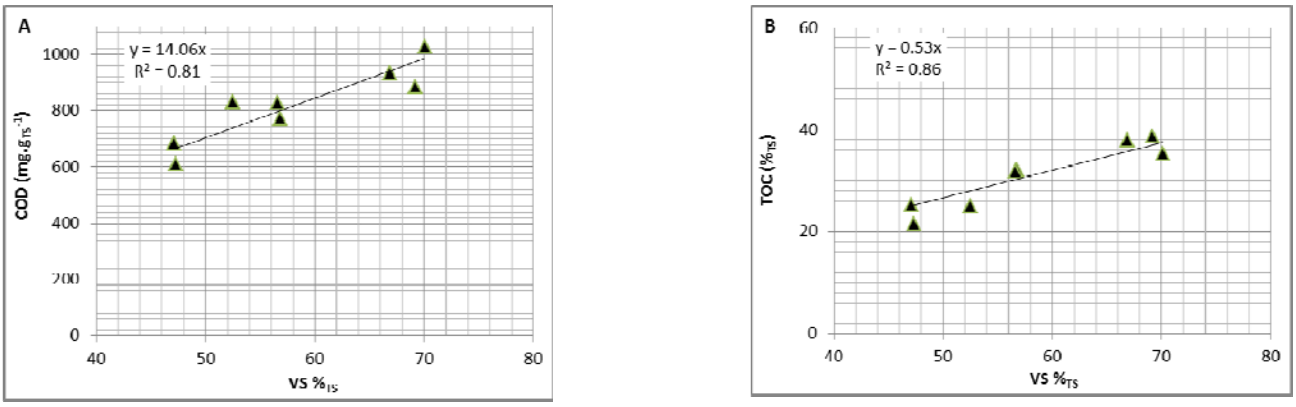


Figure 1: Comparison of total organic content determined with 3 methods (VS, COD and TOC).

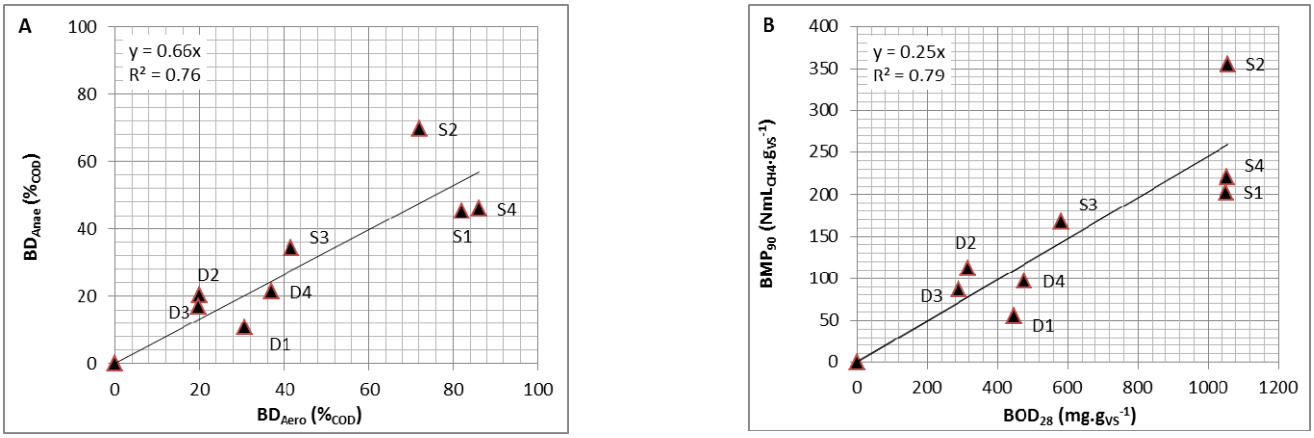
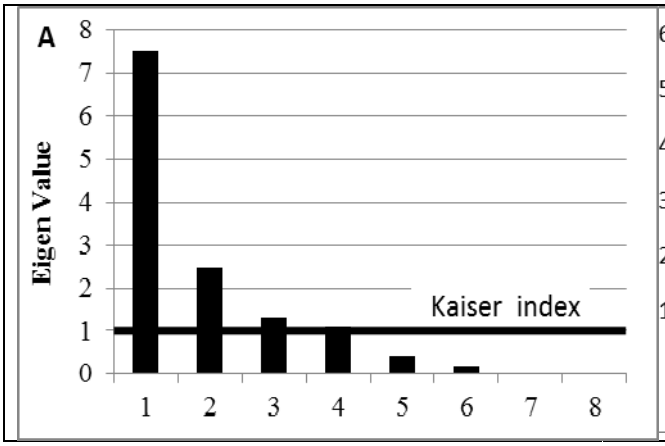


Figure 2: Linear regression between *BD$_{Ana}$ / BD$_{Aero}$* (A) and BMP$_{90}$/BOD$_{28}$ (B).



Principal Component	(%)
PC1	57.9
PC2	77.0
PC3	87.1
PC4	95.5
PC5	98.6
PC6	99.8
PC7	100.0
PC8	100.0

Figure 3: Results of the principal component analysis (PCA) in main components.

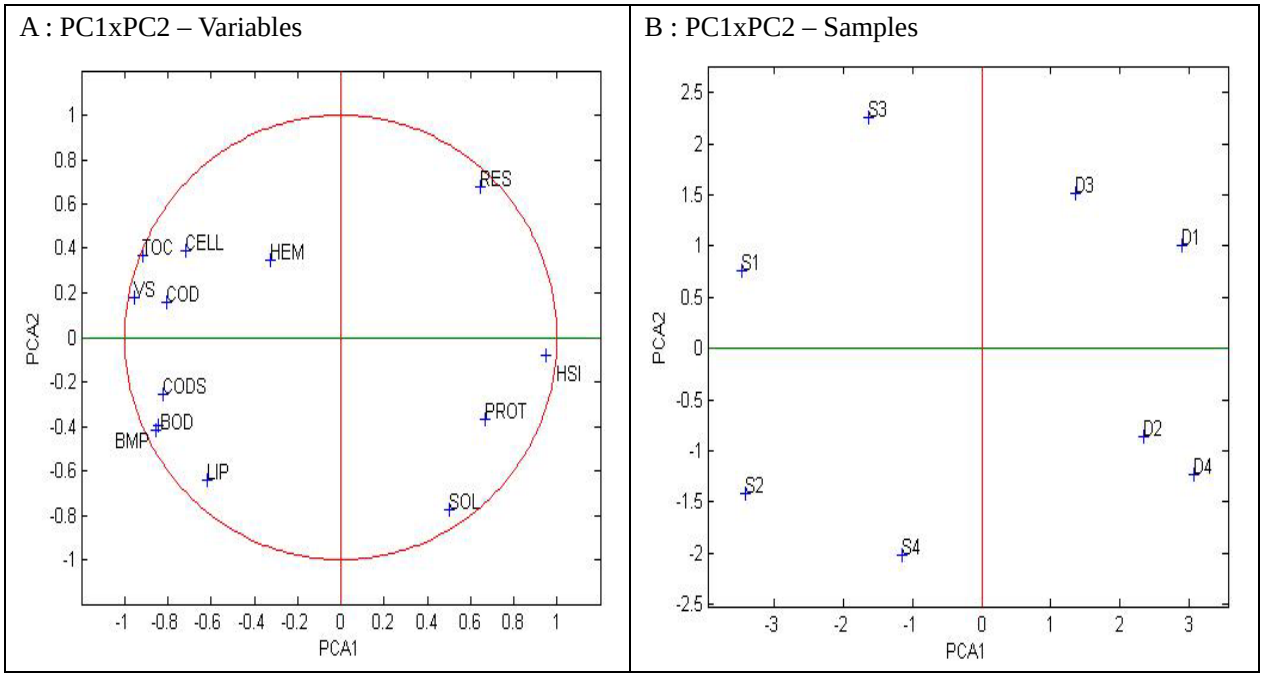


Figure 4: Plot of the loadings of the variables and samples scores with principal components 1 and 2 (PC1xPC2).