Complementary methods for the evaluation of anaerobic biodegradability of lignocellulosic biomass

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Abstract:

Lignocellulosic biomass has been regarded as a potential source for fermentation to methane. Biochemical characteristics of lignocellulosic residues are important factors for evaluating their anaerobic biodegradability. Here 14 lignocellulosic residues including 6 agricultural residues (S1 to S6) and 8 forest residues (S7 to S14) were characterized by 9 biochemical methods, principally BioMethane Potential (BMP), Biological Oxygen Demand (BOD_{28}), Enzymatic Cellulose Degradation tests (ECD), two biochemical composition analyse methods namely Van Soest fractionation method and NREL method. This large dataset was analysed by principal component analyses (PCA) to find out the correlations between parameters. Our results showed that biochemical contents in particular lignin or residual (RES) contents influence BMP, while for soluble fractions (SOL, SOC, COD, WAT and EThOH), the significance was lower. The biochemical compositions content of lignocellulosic residues obtained from the two methods were different. To some extent, BMP correlates relatively well with the content ratio of the sum of non-lignin fractions to lignin and the content ratio of cellulose to lignin. The

relationship between BMP and the lignin content of agricultural residues S1 to S6 was better than that between BMP and the RES content of S1 to S6. Agricultural residues S1 to S6 and forest residues S7 to S14 exhibit different behaviors of aerobic and anaerobic digestibility. There were two significant linear relationships between BMP and BOD_{28} of two types of residues. Moreover, a relatively strong linearity exists between ECD and the lignin content.

Keyword: anaerobic digestion, biomethane potential, lignocellulosic residues, biochemical analyses, biological oxygen demand, enzymatic hydrolysis

List of abbreviations:

ADF: concentrate acid

ADL: acid detergent

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 solid in liquid phase. 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 ($mg_{O2}.g^{-1}_{TS}$)

ECD; Enzymatic Cellulose Degradation tests (%$_{Cell}$)

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

NDF = Neutral Detergent

R1 = [SOL + HEM + CELL]/[RES]

R2 = [CELL]/[RES])

SOL: Soluble Fraction (%$_{VS}$)

TOC: Total Organic Carbon

TS: Total Solid (%$_{wS}$)

VS: Volatile Solid (%$_{TS}$)

wS: wet solid

1 Introduction

Anaerobic digestion of lignocellulosic waste is a major challenge. The utilization of renewable and abundant lignocellulosic biomass is bound to be one of the major options to minimize the dependence to fossil fuels and reduce greenhouse gases emissions in the 21st century, while avoiding competition with food products. Lignocellulosic biomass includes agriculture residues, forest residues, perennial grasses, etc (Kurian *et al*., 2013; Ruane *et al*., 2010; Ramachandra *et al.*, 2000).

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin, among which, cellulose is the most abundantly available carbohydrate polymer in nature (Imai *et al*., 2004). Despite their wide range of possible sources, biomass are remarkably uniform in many of their properties, for example, hardwood of forest residues has greater amounts of cellulose, whereas wheat straw of agricultural residues have more hemicellulose (Chandra *et al*., 2012). Furthermore their rigid structure and high lignin contents cause low digestibility and inhibit methane production. Therefore, detailed characterization of biodegradability properties of lignocellulose is required to determine the nature of different types of lignocellulosic biomass and thus to estimate the anaerobic biodegradability. Many characterization methods have been developed, such as elemental analyse, fibre analysis method determining structural carbohydrates and lignin, forage analysis method determining ADL (acid detergent lignin), ADF (acid detergent fibre) and NDF (natural detergent fibre), enzymatic hydrolysis, and anaerobic degradability test, *ie*. BioMethane Potential (BMP). Although a few studies reported the

relationship between BMP and enzymatic digestibility (Liew *et al.*, 2012) that between methane conversion efficiency and lignin content (Tong *et al.*, 1990; Mottet *et al.*, 2010), the entire quantitative relationship among all of these characteristics is still not studied.

In the present study, two types of lignocellulosic biomass were selected to determine the carbohydrate biodegradation according to the biomass resource: crop residues including two wheat straws, corn stover and corn stem, two sugarcane bagasses, and wood residues, including mix of residual softwood branches and mix of residual hardwood waste collected from a composting plant, hazel and acacia branches. The objective of this research was to compare and identify complementary methods to determine the anaerobic biodegradability of lignocellulosic biomass. At the same time, this study was conducted to attempt to correlate an intrinsic relationship among these different characteristics. The methods investigated included methane yields, carbohydrate contents with several methods, enzymatic cellulose degradation tests (ECD), and aerobic test, ie. Biochemical Oxygen Demand (BOD) on solid samples, etc.

2 Materials and Methods

2.1 Solid substrates collection and preparation

Samples used in this study were selected, offering a wide range of biochemical profiles. They exclusively included lignocellulosic residues collected from several agricultural sites, forests and green waste composting plants (Table 1). All the samples collected, about 10 kg each, were homogenized and stored at 2°C prior to sample preparation. Fresh waste samples were shredded three times with a low-speed shredder Blik®

monorotor M420 and then sieved down to 10 mm, to obtain homogenous solid samples. Afterwards, two shredding in a cutting mill Retsch® SM 200 were carried out on dry samples of 1 kg for crushing again to pass through a 4 mm mesh, and finally 1 mm mesh. All the analyses were performed in triplicates, using the same dried and crushed samples.

Table 1

2.2 Global analyses

All data on global analyses of lignocellulosic substrates are summarized in Table 2.

Organic matter quantification: Total solid (TS) and volatile solid (VS) were performed with standard methods, generally adapted from water and wastewater methods (APHA, 1998). Total organic carbon was determined by elemental analyser OI Analytical® – 1020A TOC analyser according to the standard procedure ISO 10694 (1995) by catalytic oxidation of the samples by oxygen at 950°C and infrared analysis of the CO_2 produced. The TOC was measured in the same manner after an ortho-phosphoric acid treatment of the samples (H_3PO_4, 15%) in order to eliminate inorganic carbon. The chemical oxygen demand (COD) was measured by oxidizing the crushed solid samples with a solution of potassium dichromate and sulphuric acid at a temperature of 135°C. The dichromate ions were reduced to Cr^{3+} ions which were analysed by spectrophotometry at 585 nm.

***Water soluble organic matter quantification*:** Standard leaching extraction tests were performed in triplicates to determine the leaching potential of organic matter. The liquid to solid matter (dry matter) ratio was 10. Agitation was provided during 3h by a rotary

tumbler at a speed of 10 rpm. At the conclusion of the agitation, the leachates were filtrated through a 0.45 µm pore size membrane. Filtered liquid solutions obtained were subsequently analysed for SOC and COD. These analyses were performed according to standard methods (APHA, 1998).

Table 2

2.3 Organic matter characterisation

2.3.1. Van Soest Fractionation

Fiber contents of the waste fractions were determined with the FiberCap® 2023 Manual System (FOSS®) according to the van Soest sequential extraction procedure (van Soest & Wine, 1967). The method included 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 72% sulphuric acid, 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. After each extraction, residual organic matter was quantified by calcination at 480°C, 4h in furnace (VS_{NDF}, VS_{ADF}, and VS_{ADL}). The results were calculated as mass percentages of the volatile solids (%$_{VS}$):

$SOL = 100 - VS_{NDF}$

$HEM = VS_{NDF} - VS_{ADF}$

$CELL = VS_{ADF} - VS_{ADL}$

$RES = VS_{ADL}$

2.3.2. Extractives, structural carbohydrate and lignin

The samples were extracted successively with HPLC grade water and with 99.9% (v/v) ethanol using the Dionex ASE 350 system following NREL Laboratory Analytical Procedure (NREL/TP-510-42619 - Sluiter *et al*., 2008a). Extractives free samples were used to determine cellulose, hemicellulose and lignin by according to NREL Laboratory Analytical Procedure (NREL/TP-510-42618 - Sluiter *et al*., 2008b). The samples were hydrolysed with 72% (w/w) sulphuric acid at 30°C for 1h. The acid was diluted to a final concentration of 4% (w/w) with the addition of 84 mL water, and the mixture was autoclaved at 121°C for 1h. The residue was cooled and filtered. The acid insoluble residue was dried at 105°C overnight, after which it was placed in a furnace at 575°C for 24h. The difference between the weight of dried solids and that of the ash was determined as acid insoluble lignin. Acid soluble lignin was determined by measuring the UV absorption of the acid hydrolysis supernatant at 205nm. Monomeric sugars (glucose, xylose, galactose, arabinose, mannose) were measured with a HPLC Waters® equipped with Bio-Rad Aminex HPX-87P column and refractive index detector (RID) at 85°C and 49 °C, with HPLC grade water as the mobile phase, eluting at 0.6 $mL.min^{-1}$. Cellulose and hemicellulose concentrations were calculated from the corresponding monomers.

2.4. Biodegradation measurements

Biodegradation measurements under aerobic and anaerobic conditions were both performed under batch conditions on dried, crushed and sieved solid samples (< 1 mm) and monitored using manometric methods. All results reported in Table 3 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. All data are summarized in Table 3.

Table 3

2.4.1. BOD measurement on suspended solid samples

A static respiration activity test for suspended solid samples was developed from the classical Biological Oxygen Demand (BOD) protocol for liquid samples. The BOD flasks were hermetically closed by a manometric cap to monitor pressure variations inside the bottles. 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 and was subsequently converted into BOD values. The samples were incubated for 28 days at least in the dark at 30°C under continuous magnetic stirring. The mineral medium was described previously (Massardier-Nageotte *et al.*, 2006). The aerobic biodegradability was defined as the ratio between the BOD_{28} and COD in solid sample (COD_{Tot}) as explained in Eq. 1.

$$BD_{Aero} = \frac{BODS_{28}\left(g_{O_2} \cdot kg_{TS}^{-1}\right) \times 100}{COD_{Tot}\left(g_{O_2} \cdot kg_{TS}^{-1}\right)} \quad (1)$$

2.4.1. Biomethane potential

Biochemical methane potentials (BMP) were determined following a method adapted from Angelidaki *et al.* (2009). The nutrient medium was prepared according to the

standard procedure ISO 11734 (1995) dedicated to the evaluation of the anaerobic biodegradability of organic compounds in digested sludge. Serum bottles of 600 mL were used where samples of 2 g dry solid were suspended in 400 mL of nutrient medium and 200 mL of inoculum suspension. To insure anaerobic conditions, bottles containing tested materials and nutrient medium were flushed with gas (N_2/CO_2 - 90/10 V/V) for 5 minutes after introducing inoculum. The bottles were sealed with air-tight rubber stoppers and plastic seals, and incubated for 60 days at least at 35 ± 2°C in the dark. Methane production was monitored using a Digitron® 2085P pressure transducer. The biogas was discharged regularly to prevent pressure from exceeding 2000 mbar. Gas composition was periodically analysed with an Agilent® gas micro-chromatograph with thermal conductivity detectors and equipped with a Poraplot U column for CO2 and H2S separation and a Molsieve one for O_2, N_2, and CH_4. BMP was then calculated from the recorded data and expressed in $NmL.g^{-1}$ of TS or VS, under normal temperature and pressure conditions (NTP: 273 K and 101325 Pa). The anaerobic biodegradability was expressed as the ratio between BMP production and COD_{Tot} (Eq. 2):

$$\mathrm{BD}_{Anae} = \frac{BMP\left(Nl \cdot kg_{DM}^{-1}\right) \times 100}{0.35 \times COD_{Tot}\left(g_{O_2} \cdot kg_{DM}^{-1}\right)} \qquad (2)$$

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out following the NREL procedure (NREL/TP -510-42629 - Selig *et al.*, 2008).The samples were diluted to 1.5% (w/v) in a 50mM sodium citrate buffer (pH 4.8). The cellulase (Sigma-Aldrich, 60 FPU/mL) and the β-glucosidase (Sigma-Aldrich, 246 *p*NPGU/mL) loadings were 60 FPU/g cellulose and

64 pNPGU/g cellulose, respectively. Hydrolysis experiments were conducted in a shaking water bath at 150 rpm and 50°C. After 168h, the reaction was terminated by transferring the mixtures to boiling water for 5 min to deactivate the enzymes. The supernatant of each sample was filtered through 0.22 µm filters. The soluble glucose in the enzymatic hydrolysate was measured by HPLC using a Bio-Rad HPX-87H column and refractive index detector (RID) that were maintained at 45°C and 30°C, respectively. 5 mM H_2SO_4 was used as the mobile phase, eluting at 0.6 mL.min^{-1}. The percent digestibility of the cellulose was determined as follows:

$$\%\ \text{Enzymatic}\ \textit{cellulose}\ \text{deg}\ \textit{radation}\ (ECD) = \frac{\textit{grams cellulose digested}}{\textit{grams cellulose added}} \times 100 \qquad (3)$$

2.6. Statistical approach

Parameter values from organic soluble soluble fraction in pure water SOC et COD_{Sol}, biochemical characterisations (Van Soest fractions: SOL, HEM, CELL and RES; extractives from NREL procedure : WAT (water extractives) and EThOH (ethanol extractives) ; structural carbohydrates and lignin from NREL procedure: hemicellulose, cellulose and lignin) were analysed statistically by principal component analyses (PCA). PCA is a multivariate statistical 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. PCA was used to decrease redundancies in data sets and select the more relevant parameters to estimate biomethane potential. PCA calculations were

carried out with Matlab® software.

3. Results and discussion

3.1. Descriptive approach with PCA and parameters selection

The data of organic matter content (VS, COD_{Tot}, TOC, water leachable organic fraction COD_{Sol} and SOC), biochemical characterisation of organic matter, and biological tests (BOD_{28}, BMP_{60}, and Enzymatic Cellulose Degradation ECD) are reported in Tables 2, 3 and 4. Analyses of parameters were simultaneously treated in principle component analysis for substrates S1 to S9. Substrates S10 to S14 were excluded to the PCA because of missing value for ECD and biochemical characterisation with the NREL procedure (WAT, EThOH, cellulose, hemicellulose and lignin). All input data were normalised by taking the log_{10}, extracting the mean and dividing it by the standard deviation. Selected by the criterion of Kaiser (>1), Fig. 1, the PCA resulted to four principle components explaining 41, 21, 17 and 11% of the global variance of the initial data. The co-variance of variables, corresponding to 91% of the global variance, is illustrated by the loading plots in Fig. 2. The first principle component (Fig. 2A, horizontal axis) corresponds to 41% of the global variance, associated to several parameters closed to the correlation circle, and supposed to be correctly correlated. These include residual fraction (RES) from Van Soest sequential extraction and Lignin content from the NREL acid hydrolysis, and thus, indicating covariance of these two parameters. These two parameters are found in opposite to over biochemical parameters, including cellulose, hemicellulose, and to a lesser extent Van Soest fractions HEM and

CELL. Biological parameters BMP_{60} and BOD_{28} are located in the same quarter, close together, but are taken away from the circle of correlation. Organic soluble parameters COD_{Sol}, SOC are closely positioned and to a lesser extent to Van Soest fraction SOL, explaining the second principle component (PC2) illustrated in Fig. 2A. No clear correlation with other parameters was observed with, the cellulose hydrolysis parameter (ECD), the NREL extractable fractions WAT (water extractives) and EThOH (ethanol extractives). As observed on Fig. 2B which displays the two next principle components PC3 and PC4, WAT and EThOH overlap and build together PC3 with no other significant correlations. Moreover, PC4 is clearly built by the hydrolytic parameter "Cell hydrolysis" ECD, respirometric parameter BOD_{28}, and, to a lesser extent even to BMP_{60}. Located on the axis 4, but in one more great distance of the circle of correlation, this suggests a lesser correlation between BMP_{60} and "Cell hydrolysis" ECD, compared to BOD_{28} and ECD.

Relationships between samples and principle components axis, PC1-PC2 and PC2-PC4 are displayed in Fig. 2B and 2C, respectively. Substrates are mainly distributed on PC1, with two exceptions: S8 "Acacia branches" and S6 "Sugarcane bagasse (2)" located on PC2, respectively characterised by their relative low and high COD_{Sol} and SOC contents. Difference in scores for the next seven substrates is clearly observed on PC1, with the most biodegradable substrates positioned on the left half of the axis, according to their biochemical characteristics, in opposition to RES and lignin contents. Expect for S5 "Sugarcane bagasse (1), substrate distribution on PC3 and PC4 underlines the major role

of PC4 to characterise samples (Fig. 2D), correlated to biodegradability set including BOD_{28}, Cell hydrolysis and BMP_{60}, and, in opposition to RES lignin contents.

To conclude on data set analysed with PCA, a combination of selected parameters is strongly required to characterise biodegradability in anaerobic conditions as expected. Biochemical contents in particular lignin or residual (RES) contents influence biological parameters, while for soluble fractions (SOL, SOC, COD, WAT and EThOH), the significance was lower. Moreover, ECD and BOD_{28} might be used to predict biomethane potential BMP_{60}.

Table 4

Figure 1

Figure 2

3.2. Descriptive approach with simple linear regressions on selected parameters

Results on biochemical characterisation of organic matter and biological tests are reported in Tables 3 and 4. As already mentioned in the previous paragraph with PCA, significant differences were observed among lignocellulosic substrates S1 to S9 selected for the PCA analyses.

3.2.1. Comparison of biochemical method to compositional analysis of lignocellulosic biomass

As is shown in Table 4, the content of cellulose measured by Van Soest fractionation method was higher than that measured by hydrolyse acid method NREL. Moreover, the content of SOL was also higher than the sum of the fraction WAT and EThOH, except

for corn bagasse (S2). However, the case of lignin was opposite. The content of RES is lesser than Lignin content. The content of cellulose and Van Soest fraction “CELL” in the agricultural crops residues ranging from S1 to S6 were higher than that in the forest residues S7 to S9, we obtained the same results for hemicellulose and Van Soest fraction “HEM”. On the contrary, the content of lignin and Van Soest fraction “RES” in the agricultural residues were lower compared to those of the forest residues. This could explain that the biodegradability of agricultural residues were relatively better than forest residues. CELL and HEM or cellulose and hemicellulose were predominant compositions in all of the biomass. As can be seen from the Table 4, the biochemical compositions of lignocellulosic residues obtained from the two methods were different, which might be due to the different solvents used, In addition, Van Soest Fractionation is based on gravimetry, however, NREL procedure includes the measurement of easily quantified forms derived from the compositions besides gravimetry.

3.2.2. BMP and ratio of biochemical composition

The ratio among different compositions have been calculated and compared to BMP determination. The biochemical compositions content ratio varied with the composition analyse method, in which the ratio of the sum of non-RES fractions to RES, the sum of non-lignin fractions to lignin, CELL to RES and cellulose to lignin ranged from 2.33 to 17.18, 1.65 to 6.58, 0.60 to 8.90 and 0.82 to 3.10, respectively. Fig. 3 shows that linear relationships exist between BMP and the four above ratios. Furthermore, the trend of agricultural and forest residues was concordant. It is possible to conclude that BMP

correlates relatively well with the ratio of the sum of non-lignin fractions to lignin and that of cellulose to lignin.

Figure 3

3.2.3. Biomethane potential and non-extractable fractions

Non-extractable fractions in biomasses are the content of RES and lignin in the present study. The PCA analyses clearly prove the fact that BMP correlate with the content of lignin negatively whether agricultural or forest residues Although RES and lignin content are not converted into methane, they have significant impact on the recalcitrance of the lignocellulosic biomass, thus the content of RES and lignin could have significant impact on the production of biomethane. As seen in Fig. 4A and Fig. 4B, the relationship between BMP and the lignin content of agricultural residues S1 to S6 was better than that between BMP and the RES content of S1 to S6. However, it is not yet possible to conclude that the relationship between BMP and the RES content of forest residues is better than that between BMP and the lignin content because of the uncompleted data of other substrates S10 to S14. However, it should be noted that S9 point in Fig. 4B is far away from the trend, which could be explained by the low VS content of S9.

Figure 4

3.2.4. Biomethane potential and biological tests : BOD_{28} and ECD

Fig. 5A shows that two significant linear relationships between BMP and BOD_{28} of lignocellulosic residues. Agricultural and forest residues, S1 to S6 and S7 to S14, exhibit different behaviors of aerobic and anaerobic digestibility (Fig. 6). In Fig. 5B, the

relationship of agricultural residues between BMP and ECD was not relatively strong, however, it is not possible to conclude that the linear relationship of forest residues between BMP and ECD was significant because of uncompleted data of other substrates S10 to S14. However, it seems likely that different types of lignocellulosic residues show different relationship between BMP and ECD. Our result of relation between BMP and ECD is different compared to that reported by Liew *et al.* (Liew *et al.* 2012), which might be due to differences in BMP and ECD conditions or lignocellulosic residues used. As seen in Fig. 5C, except for S9, which has a low VS content, ECD of agricultural and forest residues decreased as the content of lignin increased, which is in general agreement with that reported by Masarin *et al.* (Masarin *et al.*, 2011)

Figure 5

Figure 6

4. Conclusions

The PCA data showed that biochemical compositions content in particular lignin or residual (RES) contents influence BMP, while for soluble fractions (SOL, SOC, COD, WAT and EThOH), the significance was lower. The biochemical compositions of lignocellulosic residues obtained from the two methods were different, which might be due to different measurement conditions. Among the relationships between BMP and other biochemical parameters, it is possible to conclude that BMP correlates relatively well with the ratio of the sum of non-lignin fractions to lignin and that of cellulose to lignin. The relationship between BMP and the lignin content of agricultural residues S1

to S6 was better than that between BMP and the RES content of S1 to S6. Agricultural residues S1 to S6 and forest residues S7 to S14 exhibit different behaviors of aerobic and anaerobic digestibility. There were two significant linear relationships between BMP and BOD_{28} in different types of residues. On the other hand, it seems likely that different types of lignocellulosic residues show different relationship between BMP and ECD. There was a relatively strong linearity between ECD and the lignin content. Although results showed that BMP and other characteristics of lignocellulosic residues were interrelated, for instance, BOD_{28}, ECD, biochemical compositions, it is not yet possible to predict BMP of lignocellulosic residues because of different types of residues, especially for the low VS content residues. Therefore, further study is needed to investigate more sorts of agricultural and forest residues and intrinsic differences of nature among different types of lignocellulosic residues.

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Tables

Table 1. Substrates designation, origin, and category.

#	Designation	Origin	Category
S1	Corn stover	France –Picardie	Agricultural residue
S2	Corn bagasse	France –Picardie	Agricultural residue
S3	Wheat Straw (1)	France –Picardie	Agricultural residue
S4	Wheat Straw (2)	France –Picardie	Agricultural residue
S5	Sugarcane bagasse (1)	Haïti	Agricultural residue
S6	Sugarcane bagasse (2)	Brasil	Agricultural residue
S7	Hazel branches	France – Rhône-Alpes	Sylvicultural residue
S8	Acacia branches	France – Rhône-Alpes	Sylvicultural residue
S9	Mix residual wood (1) from composting plants	France – Rhône-Alpes	Residues from green waste composting plants
S10	Mix residual wood (1) from composting plants	France – Rhône-Alpes	Residues from green waste composting plants
S11	Hardwood (*Pinus radiata*)	France – Rhône-Alpes	Sylvicultural residue
S12	Green waste Mix 1 (branches)	France – Rhône-Alpes	Biowaste
S13	Green waste Mix 2 (branches)	France – Rhône-Alpes	Biowaste
S14	Green waste Mix 3 (branches)	France – Rhône-Alpes	Biowaste

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

#	Designation	VS ($\%_{TS}$)	COD_{Tot} ($g_{O2}.kg^{-1}_{TS}$)	TOC ($\%_{TS}$)	COD_{Sol} ($g_{O2}.kg^{-1}_{VS}$)	% COD_{Sol} ($\%_{CODTot}$)	SOC ($g_{OC}.kg^{-1}_{VS}$)	% SOC ($\%_{TOC}$)
S1	Corn stover	95.5 ± 0.3	1180 ± 5	45.6 ± 2.0	129.2± 2.7	9.6	52.1	10.9
S2	Corn bagasse	89.4 ± 1.6	1150 ± 80	46.3 ± 1.5	115.3± 0.6	8.4	47.7	9.05
S3	Wheat Straw (1)	94.9 ± 0.1	1220 ± 4	51.5 ± 2,4	76.2 ± 0.4	5.7	30.7	5.4
S4	Wheat Straw (2)	91.5 ± 0.2	1250 ± 15	42.3 ± 1.1	81.5± 0.6	5.8	34.4	7.4
S5	Sugarcane bagasse (1)	96.8 ± 0.2	1260 ± 15	50.0 ± 1.9	280.3±0.9	20.0	71.5	12.8
S6	Sugarcane bagasse (2)	93.8 ± 0.2	1240 ± 15	44.5 ± 2.4	15.1± 0.6	1.1	6.1	1.3
S7	Hazel branches	97.3 ± 0.3	1345 ± 20	46.6 ± 0.9	59.7±0.3	4.1	23.2	4.8
S8	Acacia branches	95.7 ± 0.1	1300 ± 50	45.2 ± 2.0	113.7±0.4	8.1	47.0	9.9
S9	Mix residual wood (1)	62.7 ± 2.5	990 ± 9	49.5 ± 2.7	41.6±0.3	2.6	15.3	1.9
S10	Mix residual wood (2)	78.0 ± 2.2	1080 ± 50	40.1 ± 0.8	21.9±0.3	1.5	8.2	1.5
S11	Hardwood	98.3 ± 1.2	1370 ± 15	49.2 ± 2.5	28.2 ± 0.4	1.7	5.4	1.1
S12	GW Mix (1)	61.8 ± 0.6	889 ± 19	41.1 ± 5.9	183.3 ± 4.0	12.7	70.5	10.6
S13	GW Mix (2)	50.4 ± 1.9	756 ± 10	35.1 ± 1.1	110.5 ± 1.2	7.4	48.0	6.8
S14	GW Mix (3)	78.1 ± 0.1	972 ± 17	42.8 ± 7.0	234.3 ± 4.2	18.8	86.5	15.8

Table 3. Bioreactivity analyses of substrates: enzymatic cellulose degradation (ECD), biological oxygen demand (BOD_{28}), and biomethane potential (PBM_{60}).

#	Designation	ECD ($\%_{Cellulose}$)	BOD_{28} ($mg_{O2}.g^{-1}_{TS}$)	BOD_{28} ($mg_{O2}.g^{-1}_{VS}$)	BD_{Aero} ($\%_{COD}$)	BMP_{60} ($NmL.g^{-1}_{TS}$)	BMP_{60} ($NmL.g^{-1}_{VS}$)	BD_{Ana} ($\%_{COD}$)
S1	Corn stover	35.8±1.4	893 ± 8	935 ± 9	75.9	292 ± 3	306 ± 3	70.9
S2	Corn bagasse	25.6±0.6	889 ± 2	994 ± 2	77.5	315 ± 16	353 ± 18	78.5
S3	Wheat Straw (1)	19.7±1.3	787 ± 28	829 ± 30	64.6	264 ± 2	278 ± 2	61.8
S4	Wheat Straw (2)	20.3±0.2	871 ± 5	952 ± 5	69.8	296 ± 14	324 ± 15	67.8
S5	Sugarcane bagasse (1)	9.1±1.5	750 ± 22	775 ± 24	59.4	244 ± 5	252 ± 5	55.2
S6	Sugarcane bagasse (2)	15.0±0.4	620 ± 39	660 ± 41	50.0	211 ± 21	225 ± 22	48.6
S7	Hazel branches	8.8±0.5	482 ± 24	495 ± 25	35.9	95 ± 2	97 ± 2	20.2
S8	Acacia branches	22.8±0.8	835 ± 8	873 ± 8	64.5	145 ± 2	151 ± 2	31.9
S9	Mix residual wood (1)	13.4±2.2	433 ± 40	690 ± 64	43.9	62 ± 4	99 ± 6	18.0
S10	Mix residual wood (2)	ND	375 ± 23	481 ± 29	34.8	55 ± 5	70 ± 6	14.6
S11	Hardwood	ND	129 ± 11	131 ± 12	9.4	16 ± 1	16 ± 1	3.3
S12	GW Mix (1)	ND	195 ± 14	316 ± 10	21.9	39 ± 4	64 ± 7	12.5
S13	GW Mix (2)	ND	360 ± 23	714 ± 45	47.6	69 ± 5	137 ± 10	26.1
S14	GW Mix (3)	ND	678 ± 6	868 ± 8	69.8	138 ± 13	177 ± 16	40.6

ND : not determined

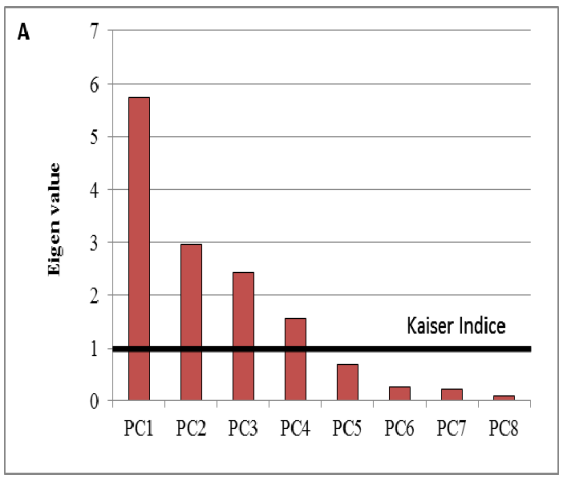
Table 4. Biochemical analyses of substrates.

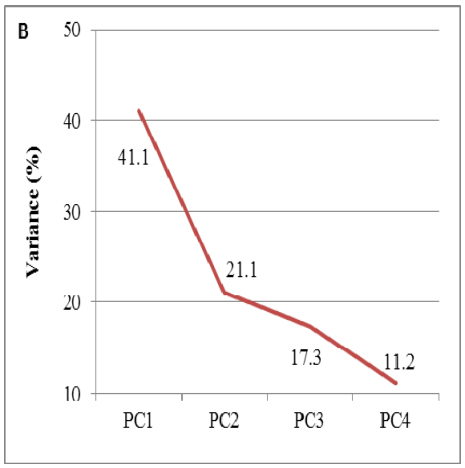
		Van Soest fiber extraction				NREL carbohydrates extraction				
#	**Designation**	**SOL (%vs)**	**HEM (%vs)**	**CELL (%vs)**	**RES (%vs)**	**WAT (%vs)**	**EThOH (%vs)**	**Cellulose (%vs)**	**Hemicellulose (%vs)**	**Lignin (%vs)**
S1	Corn stover	14.6 ± 1.5[a]	17.6 ± 1.2[a]	56.2 ± 2.1[a]	11.6 ± 0.5[a]	1.5 ± 0.6	1.4 ± 0.1	35.4 ± 0.7	37.6 ± 0.9	11.5 ± 0.1
S2	Corn bagasse	5.5 ± 2.1	40.0 ± 2.8	49.0 ± 0.9	5.4 ± 0.2	8.4 ± 0.3	3.9 ± 0.6	37.8 ± 0.8	24.5 ± 0.4	12.2 ± 0.6
S3	Wheat Straw (1)	12.2 ± 0.3	31.6 ± 0.5	50.1 ± 2.5	6.1 ± 2.3	8.5 ± 0.1	2.8 ± 0.4	37.3 ± 0.1	30.2 ± 1.3	13.8 ± 0.9
S4	Wheat Straw (2)	13.3 ± 0.9	28.2 ± 1.1	50.9 ± 0.5	7.6 ± 0.4	7.6 ± 0.2	3.0 ± 0.2	38.2 ± 0.4	23.0 ± 0.2	14.3 ± 2.4
S5	Sugarcane bagasse (1)	21.8 ± 0.3	23.1 ± 1.0	44.1 ± 4.4	11.0 ± 3.0	2.8 ± 0.5	1.7 ± 0.4	39.3 ± 0.4	25.6 ± 0.2	17.7 ± 0.2
S6	Sugarcane bagasse (2)	5.2 ± 0.9	36.7 ± 0.7	51,7 ± 0.4	6.4 ± 0.1	1.0 ± 0.1	1.4 ± 0.2	41.3 ± 0.9	27.0 ± 0.4	16.2 ± 2.2
S7	Hazel branches	10.6 ± 0.8	15.4 ± 0.9	55.3 ± 0.5	18.7 ± 0.4	5.0 ± 0.2	3.0 ± 0.1	31.6 ± 0.2	16.4 ± 1.4	20.4 ± 0.4
S8	Acacia branches	18.2 ± 0.8	17.6 ± 1.3	48.1 ± 1	16.1 ± 0.3	8.1 ± 0.7	4.0 ± 0.4	34.4 ± 0.4	12.1 ± 0.3	16.0 ± 0.5
S9	Mix residual wood (1)	13.7 ± 2.7	14.9 ± 4.0	45.0 ± 2.3	26.3 ± 0.9	4.5 ± 0.3	3.7 ± 0.2	24.3 ± 2.4	16.3 ± 1.3	29.5 ± 0.8
S10	Mix residual wood (2)	12.1 ± 0.1	13.8 ± 1.5	46.8 ± 2.3	28.0 ± 0.8	ND	ND	ND	ND	ND
S11	Hardwood	5.0 ± 0.2	15.0 ± 0.3	50.0 ± 0.8	30.0 ± 1.0	ND	ND	ND	ND	ND
S12	GW Mix (1)	31.8 ± 0.3	5.2 ± 2.4	38.4 ± 4,2	24.5 ± 0.6	ND	ND	ND	ND	ND
S13	GW Mix (2)	36.8 ± 0.5	15.8 ± 1.0	18.4 ± 1,1	29.0 ± 1.8	ND	ND	ND	ND	ND
S14	GW Mix (3)	42.9 ± 0.8	14.5 ± 0.8	29.0 ± 1,2	13.6 ± 1.5	ND	ND	ND	ND	ND

ND : not determined

a : Medic *et al.*, 2012.

Figures





Principal componente	Cumulative variance (%)
PC1	41.1
PC2	62.2
PC3	79.5
PC4	90.7
PC5	95.7
PC6	97.6
PC7	99.4
PC8	100.0

Figure 1: Results of the analysis in main components.

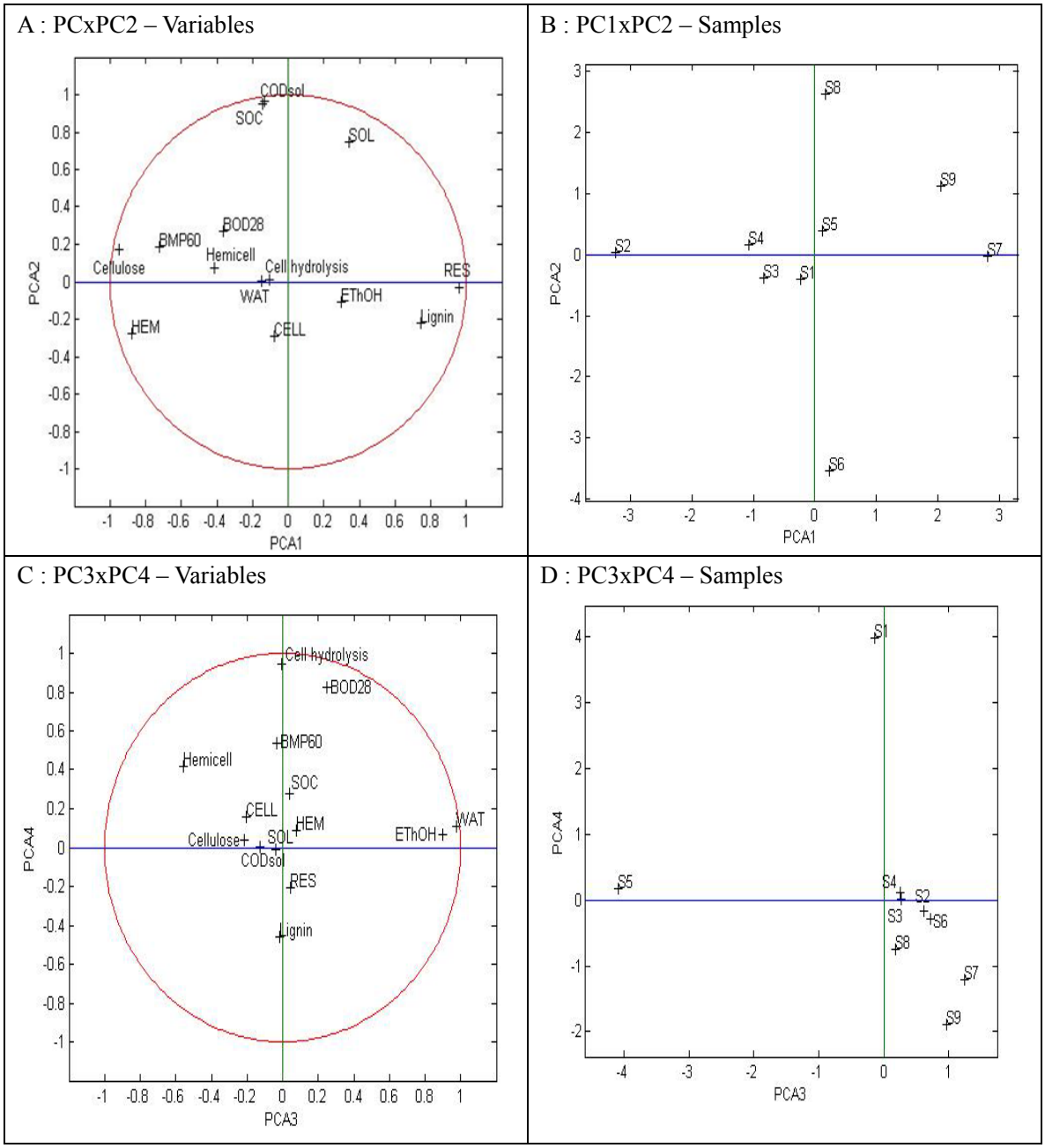


Figure 2: Plot of the loadings of the variables (A and C) and samples scores (B and C) with principal components PC1xPC2, and PC3xPC4.

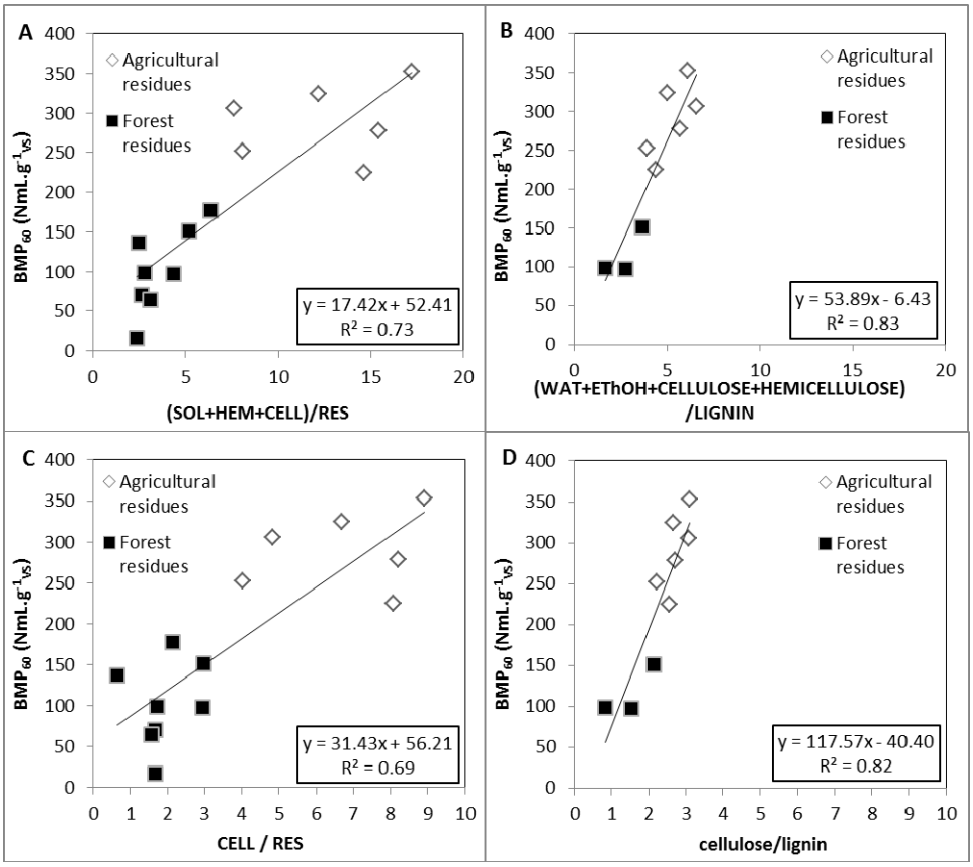


Figure 3: Relationship between BMP_{60} and ratio of biochemical composition.

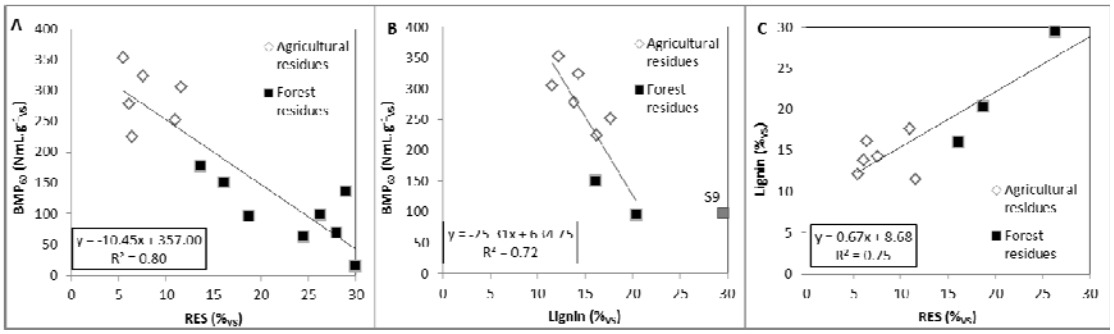


Figure 4: Relationship between BMP_{60} and RES or Lignin content: BMP and RES (%vs) (A); BMP and Lignin (%vs) (B); RES (%vs) and Lignin (%vs) (C).

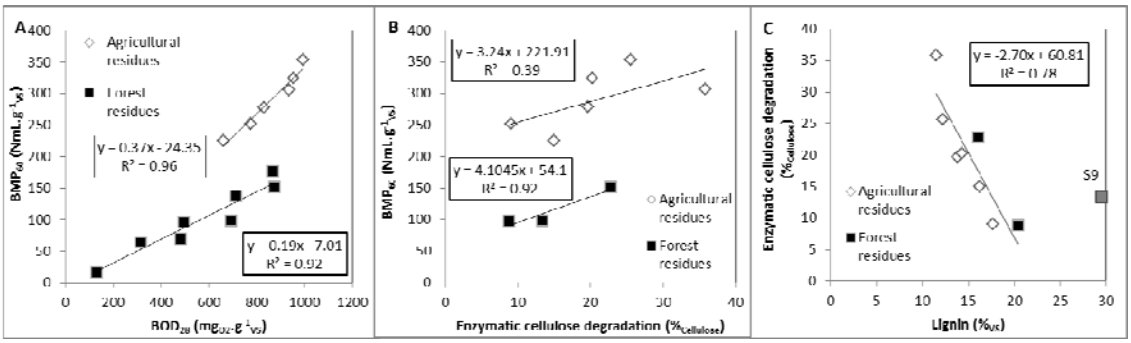


Figure 5: Relationship between BMP_{60} and BOD_{28} (A), BMP and Enzymatic cellulose degradation (ECD) ($\%_{Cellulose}$) (B), and ECD and Lignin ($\%_{VS}$) (C).

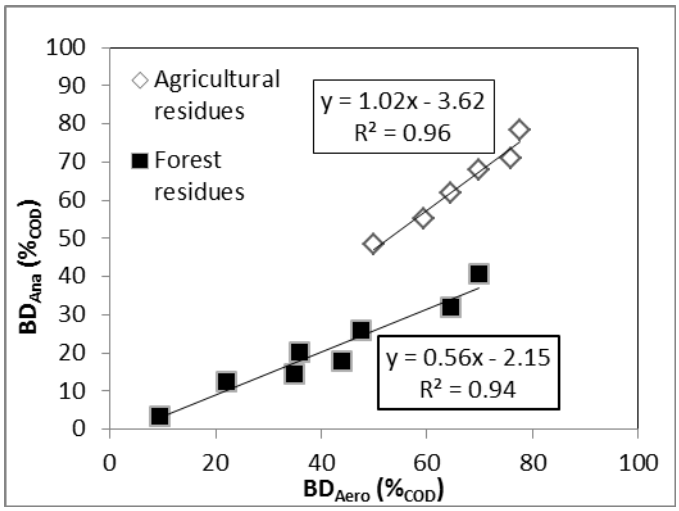


Figure 6: Relationship between anaerobic biodegradation BD_{Ana} (%COD), and BD_{Aero} (%COD).