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An improved procedure to assess the organic biodegradability and the biomethane potential of organic wastes for anaerobic digestion

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Abstract

In this study a fractionation procedure was developed and applied to evaluate the potential of some organic wastes (2 cattle manures and 2 catch crops, fresh and after ensiling) for anaerobic digestion. This procedure was based on water extraction of the raw sample, which enabled to evaluate the contributions of water-soluble and particulate phases to the investigated properties. Biomethane potential (BMP) and chemical oxygen demand (COD) were determined and used to assess the anaerobic biodegradability of raw materials. Analysis of structural carbohydrates, total Kjeldahl nitrogen, water-soluble carbohydrates, volatile fatty acids and pH were also included to explain the main phenomena involved in methane production from the tested biomasses. Results show that the origin and the preparation mode had a significant impact on BMP distribution. Based on a COD balance, the biodegradability of the various feedstocks ranged from 45% to 75%. Biodegradability of fresh materials was negatively correlated with the sum of structural carbohydrates and lignin content. Among the feedstock used, the water-soluble phase represented 8-69% of the total COD and 7-46% to the total BMP. Solubilization of organic matter during ensiling was due to the production and accumulation of organic acids from particulate carbohydrates and organic nitrogen. This procedure detects kinetic and biodegradability differences among biomasses and thus it can be useful for the design of anaerobic digestion plants. Furthermore, it can be applied to evaluate the efficiency of biomass pretreatments.

Keywords: Anaerobic digestion, Organic wastes, Biomass characterization, Organic matter leaching, Biodegradability, Biomethane production, Biodegradability rate, Methane yield.

Introduction

Over the last decades, anaerobic digestion (AD) has become an outstanding alternative to produce green energy. This process is a seductive solution not only for the treatment of agricultural and agro-industrial organic waste, but also for the management of municipal wastewater sludge, residual or source-sorted municipal solid waste. In this context, AD answers to two complementary issues: the treatment of organic waste, as well as the production of renewable energy. Biogas and biomethane are produced by biological pathway of methanogenesis. Without oxygen, biomass is hydrolyzed and monomers converted to water, carbon dioxide and methane. Thereafter, biogas can be used in combined heat and power (CHP) to produce energy and electricity, or purified and upgraded as biomethane for injection in urban natural gas grid, or used as biofuel for vehicles. For farm-scale digesters, the solid residues are generally post-treated for drying and composting, and used as organic amendment. Due to the diversity of feedstocks that can be used for biogas production, there is a growing interest in developing characterization procedures to assess their potential and suitability (Carlsson *et al.*, 2012; Kianmehr *et al.*, 2010; Montgomery and Bochmann, 2014). Moreover, these feedstocks undergo different stages, or unit operation, before entering the AD reactor, such as storage and pretreatments. Optimizing these steps requires an adequate characterization of their effect, including detailed data on specific properties such as methane yield, biochemical composition and biodegradability.

A large variety of substrates and organic residues may, therefore, be treated by anaerobic digestion (AD), including agricultural waste and green waste, sludge from wastewater treatment, source-sorted biowaste or residual municipal solid waste, food and food-processing waste (Bayard *et al.*, 2016). The most common parameter for characterization is the biomethane potential (BMP), which corresponds to the amount of

methane produced per mass unit of total solids (TS) or volatile solids (VS). Nevertheless, BMP is not enough to predict the degradation rate in AD process (Bayard *et al.*, 2018; Schievano *et al.*, 2008; Xu *et al.*, 2014). Many experimental studies have been conducted to correlate BMP and biochemical characteristics of organic substrates. Among these, lignin content is known to be negatively correlated with anaerobic biodegradability (BMP) (Buffiere *et al.*, 2008, 2006; Liu *et al.*, 2015; Triolo *et al.*, 2012). Under process conditions, biomass conversion to methane strongly depends on the accessibility of organic compounds to external enzymes (Jeffries, 1994, 1990; Jimenez *et al.*, 2015). Indeed, hydrolysis of particulate matter is considered as the rate-limiting step during anaerobic digestion of solid organic substrates, and its intensification leads to a better digestion performance (Shin *et al.*, 2001; Tanaka *et al.*, 1997; Vavilin *et al.*, 1996). Organic compounds such as cellulose, lignin, cutin or keratin are slowly or non-biodegradable compounds under anaerobic conditions while polymers such as starch and hemicellulose are assumed to be more readily biodegradable. Moreover, some biodegradable compounds may be less available since they are incorporated into complex, hardly biodegradable structures, such as lignocellulose or microbial cells (Gonzalez-Estrella *et al.*, 2017; Jeffries, 1994, 1990; Patinvoh *et al.*, 2017).

Biomass storage before anaerobic digestion is also an issue: the wide diversification of AD inputs and the need for continuous feeding of AD plants throughout the year make necessary to store collected agricultural/industrial waste or catch/energy crops for prolonged period (Teixeira Franco *et al.*, 2016a). The nature of the feedstock, the presence of air and the duration are the main parameters for the success of the long term storage (McDonald *et al.*, 1991). Regarding BMP conservation, ensiling (or confined storage) is a good solution to store wet biomass (Teixeira Franco *et al.*, 2017a, 2017b).

Pre-treatment processes have been developed over the last three decades to improve methane production in AD plants. Several reviews have recently been published with full description of pre-treatment technologies (Ariunbaatar *et al.*, 2014; Carlsson *et al.*, 2012; Carrere *et al.*, 2016; Elliott and Mahmood, 2012; Hendriks and Zeeman, 2009; Montgomery and Bochmann, 2014; Paudel *et al.*, 2017). It is well established that the

expected effects are i) the improvement of the methane yield and ii) the improvement of the methane production rate. As reviewed by Carlsson *et al.* (2012), the main targeted effects of pre-treatment are particle size reduction, solubilisation, and biodegradability enhancement. For instance, mechanical pre-treatments are commonly used for the reduction of particle size, and increase substrate surface (Hartmann *et al.*, 2000; Tsapekos *et al.*, 2015; Vavilin *et al.*, 1996). Combination of grinding, milling and chipping can significantly increase the methane yield because organic compounds become more available to enzymatic hydrolysis (Vavilin *et al.*, 1996; Ward *et al.*, 2008). In addition, the efficiency of pre-treatments can be highly different depending on the characteristics of the substrates and of the pre-treatment parameters (Ariunbaatar *et al.*, 2014). Moreover, it is difficult to establish a systematic comparison of the effects of a given pre-treatment. The main reasons for this are the diversity of substrates and the lack of common/standardized protocols for the evaluation (Carlsson *et al.*, 2012; Kianmehr *et al.*, 2010).

In the present work, an improved fractionation procedure is proposed to explain more in details the distribution of biodegradability and the methane yield (BMP) of feedstocks for anaerobic digestion. This procedure was complemented with the analysis of chemical oxygen demand (COD), structural carbohydrates, total Kjeldahl nitrogen (TKN), water-soluble carbohydrates (WSC), volatile fatty acids (VFA) and pH. Additionally, the monitoring of both water-soluble and particulate fractions of the different studied properties was performed, which is another originality of this study. Trials were carried out with two different types of catch crops and cattle manures. Nevertheless, this procedure should be suitable for other types of biomass, such as energy crops or urban organic waste, and appropriate to study storage conditions and the effect of substrate pretreatment on AD. The main aim of this work is to provide a useful tool to evaluate potential inputs for AD and to optimize the design of biogas plants. For this purpose, several agricultural fresh and ensiled feedstocks are being selected to design and test the fractionation procedure.

Material and methods

Raw materials

Two different types of catch crop and fresh cattle manure were collected from an agricultural site near Lyon (France). These feedstocks were chosen due to its major relevance in farm-scale AD, especially in France. Catch crop 1 (CC1) was a mixture of triticale, peas, vicia and fodder radish, and it was chopped at 4 cm maximum length at harvesting. Catch crop 2 (CC2) was a mixture of sunflower, sorghum, peas, vicia and *Trifolium alexandrinum*, and it was shredded with a BLIK BB350 rotary shear crusher before use (8 mm output particle size according to the manufacturer). Cattle manure 1 (M1) and Cattle manure 2 (M2) were collected from the same site but on different seasons of the year (March and October, respectively). Samples were stored at 4°C before further use.

The characterization procedure described in this work was applied to both fresh and ensiled raw materials. Confined storage (ensiling) was performed at 25 ± 2 °C in 3.5 L airtight round plastic storage drums during 3 months for catch crops and 4 months for cattle manure. Nomenclature and description of feedstocks are summarized in Table 1. Ensiling was tested for several reasons. First, it is a common practice for storage of herbaceous AD feedstocks (catch and energy crops). Since several biological phenomena occur during ensiling, biochemical properties may be extensively modified with biomass storage. In some cases, ensiling can be even considered as a biological pretreatment due to its positive impact on the methane potential of stored feedstocks (Janke *et al.*, 2019; Vervaeren *et al.*, 2010). Therefore, by testing ensiled feedstocks, we assess the applicability of the proposed analytical procedure to feedstocks with different biochemical properties, management practices and pretreatments before AD.

Moreover, at field scale, manure is rather stored in an open-air mode, which leads to considerable loss of organic matter and BMP (Teixeira Franco *et al.*, 2018b, 2017b). By storing manure under confined conditions, the objective was to evaluate if its BMP could be more efficiently preserved (for simplicity, confined conditions are referred to as “ensiling” in the present paper). It has to be mentioned however that

the evolution of the total mass, TS, VS, BMP during the ensiling process were also measured for all the experiments. Since the impact of the ensiling conditions on the global efficiency of the process have been investigated in details (Teixeira Franco *et al.*, 2016b, 2017a, 2017b, 2018a), the present paper will rather focus on the interest of the proposed experimental procedure.

Table 1 Nomenclature and properties of raw materials

Biomass	Preparation	Nomenclature	TS (%)	VS (%TS)	pH
Catch crop 1	Fresh	CC1-F	18.2±0.3	89.2±0.7	7.20
	Ensiled	CC1-E	16.8±0.3	90.0±0.4	5.41
Catch crop 2	Fresh	CC2-F	10.1±0.13	83.5±1.4	6.35
	Ensiled	CC2-E	8.10±0.3	78.9±4.1	5.59
Cattle manure 1	Fresh	M1-F	12.8±0.07	79.9±0.17	7.94
	Ensiled	M1-E	9.12±0.08	70.6±0.10	8.40
Cattle manure 2	Fresh	M2-F	11.6±0.12	82.2±0.20	7.70
	Ensiled	M2-E	9.34±0.12	77.4±0.04	7.82

Experimental procedure and chemical analysis

The developed fractionation procedure (**Erreur ! Source du renvoi introuvable.**) was based on water extraction of the raw sample (leaching test), separation and analysis of its different fractions. Leaching test is based on the NF EN 12457-4 (AFNOR, 2002) and it was performed with a standard 10:1 water/TS ratio during 2 h under constant bottle rotation at room temperature. Phase separation was done by centrifugation (5000 G; 10 min), followed by 0.7 mm particle size filtration. Finally, the particulate phase was dried at 70 °C until constant weight, and grounded with a Retsch SM 200 cutting mill and a bottom sieve with an aperture size of 2 mm. Raw and water-soluble samples were stored at 4 °C until analysis and particulate ones were stored at and -20 °C.

Raw sample (RS) was analyzed for its TS, VS and BMP. For the water-soluble phase, besides TS, VS and BMP, pH, WSC, VFA, COD, TKN, and ammonia nitrogen (NH₃-N) fractions were determined. Particulate solid was analyzed for its TS, VS, COD, TKN, and cell wall constituents. In order to assess the distribution of the various components of interest, some properties were determined through mass balances. For instance, the BMP of the particulate phase ($L_{STP}/kgVS_{RS}$) was estimated as the difference between the BMP of the raw material and the BMP of the water-soluble phase ($L_{STP}/kgVS_{RS}$). On the other hand, COD and TKN of the raw sample were estimated as the sum of the water-soluble and particulate contributions (both expressed in $kg/kgVS_{RS}$).

The biodegradability (BD) of each fraction was calculated from BMP and COD values considering the theoretical BMP of 0.35 $L_{STP}/kgCOD$ (Buswell and Mueller, 1952):

$$BD (\%) = \frac{BMP[L_{STP}/kgVS]}{COD [kg/kgVS] \times 0.35}$$

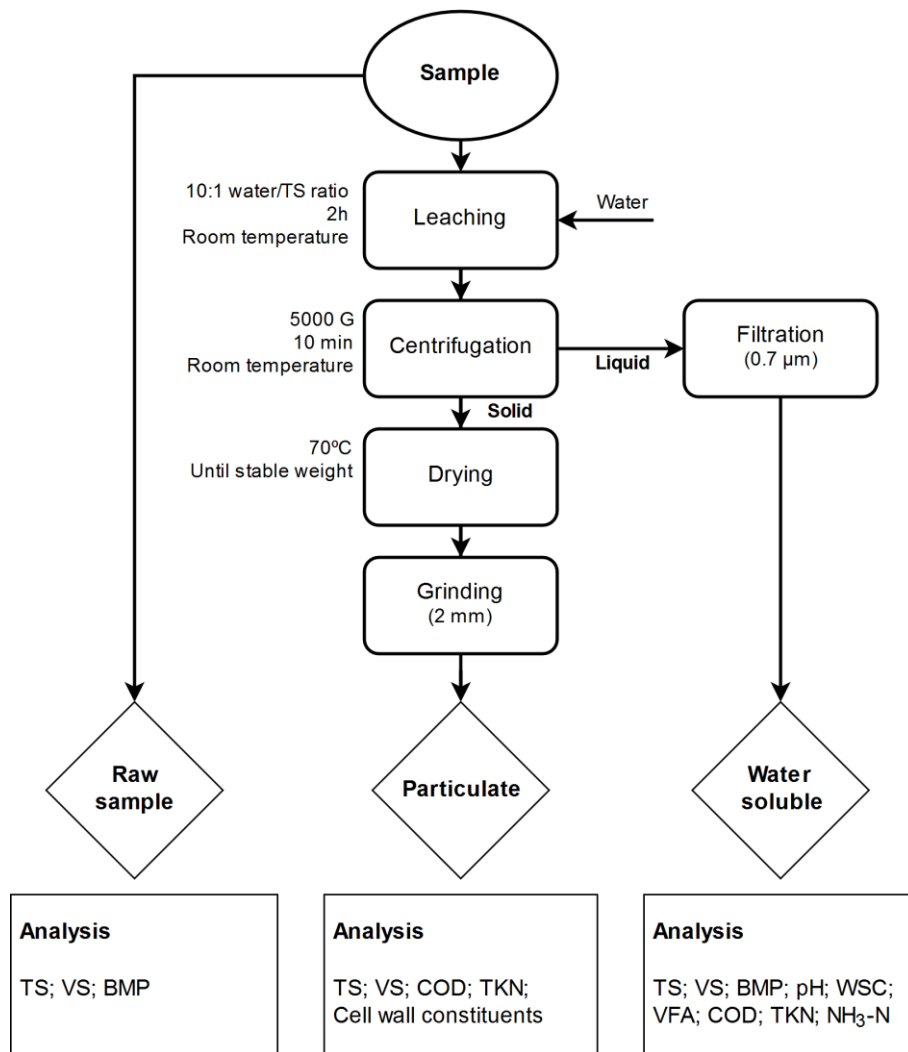


Fig. 1 Flowchart of the experimental methodology

TS was measured by oven drying at 105 °C during 24 h and VS was subsequently burned for 2 h at 550 °C. Since TS and VS contents are underestimated due to the loss of volatile compounds during the drying tests (Kreuger *et al.*, 2011), data was corrected according to the volatilization coefficients at 100 °C (Porter and Murray, 2001). pH was measured by a Consort C3020 device with a SP10B pH-electrode. WSC, lactic acid and formic acid contents were determined with high performance liquid chromatography (LC Module 1

plus, Waters) equipped with a Supelcogel™ C-610H column (300 x 7.8 mm, Sigma-Aldrich), both refractive index (RID) and UV detectors and operating with H₃PO₄ 0.1%v as solvent (flow rate of 0.5 mL/min). WSC content was estimated as the sum of glucose, xylose, galactose, mannose, arabinose and cellobiose and it was determined using the RID detector. Lactic acid and formic acid contents were obtained with the UV detector (210 nm). Acetic, propionic, butyric, valeric and caproic acids content were analyzed by gas chromatography (Shimadzu Corp.) equipped with a HP-FFAP fused silica capillary column (30 m x 0.25 mm, Agilent Technologies), a flame ionization detector and using H₂ as carrier gas. Total VFA was calculated as the sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acids. TKN and NH₃-N were determined through the procedure described in the NF EN 25663 standard (AFNOR, 1994). COD of water-soluble phase was determined through the colorimetric HACH procedure (method 8000). COD of particulate phase was measured by the Walkley and Black (1934) modified method, based on the NF ISO 14235 international standard (ISO, 1998). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed through Van Soest and Wine (1967) modified extractions method based on FD U44-162 standard (AFNOR, 2016). It was conducted on aliquots of powdered dry samples corresponding to 2 g of volatile solids. At each step, the residual solids were dried and weighed and analysed for VS contents. Four types of organic constituents were quantified, namely: (1) neutral detergent soluble compounds (SOL) extracted at the first step by a neutral detergent aqueous solution (NDF); (2) Hemicelluloses-like (HEM) extracted with a dilute acidic aqueous detergent solution, calculated as NDF minus ADF; (3) Cellulose-like (CELL) extracted with a concentrated 72% sulfuric acid solution, calculated as ADF minus ADL; and (4) lignin-like residual organic matter (LIG) which was not extracted in the procedure, corresponding to ADL.

The BMP tests followed the guidelines provided by the international working group on harmonization of BMP measurement (Holliger *et al.*, 2016). They were conducted at 35 °C using glass vessels of 2 L for raw sample, in order to ensure sample representativity, and 0.1 L for water-soluble phase in order to limit the

amount of sample needed. Vessels were filled with 5 g/L VS of sample, and 10 g/L VS inoculum (inoculum/substrate = 2) and a sufficient volume of a mineral solution to achieve 60% of the total volume of the vessel. The inoculum used (TS 2.0-3.3% wt; VS 1.4-2.2% wt) was a digested sludge originating from the wastewater treatment plant of La Feyssine, Lyon, France. The mineral solution, which contains essential elements for microbial growth and also gives the solution a buffer able to control any pH adjustments, was prepared according to the recommendations of ISO 11734:1995 standard (ISO, 1995). Once filled, reactors were purged with a N₂/CO₂ mixture (80/20% v) for about 5 minutes, sealed and equilibrated at 35 °C. Blanks with inoculum and mineral solution were performed for each batch series in order to correct the BMP from residual methane production of the inoculum. All tests were performed in triplicates. Biogas production was determined by pressure measurement using a Digitron precision manometer. Biogas was released when the pressure exceeded 1200 hPa. Gas composition (CH₄, H₂, N₂, O₂, H₂O and H₂S) was analysed using an Agilent 3000 micro gas chromatography with thermal conductivity detector (GC-TCD). Molsieve 5A (14 m length; pore size: 5 Å) and PoraPlot A (10 m length; 0.320 mm ID) columns were used as stationary phases for GC-TCD, with Argon and Helium as carrier gases, respectively. According to the guidelines, BMP was considered achieved when daily biogas production represented less than 1% of the total volume of biogas produced.

Results and discussion

Distribution of methane yield (BMP) and biodegradability (BD)

The BMP values varied widely within the set of tested raw materials (Table 2). Fresh catch crops had a BMP of 210-335 L_{STP}/kgVS_{RS}, while BMP of fresh cattle manures was 257-288 L_{STP}/kgVS_{RS}. The preparation mode also had an impact on the BMP of the feedstocks. Ensiling had a positive effect on the methane production of catch crops. On the contrary, BMP of cattle manure decreased after long-term ensiling. This reflects the relevance of controlling storage and preparation of inputs before biogas

production. Also, it is worth mentioning that BMP of ensiled biomass may be overestimated, since organic losses during storage are not taken into account.

Table 2 Biomethane potential of feedstocks and its phase distribution

	CC1-F	CC1-E	CC2-F	CC2-E	M1-F	M1-E	M2-F	M2-E
Raw sample								
BMP (L _{STP} /kgVS _{RS})	210±14	300±12	335±34	410±7	288±14	255±7	257±6	217±2
Water-soluble phase								
BMP (L _{STP} /kgVS _{RS})	20±0.6	72±5.0	41±2.3	190±28	77±1.1	38±0.3	43±4.0	48±1.8
% BMP _{RS}	7.3	24.1	12.3	46.3	26.9	15.1	16.6	22.2
Particulate phase								
BMP (L _{STP} /kgVS _{RS})	250±20	228±24	294±46	220±37	210±13	217±8	215±27	169±8
% BMP _{RS}	92.7	75.9	87.7	53.7	73.1	84.9	83.4	77.8

Likewise, distinct distributions of BMP were found among the feedstocks. Indeed, contribution of water-soluble phase to the BMP of the raw sample ranged from 7% to 46%. For catch crops, the ensiling process leads to a general increase of the contribution of the soluble fraction to the total BMP (from 7.3 to 24.1 % and from 12.3 to 46.3% for CC1 and CC2 respectively). The water-soluble phase mostly contains non-lignocellulosic and simple compounds that can be transformed in methane afterwards, including different types of water-soluble carbohydrates, volatile fatty acids, or even soluble amino acids. These soluble compounds are more quickly converted into methane than particulate ones (Pavlostathis and Giraldo-Gomez, 1991; Shrestha *et al.*, 2017). Therefore, feedstocks with higher contribution of water-soluble phase to the BMP will theoretically have a faster degradation during AD, as will be discussed later. In addition, soluble compounds are more easily diffused into the reactor, improving feedstock's accessibility to the degrading microorganisms. Therefore, the analysis of BMP distribution provides crucial information

concerning degradation kinetics, which is essential for feedstock selection, pretreatment operation, and design of AD plants.

Moreover, the distribution of BMP was affected by ensiling, demonstrating that management practices of feedstocks before AD may have a significant influence on organic matter structure. On the one hand, for catch crops the contribution of water-soluble phase to the BMP increased with ensiling. This may be explained by the hydrolysis of complex carbohydrates/proteins and their subsequent accumulation in the form VFA after lactic fermentation. These are well documented phenomena in good quality silages (at low pH conditions) (Desta *et al.*, 2016; McDonald *et al.*, 1991; Menardo *et al.*, 2015; Pakarinen *et al.*, 2011; Teixeira Franco *et al.*, 2017a). On the other hand, while testing cattle manure it is difficult to distinguish a specific impact of confined storage on the phase distribution of BMP. This is attributed to the fact that manure evolves differently during storage and it is highly degraded due to its inadequate conservation properties (lack of WSC, high buffering capacity, etc.) (Teixeira Franco *et al.*, 2018b, 2018a, 2017b).

It should be noticed that ensiling is sometimes considered as long-term biological pretreatment due to its impact on BMP and biomass properties (Ambye-Jensen *et al.*, 2013; Gallegos *et al.*, 2017; Larsen *et al.*, 2017; Teixeira Franco *et al.*, 2016a). Thus, these results suggest that this type of multiphase analysis may also provide important data on the efficiency of various pretreatments, either concerning the solubilization of organic matter or the improvement of degradation kinetics.

From the collected data on BMP tests, it is possible to evaluate the individual methane production kinetics between the raw and soluble fraction of a given sample. In all our tests, the methane production (after deduction of inoculum methane production) was well represented by a 1st order kinetics. The interesting output was that for all the dataset presented, the 1st order constant k ranged from 0.1 to 0.18 d⁻¹ for raw samples, and from 0.25 to 0.39 d⁻¹ for the soluble fraction. However, the comparison of the BMP kinetics for long-term tests like ensiling is subject to doubts, since the anaerobic inoculum used for the BMP tests

would probably not have a similar activity. In other word, it is advised to use the same inoculum to compare the effect of a given pre-treatment on the BMP production kinetics (Buffière *et al.*, 2018).

COD balance

The results of COD analysis and its phase distribution are shown in Table 3. COD of raw sample was 1204-1553 g/kgVS_{RS} for the tested feedstocks.

Table 3 Chemical oxygen demand of feedstocks and its phase distribution. WSC and VFA contents (COD basis) are presented in the water-soluble phase

	CC1-F	CC1-E	CC2-F	CC2-E	M1-F	M1-E	M2-F	M2-E
Raw sample								
COD (g _{O2} /kgVS _{RS})	1280	1527	1296	1553	1476	1204	1314	1310
Water-soluble phase								
COD (g _{O2} /kgVS _{RS})	100	464	184	1074	275	146	133	183
% COD _{RS}	7.8	30.4	14.2	69.2	18.6	12.1	10.2	14.0
WSC (% COD _{RS})	0.10	0.11	9.90	0.09	0.00	0.00	0.00	0.16
VFA (% COD _{RS})	3.1	16.0	0.72	18.3	2.2	2.3	5.8	10.6
Particulate phase								
COD (g _{O2} /kgVS _{RS})	1180	1063	1112	479	1201	1058	1181	1127
% COD _{RS}	92.2	69.6	85.8	30.8	81.4	87.9	89.8	86.0

However, as for the methane potential, COD distribution varied greatly depending on the feedstock. The COD of the water-soluble phase was 100-1074 g/kgVS_{RS}, which represents a contribution to the total COD of 8-69%. Ensiled catch crops have the highest water-soluble COD. In fact, for these feedstocks there was

a vast production and accumulation of VFA during ensiling. Likewise, VFA content increased during ensiling of Cattle Manure 2 (M2). In such cases, VFA produced during ensiling were far larger than initial WSC (even neglecting COD yield of endogenous bacteria). This means that part of VFA was a product of the hydrolysis (and fermentation) of particulate organic matter, as previously suggested.

It should be mentioned that it was not possible to establish a fully detailed COD balance of the water-soluble phase. In fact, the measured WSC and VFA accounted for 12% to 77% of the total water-soluble COD. The analysis of other compounds, such as alcohols, oligosaccharides or soluble amino acids, should be carried out in order to improve the description of the water-soluble COD.

Structural carbohydrates and TKN balance

Table 4 shows a mass balance of the organic matter with a focus on the particulate fraction determined from the Van Soest analysis (on a VS basis of the raw sample since the COD of the lignocellulosic fractions are not known).

Table 4 VS balance of feedstocks, with a focus on the particulate organic matter and structural carbohydrates. Results are expressed on % VS

	CC1-F	CC1-E	CC2-F	CC2-E	M1-F	M1-E	M2-F	M2-E
Water-Soluble	8.6	25.7	15.6	68.5	18.0	19.5	16.6	22.2
SOL	19.7	20.0	49.0	16.9	16.5	14.6	20.7	23.3
Particulate								
HEM	13.2	9.5	11.3	2.6	32.5	27.7	28.4	22.4
CEL	40.2	33.4	20.4	9.8	26.8	28.0	25.7	21.4
LIG	18.2	11.4	3.8	2.1	6.1	10.1	8.5	11

Besides, the structure of organic matter strongly depends on either if the biomass is conserved through ensiling or not. Indeed, for all feedstocks there was a decrease of the lignocellulosic fraction during ensiling. According to previous works, this degradation is due to the enzymatic or acid hydrolysis that may occur during biomass preservation (Dewar *et al.*, 1963; McDonald *et al.*, 1991). Moreover, the degradation products of these reactions should be water-soluble compounds, since no increase of the non-structural particulate fraction (SOL) was recorded with ensiling.

TKN of raw samples varied from 25.8 g/kgVS to 84.1 g/kgVS (results not shown) and no correlation was found either for the type of biomass or the mode of preparation. This evidenced a great variability of the protein and amino acids content among organic wastes.

Furthermore, TKN composition clearly depended on the nature of the feedstock, as illustrated in Fig. 2. First, TKN was mostly present in the form organic nitrogen (soluble and particulate) for fresh materials. This was especially true for catch crops, in which ammonia content was less than 7% of raw sample TKN. For fresh manures, ammonia nitrogen was 21-32% of raw sample TKN. The presence of ammonia is an indicator of biomass (protein) hydrolysis (McDonald *et al.*, 1991; Teixeira Franco *et al.*, 2016a) and may lead to bacterial inhibition (Batstone *et al.*, 2002; Chen *et al.*, 2014; Yenigün and Demirel, 2013). This issue should be more significant for the operation of digesters using cattle manure, since its low level of VFA may not contribute to mitigate the effects of NH₃ on the pH.

Likewise, ensiling had a marked impact on the distribution of nitrogen between the fractions. The particulate fraction of TKN decreased with ensiling, especially for catch crops. This was attributed to the slow hydrolysis of protein during storage, followed by a subsequent fermentation of amino acids, producing a pool of VFA, H₂ and ammonia nitrogen (Batstone *et al.*, 2002; McDonald *et al.*, 1991). In summary, this fractionation procedure enabled to show that not only structural carbohydrates but also proteins and amino

acids are degraded during the storage before AD. All these results clarify the solubilization of COD and BMP observed for some raw materials.

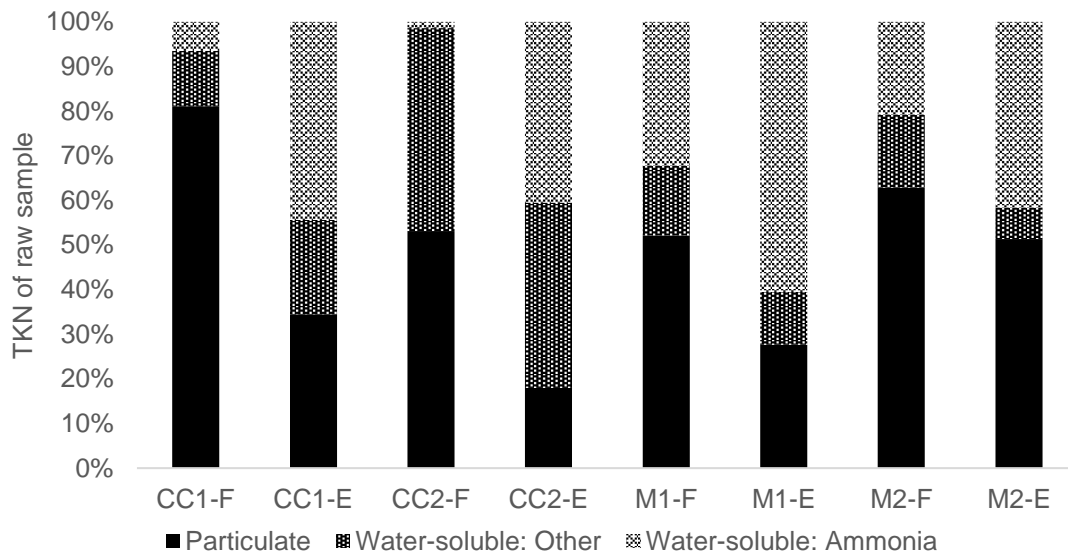


Fig. 2 TKN balance of feedstocks

Organic biodegradability

The impact of the results discussed above on the biodegradability of organic matter is shown in Fig. 3. It presents the different biodegradable (BD) and non-biodegradable (NDB) contributions of water-soluble and particulate fractions to the COD of the raw sample.

Total biodegradability varied among the fresh feedstocks: it was 47% for CC1-F, 74% for CC2-F and 56% for both M1-F and M2-F. From the VS balance presented in Table 4, biodegradability of fresh materials was negatively correlated with the sum of structural carbohydrates and lignin content. This is in agreement with previous studies that have demonstrate a negative impact of lignin content on methane production by anaerobic digestion (Buffiere *et al.*, 2006; Jeffries, 1994; Liu *et al.*, 2015; Triolo *et al.*, 2011).

On the one hand, the biodegradability of the particulate COD fraction was 59% in average. The non-biodegradable (NBD) fraction corresponded to the lignocellulosic compounds that are not degraded during the BMP tests (Jeffries, 1994), and to the COD used for bacterial growth (biomass yield), which is a small amount. The biodegradability of the particulate phase was closely linked to the lignin content. On the other hand, the biodegradability of water-soluble phase was 65% in average. It is unlikely that all water-soluble NBD corresponds to the COD used for bacterial growth (the biomass yields suggested in the Anaerobic Digestion Model 1 is around 10% on a COD basis (Batstone *et al.*, 2002). Therefore, this suggests that an important part of the organic matter present in the water-soluble phase is not biodegradable, which was unexpected.

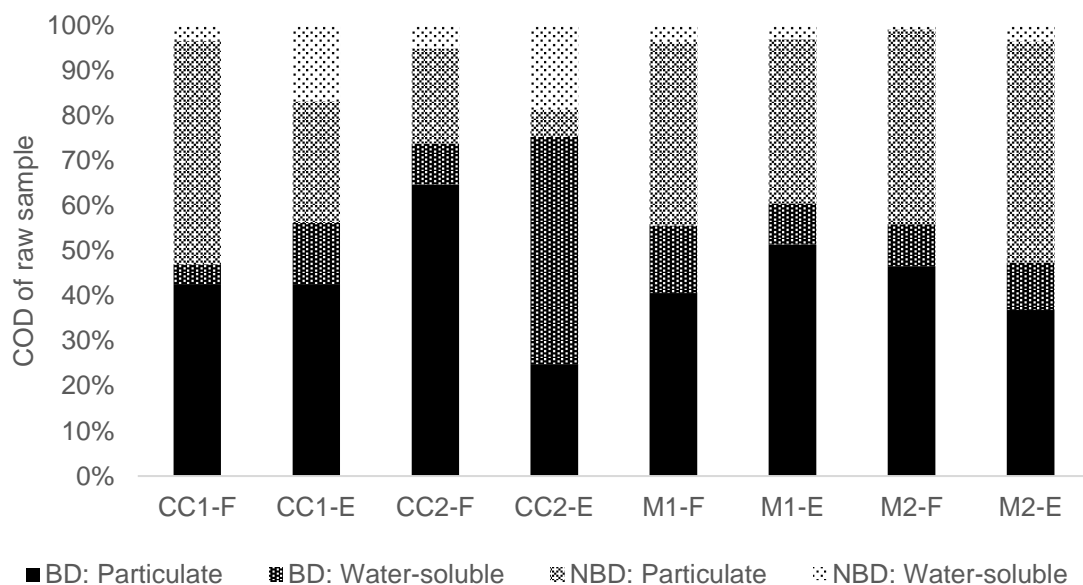


Fig. 3 Organic biodegradability of feedstocks and its phase distribution on a COD basis. BD stands for biodegradable; NBD stands for non-biodegradable

Finally, considerable differences in biodegradability were found for some feedstocks before and after ensiling. This was especially true for Catch Crop 1 and Cattle Manure 2. In the case of CC1, ensiling had a

positive impact on biodegradability (+19%). On the contrary, biodegradability of M2-E was 19% lower than that of M2-F. This demonstrates how the efficiency of biomass management before AD is important to safeguard the energetic content of organic matter as well as its biodegradability.

Conclusions

A fractionation procedure based on the separation of a water soluble and a particulate fraction was successfully applied to assess and explain the organic biodegradability and the biomethane potential of different catch crops and cattle manures. This procedure evidenced a significant impact of the origin of biomass and its management conditions on the BMP and the biodegradability rates. Furthermore, biodegradability of fresh materials was negatively correlated with the sum of (hemi-) cellulose and lignin content.

Likewise, distinct distribution of COD and BMP were found among feedstocks: contribution of the water-soluble phase was 8-69% to the COD and 7-46% to the BMP of the raw sample. The highest water-soluble contributions corresponded to the ones of efficient ensiled biomass. Solubilization of organic matter during ensiling was mainly due to the production and accumulation of organic acids from particulate carbohydrates and organic nitrogen. This procedure enabled to compare different strategies or unit operation during the preparation of feedstocks for AD. However, additional methods are required for the identification of the soluble non-biodegradable compounds. In addition, this procedure may be useful to detect and explain kinetic (methane production rate) and biodegradability differences among biomasses before and after pretreatments.

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