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

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Abstract

In this study a methodology was developed and applied to evaluate the potential of several organic wastes (cattle manures and catch crops) for anaerobic digestion. These procedure was based on water extraction of the raw sample, which enabled the measurement of the contributions of water-soluble and particulate phases to the studied 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 biomass source and its preparation mode had a significant impact on BMP. Likewise, biodegradability rate of feedstocks varied from 45% to 75%. Biodegradability of fresh materials was negatively correlated with the sum of structural carbohydrates and lignin content. Distinct distribution of COD and BMP were found among feedstocks. Indeed, contribution of the water-soluble phase was 8-69% to the total COD and 7-46% to the total BMP. The highest water-soluble contributions corresponded to the ones of efficient ensiled biomass. Solubilization of organic matter during ensiling was due to the production and accumulation of organic acids from particulate carbohydrates and organic nitrogen. This methodology 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 for other feedstocks and be used to evaluate the efficiency of biomass pretreatments.

Keywords: Anaerobic digestion, Organic wastes, Biomass characterization, Organic matter leaching, Biodegradability, Biomethane production

1. 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 residual municipal solid waste or urban bio-waste after selective collection. 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. The solid residues are generally post-treated for drying and composting, and used as organic amendment in agriculture.

AD processes can work under wet or dry operating conditions. The type of system used and the design of the biogas plant is mostly based on the properties of the inputs. Commonly, this characterization is mostly done through the analysis of 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. Biomass conversion to methane in liquid and solid AD processes can be assumed to be strongly dependent of the accessibility of organic compounds to microbial population. Bio-accessibility is supposed to be linked to the biochemical composition, but also to the water solubility of organic solid particles [1]. Due to the wide diversity of feedstock that can be used for biogas production, there is today a growing interest on developing characterizing procedures that provide a full picture on the potential and suitability of inputs for the different AD systems.

In the present work, an improved methodology is proposed to assess the biodegradability rate (BD) and the 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 one of the originalities 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. 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.

2. Material and methods

2.1. Raw materials

Two different types of catch crop and fresh cattle manure were collected from an agricultural site near Lyon (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 chopped to theoretical particle size of 8 mm before use. Cattle manure 1 (M1) and Cattle manure 2 (M2) were collected from the same site but on different seasons of the year. Samples were stored at 4°C before further use.

The experimental characterization described in this work was applied for both fresh and ensiled raw materials. 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.

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

2.2. Experimental procedure and chemical analysis

The developed methodology (Fig. 1) was based on water extraction of the raw sample (leaching), separation and analysis of its different fractions. Leaching test was performed with a standard 10:1 water/TS ratio during 2 h under constant bottle rotation. 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 ground at 2 mm theoretical length. Raw sample/water-soluble and particulate samples were stored at 4 °C and -20 °C, respectively, until analysis.

Raw sample (RS) was analyzed for its TS/VS content and BMP. For the water-soluble phase (WS), besides TS/VS content and BMP, pH, WSC, VFA, COD, TKN and ammonia nitrogen (NH_3-N) fractions were determined. Particulate solid (P) was analyzed for its TS/VS, COD, TKN and cell wall constituents.

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/VS contents are underestimated due to the loss of volatile compounds during the drying tests [2], data was corrected according to the volatilization coefficients at 100 °C suggested by Porter and Murray [3]. 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 H_3PO_4 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 UV detector (210 nm). Lactic acid and formic acid contents were obtained with the RID detector. 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 H_2 as carrier gas. Total VFA was calculated as the sum of lactic, formic, acetic, propionic, butyric, valeric and caproic acids. TKN and NH_3-N were determined through the procedure described in the NF EN 25663 standard [4]. COD of water-soluble phase was determined through the colorimetric HACH procedure (method 8000). COD of particulate phase was measured by a Walkley and Black [5] modified method, based on the NF ISO 14235 international standard [6]. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed through Van Soest and Wine [7] modified extractions method based on FD U44-162 standard [8]. Hemicellulose content was calculated as NDF minus ADF; cellulose as ADF minus ADL and; lignin as approximatively equal to ADL

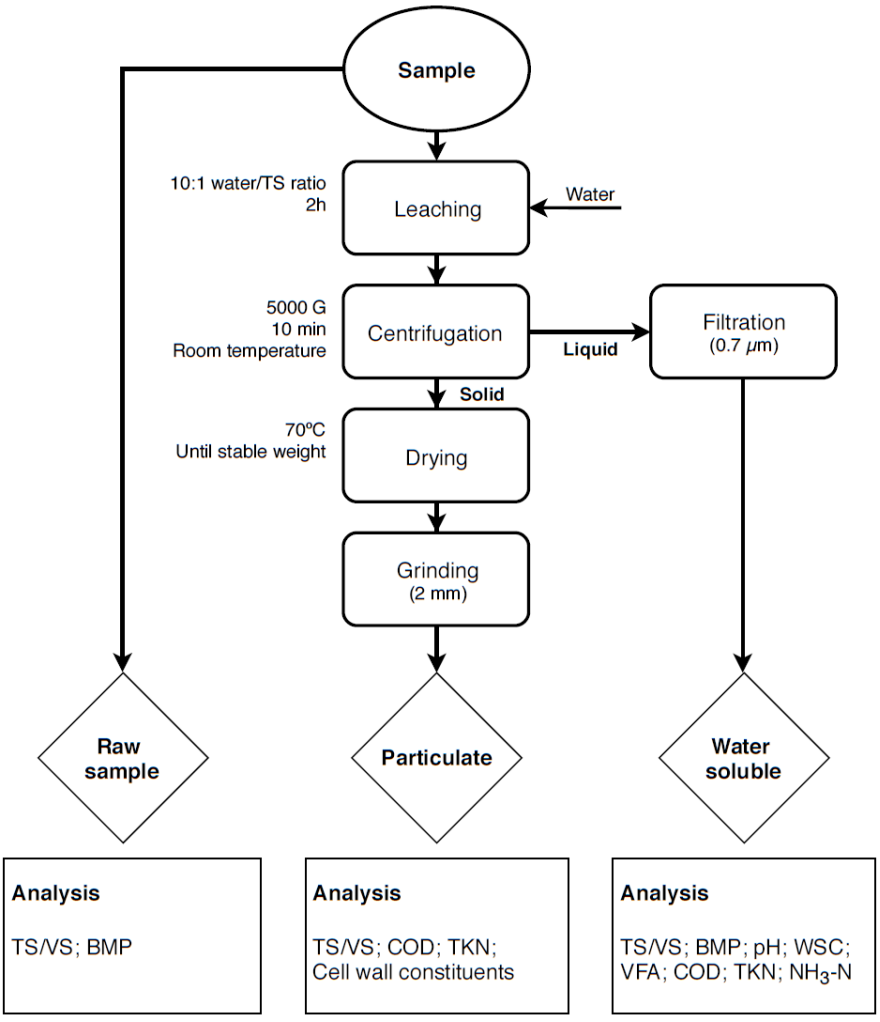

Fig. 1 Flowchart of the experimental methodology

The BMP tests followed the guidelines provided by Holliger *et al.* [9] and were conducted in a temperate room at 35 °C using glass vessels of 2 L for raw sample and 0.1 L for water-soluble phase. Vessels were filled with 5 g VS of sample, inoculum so as to keep a substrate/inoculum VS ratio of 0.5 and a certain 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 to 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 [10]. Once filled, reactors were purged with a N_2/CO_2 mixture (80/20%v) for about 5 minutes, sealed and equilibrated at 35 °C. Blanks with only 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 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. BMP was considered achieved when daily biogas production represented less than 1% of the total volume of biogas produced.

In order to assess the phase distribution of the various components of interest, non-measured properties were determined with the following mass balances:

$$BMP_P[L_{STP}/kgVS_{RS}] = BMP_{RS}[L_{STP}/kgVS_{RS}] - BMP_{WS}[L_{STP}/kgVS_{RS}] \quad (1)$$

$$COD_{RS}[kg/kgVS_{RS}] = COD_{WS}[kg/kgVS_{RS}] + COD_P[kg/kgVS_{RS}] \quad (2)$$

$$TKN_{RS}[kg/kgVS_{RS}] = TKN_{WS}[kg/kgVS_{RS}] + TKN_P[kg/kgVS_{RS}] \quad (3)$$

Finally, the biodegradability of each fraction was calculated from BMP and COD values considering the theoretical BMP of 0.35 L_{STP}/kg_{COD} [11,12], as described below:

$$BD\ (\%) = \frac{BMP[L_{STP}/kgVS]}{COD\ [kg/kgVS] \times 0.35} \quad (4)$$

3. Results and discussion

3.1. Distribution of biomethane potential

The BMP values varied widely within the set of tested raw materials (Table 2). Fresh catch crops had a BMP of 270-335 $L_{STP}/kgVS_{RS}$, 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.

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%. In the water-soluble phase it can be found most of non-lignocellulosic and simple compounds that can be transformed in methane afterwards. This comprises all different types of water-soluble carbohydrates, volatile fatty acids, or even soluble amino acids. These compounds do not need to pass through hydrolysis, usually named as the rate-limiting step during biogas production from complex biomass [13,14]. Therefore, feedstocks with higher contribution of water-soluble phase to the BMP will theoretically have a faster degradation during AD. 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 and design of AD plants.

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 ($L_{STP}/kgVS_{RS}$)	270±14	300±12	335±34	410±7	288±14	255±7	257±6	217±2.0
Water-soluble phase								
BMP ($L_{STP}/kgVS_{RS}$)	20±0.6	72±5	41±2.3	190±28	77±1.1	38±0.3	43±4	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

Moreover, the distribution of BMP was affected by ensiling, demonstrating that management practices of feedstocks before AD may have a significant impact on organic matter structure and methane production kinetics. 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) [15–19]. On the other hand, while testing cattle manure it is difficult to distinguish a specific impact of ensiling on the phase distribution of BMP. This should be related 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.) [20–22].

It should be noticed that ensiling is sometimes considered as long-term biological pretreatment due to its impact on BMP and biomass properties [23–26]. 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.

3.2. COD balance

The results of COD analysis and its phase distribution are shown in Table 3. COD of raw sample was 1204-1533 g/kgVS_{RS} for the tested feedstocks. This range is less noteworthy that the one found for the BMP. Therefore, significant variations on the biodegradability among biomasses are expected.

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	9.9	0.09	0.0	0.0	0.0	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%. Furthermore, the highest water-soluble COD values correspond to the ones of ensiled catch crops. 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 was 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 represented from 12% to 77% of the total water-

soluble COD. The analysis of other potential compounds, such as alcohols, oligosaccharides or soluble amino acids, should be carried out in order to improve the description of the water-soluble COD.

3.3. 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. This data is presented on VS basis of the raw sample since the DCO 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
Particulate	NDF+W	19.7	20.0	49.0	16.9	16.5	14.6	20.7	23.3
	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

The results show that the lignocellulosic composition varied according to the biomass used. Catch crop 1 had 53%VS of (hemi-) cellulosic compounds and high lignin content (18%VS). Catch crop 2 had far lower content of structural carbohydrates (31%VS) and lignin (4%VS). Regarding cattle manures, similar particulate distribution was found: 54-56%VS of (hemi-) cellulosic compounds and 6-9%VS of lignin content.

Besides, the structure of organic matter strongly depend 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 [16,27]. Moreover, the degradation products of these reactions should be water-soluble compounds, since no increase of the non-structural particulate fraction (NDF+W) 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 evidences a great variability of the protein and amino acids content among organic wastes.

Furthermore, TKN structure clearly depends on the nature of the feedstock, as illustrated in Fig. 2. First, it is shown that TKN is mostly present in the form organic nitrogen (soluble and particulate) for

fresh materials. This is especially true for catch crops, in which ammonia content represents less than 7% of raw sample TKN. For fresh manures, NH_3-N was 21-32% of raw sample TKN. It is relevant to notice that ammonia nitrogen is an indicator of the state of biomass evolution [16,25] and may lead to bacterial inhibition phenomena [28–30]. 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 NH_3 on the pH.

Likewise, biomass management through ensiling had a marked impact on the phase distribution of nitrogen. In fact, the particulate fraction of TKN decreased with ensiling, being this phenomenon more evident for the structure of catch crops. In this phase, it can be found the proteins that are slowly hydrolyzed during storage. These are subsequently fermented together with the amino acids present in the biomass, producing a pool of VFA, H_2 and NH_3 [16,30]. This explains the higher concentrations of NH_3 on ensiled crops. In summary, this shows that not only structural carbohydrates but also proteins and amino acids are degraded during the preservation of resources before AD. All these results clarify the solubilization of COD and BMP observed for some raw materials.

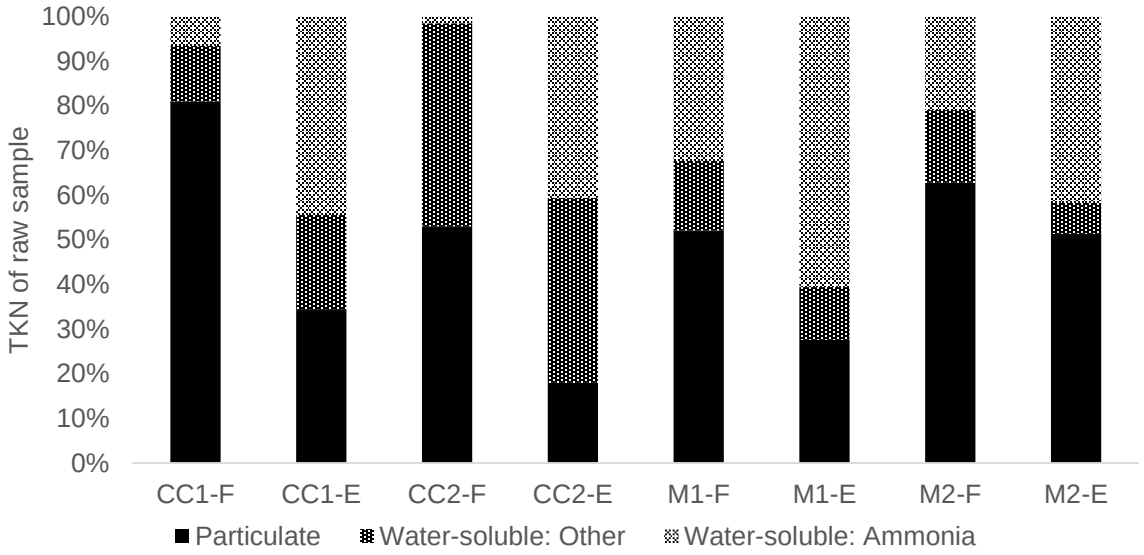

Fig. 2 TKN balance of feedstocks

3.4. Organic biodegradability

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

Total biodegradability rate during BMP tests varied among the fresh feedstocks: it was 47% for CC1-F, 74% for CC2-F and 56% for both M1-F and M2-F. Comparing these results with the VS balance presented in Table 4, it can be observed that the biodegradability of fresh materials is 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 [31–34].

Regarding the biodegradability of each DCO fraction, on the one hand the biodegradability of particulate was on average 59%. The NBD fraction should correspond to the lignocellulosic compounds that are not bio-accessibility, as well as to the DCO yield used for bacterial growth. Also, as seen for the raw sample, the DB of particulate is closely linked to the lignin content. On the other hand, the biodegradability of water-soluble phase was in average 65%. It is unlikely that all water-soluble NBD corresponds to the yield used for bacterial growth (taking into account the bacterial yields suggested in the Anaerobic Digestion Model 1 [30]). Therefore, this suggests that there is some organic matter in the water-soluble that is not bio-accessible, which was unexpected.

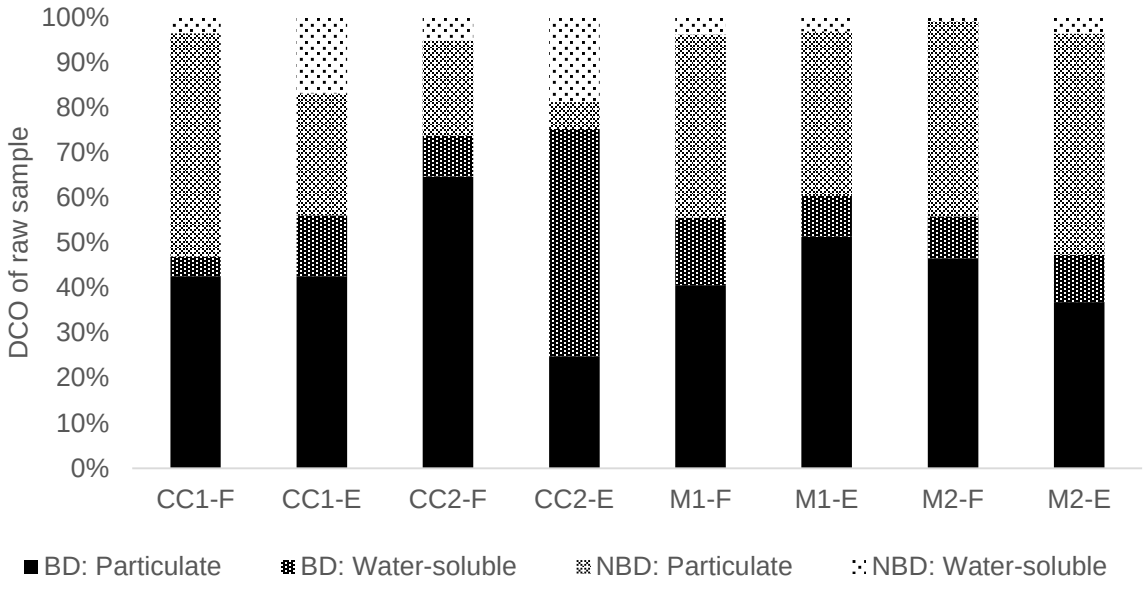

Fig. 3 Organic biodegradability of feedstocks and its phase distribution on a DCO basis. BD stands for biodegradable; NBD stands for non-biodegradable

Finally, considerable differences in biodegradability rate 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, the ensiling action had a relative positive impact on biodegradability of about 19%. On the contrary, biodegradability of M2-E was 19% lower than the one 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.

4. Conclusions

A complete methodology was successfully applied to assess 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 due to the production and accumulation of organic acids from particulate carbohydrates and organic nitrogen. This demonstrates that management practices of feedstocks before anaerobic digestion have a significant impact on organic matter structure and methane production kinetics. Moreover, one can conclude that this type of multiphase analysis may also provide important data on the efficiency and comparison of various pretreatments.

In brief, this methodology detects kinetic and biodegradability differences among biomasses and thus it can be useful for the design of anaerobic digestion plants. Finally, it can be applied for other types of biomass and be used to determine their suitability for anaerobic processes.

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