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Understanding the role of mechanical pretreatment before anaerobic digestion: lab-scale investigations

Helen Coarita Fernandez¹, Pierre Buffiere¹ and Rémy Bayard^{1*}

¹Univ. Lyon, INSA Lyon, DEEP Laboratory, EA7429, F-69621 Villeurbanne cedex, France

*Corresponding author: Rémy Bayard; Tel.: +334 72 43 87 53; E-mail:remy.bayard@insa-lyon.fr

Abstract

Three successive mechanical pretreatments were applied at lab-scale in order to mimic the main functions of industrial mechanical pretreatments used for feedstock preparation in anaerobic digestion: particle size reduction (shredding), homogenization (mixing) and fiber alteration (blending). In parallel, full-scale mechanical devices have been investigated (two hammer mills and one chain mill). A physical and biochemical characterization was undertaken before and after each pretreatment. The results at lab-scale revealed that shredding reduced the size of coarse particles, smoothly increased solubilisation but did not affect much the methane yield. Mixing further improved the solubilisation and the water retention capacity of the investigated products. The most important effect was the improvement of the methane production rate rather than the methane yield. The results on full-scale pretreatments revealed that they behave as a combination of each function. Principal component analysis enabled to assign each full-scale device according to its effect on the investigated parameters.

Keywords: anaerobic digestion, agricultural waste, mechanical pretreatment, biomass characterization, Biochemical methane potential.

1. Introduction

In France, about 65% of anaerobic digestion sites use agricultural waste, green waste or the organic fraction of municipal waste as feedstock. Indeed, France produces a

1 considerable amount of manure and crop residues (André et al., 2018). In Europe, over
2 70% of the biogas produced comes from anaerobic digesters of agricultural wastes,
3 manure and energy crops (Scarlat et al., 2018). This feedstock is mainly composed of
4 lignocellulosic material, which is hard to handle and to degrade biologically. In addition
5 it often causes mechanical troubles in the Anaerobic Digestion (AD) plants due to
6 clogging issues (Mönch-Tegeder et al., 2014a; Tsapekos et al., 2016). Pretreatment
7 operations are often proposed to improve agricultural biomass conversion to methane
8 (Zheng et al., 2014). They enable a better handling of ligno-cellulosic biomass,
9 including mixing of the co-substrates and pumping operations. Size reduction and
10 alteration of the lignocellulosic structure are also expected to facilitate the action of
11 microorganisms (Carrere et al., 2016; Kratky and Jirout, 2011).

12 In the agricultural sector, mechanical pretreatments are the most applied operations.
13 There is a wide range of mechanical technologies (ball mills, disc mills, chain mills,
14 etc.), the hammer and knife mill being the most used (Kratky and Jirout, 2011). Among
15 their advantages, they are easy to use, they do not present risk of inhibitors production
16 (Carrere et al., 2016; Mönch-Tegeder et al., 2014b) and do not require the use of costly
17 operations like rising the temperature or adding chemicals (Victorin et al., 2020).

18 Concerning pure mechanical effects, particle size reduction is the most common criteria
19 used to qualify the efficiency of mechanical operations (Bruni et al., 2010b; Herrmann
20 et al., 2012). Mönch-Tegeder et al. (2014a,b) found a significant particle size reduction
21 applying a cross flow grinder at full-scale. Likewise, the evaluation of a two-hammer
22 mill at full scale showed the particle size reduction mainly occurred for the larger fibers
23 (Coarita Fernandez et al., 2020a). Herrmann et al. (2012) found an increase about 20%
24 of thinner particles and a reduction of more than 50% of coarser particles applying a

1 laboratory chopper as mechanical pretreatment. In addition, they made a farm-scale
2 evaluation with different precision forage harvesters. In this case, the particle size
3 reduction varied between 1.2 to 5.6 times the nominal chopping lengths. In addition,
4 particle size reduction may lead to an homogenization of organic matter which enables a
5 better handling of substrate and prevents from layer formation (Mönch-Tegeder et al.,
6 2014a). Additionally, it improves the substrate flow properties (Mönch-Tegeder et al.,
7 2015). Mechanical pretreatments also affects the structure of the lignocellulosic
8 compounds (Hartmann et al., 2000; Paudel et al., 2017). The breakdown of
9 lignocellulosic compounds exerts a positive effect on the methane production. Some
10 authors (Menardo et al., 2012; Mönch-Tegeder et al., 2014b; Tsapekos et al., 2015)
11 described an enhancement of the biochemical methane potential (BMP) from 25% to
12 83.5% for diverse lignocellulosic substrates; others (Bruni et al., 2010a; Kalamaras and
13 Kotsopoulos, 2014) reported no significant differences between raw and mechanically
14 pre-treated samples. In short, the literature reveals the positive effect of mechanical
15 pretreatments on methane production, but the results are mitigated by the type of
16 substrates and mechanical devices used (Gallegos et al., 2017; Victorin et al., 2020).
17 Particle size reduction is often associated to an increase of the biochemical methane
18 potential (De la Rubia et al., 2011; Paudel et al., 2017; Sharma et al., 1988). However, it
19 is not clear if this effect is only due to size reduction, or to a combination of other
20 effects, like mechanical destruction of the lignocellulosic structure (Paudel et al., 2017).
21 Solubilisation is also an important effect of mechanical treatments (Carlsson et al.,
22 2012; Carrere et al., 2016). However, we observed that it much probably affects the rate
23 of methane production rather than the intrinsic biodegradability of the lignocellulosic
24 compounds (Coarita Fernandez et al., 2020b).

1 In this context, it is clear that mechanical pretreatments improve the physical and
2 biochemical characteristics of agricultural feedstock. However, to our knowledge, there
3 are still very few objective criteria enabling to qualify separately the different effects of
4 mechanical pretreatments. Indeed, mechanical operations are a combination of functions
5 like size reduction, mixing and fiber breakdown. The purpose of the present work was
6 to investigate these functions separately at lab-scale: a shredding operation, which
7 aimed at reducing the particle size; a mixing pretreatment in order to homogenize the
8 organic matter; and a tougher operation of blending which it is deemed to a deeper
9 breakdown of fibers. We applied these three operations sequentially. We aimed to check
10 up the functions of every pretreatment and associate them with the main expected
11 effects. To achieve this objective, we carried out a physical and a biochemical
12 characterization of raw and pre-treated samples. We applied this procedure to three
13 types of feedstock: cattle manure, maize and triticale silage and a mixture of cattle
14 manure and maize + triticale silage. In parallel, three full-scale mechanical devices were
15 investigated with the same characterization procedure. The objective was to understand
16 their mode of action according to the function assigned to the lab-scale devices.

17
18
19

20 **Table 1:** Samples and Mechanical pretreatment tests carried out in lab-scale and full-scale.
21

Type	Sampling site	Pretreatment	Code
Lab-scale mechanical pretreatments			
M1 – Cattle manure	Farm, Mont du Lyonnais (Rhône, France)	Untreated	M1
		Shredding (I-PT)	M1-I-PT
		Shredding and mixing (II-PT)	M1-II-PT
		Shredding, mixing and blending (III-PT)	M1-III-PT
		Untreated	M2
		Shredding (I-PT)	M2-I-PT

M2-Cattle manure	Farm, Ennezat (Puy de Dôme, France)	Shredding and mixing (II-PT)	M2-II-PT
		Shredding, mixing and blending (III-PT)	M2-III-PT
S – Maize and triticale silage	Farm, Ennezat (Puy de Dôme, France)	Untreated	S
		Shredding (I-PT)	S-I-PT
		Shredding and mixing (II-PT)	S-II-PT
		Shredding, mixing and blending (III-PT)	S-III-PT
SM – Mixture of cattle manure and silage	Farm, Ennezat (Puy de Dôme, France)	Untreated	SM
		Shredding (I-PT)	SM-I-PT
		Shredding and mixing (II-PT)	SM-II-PT
		Shredding, mixing and blending (III-PT)	SM-III-PT
Full-scale mechanical pretreatments			
M3 – Cattle manure	Farm, La Bouzule, Meurthe et Moselle, France	Untreated	M3
		Mobile Hammer mill	HM3
M4 – Cattle manure	Farm, confidential	Untreated	M4
		Fixed Hammer mill	HM4
SM – Mixture of cattle manure and silage	Farm, Ennezat (Puy de Dôme, France)	Untreated	SM
		Chain mill	CH-SM

1

2. Material and methods

2.1. Lab-scale feedstock and pretreatments

4 We have selected 4 agricultural feedstock from 2 farms (see Table 1).

- 5 - M1 is cattle manure from a farm located nearby Lyon (Monts du Lyonnais,
- 6 Rhône, France);
- 7 - M2 is cattle manure from a farm located in Ennezat (Puy de Dôme, France);
- 8 - S is a mixture of maize and triticale silage from the same farm than M2;
- 9 - SM is a mixture of silage S and cattle manure M2 used to feed the AD plant.

10 According to the plant operator, the proportion of silage and manure was 1:3 in
11 fresh weight.

12 All samples were stored at 4°C on the day of sampling, and processed within 7 days.

13 However, the SM sample was suspected to be unstable even at 4° and it was also
14 characterized immediately after sampling.

We applied three successive mechanical pretreatments: shredding (I-PT code in Table 1), shredding and mixing (II-PT) and shredding, mixing and blending (III-PT). The specifications for each lab scale device are listed on the Table 2. II-PT and III-PT were carried out with water addition in a 10:1 Water content / Total Solids (TS) ratio which corresponds to the same proportion for performing the substrate characterization (Teixeira Franco et al., 2019).

Table 2. Lab-scale devices used for mechanical pretreatments.

Device	Manufacturer	Characteristics	Others
Shredding	BLIK BB350	Rotary shear knife mill	8 mm output particle size (according to fabricant) Three times passage
Mixing device	H. KOENING	9 speed 5L capacity	Middle speed used during five minutes
Blender	WARING Commercial	7 speed 1L capacity	Middle speed used during five minutes

These three successive pretreatment operations were chosen in order to mimic existing functions:

- a knife mill for I-PT (low speed), whose role is to reduce the particle size;
- a pulping operation for II-PT, whose role is to favor the extraction of organic compounds into the liquid phase;
- a fine blending operation for III-PT (like wet grinders in industrial applications), whose role is to alter the lignocellulosic structure and to create an homogeneous liquid slurry.

2.2. Full scale feedstock and pretreatments

We have selected 3 sites of AD plants managing agricultural residues and using mechanical pretreatments:

- The first investigated site was an on-farm AD plant (La Bouzule, Meurthe-et-Moselle, France) treating cattle manure (M3). The mechanical pretreatment was a mobile

1 hammer mill (Noremat Valormax BA915D). The sample taken after the pretreatment is
2 referred to as HM3.

3 - The second site was an on-farm AD plant also treating cattle manure (M4). The
4 mechanical pretreatment was a fixed (on line) hammer mill. The sample taken after the
5 pretreatment is referred to as HM4. The device used cannot be disclosed for
6 confidentiality reasons.

7 - The third site was the METHELEC AD plant located in Ennezat (France). The
8 mechanical pretreatment was a X-Chopper chain mill (Xergi). The incoming feedstock
9 was the same mixture of silage and cattle manure used for lab-scale pretreatments (SM).
10 The sample taken after the pretreatment is referred to as CH-SM.

11 **2.3. Sample characterization procedure**

12 Each sample (before and after pretreatment) was characterized according to the method
13 proposed by Teixeira Franco et al. (2019). This procedure consists in separating the
14 liquid and the particulate fraction after a leaching test with 10:1 water/TS proportion
15 during 2h under a constant bottle rotation. Afterwards, samples were centrifuged (20
16 min at 5000 G) and filtered at 1.2 μm . The particulate fraction (remaining solid) was
17 dried (70°C) during three days. We used the liquid phase (supernatant) for further
18 analysis. Analyses were performed for the raw sample, water soluble and particulate
19 fraction:

20 - *Raw sample*: Measurement of Total solids (TS), Volatile Solids (VS),
21 Biochemical Methane Potential (BMP), particle size distribution, water retention
22 capacity (WRC);

23 - *Water soluble*: Measurement of Total solids (TS), Volatile Solids (VS), BMP,
24 pH, , Chemical Oxygen Demand (COD);

1 - *Particulate*: Total solids (TS), Volatile solids (VS), Chemical Oxygen
2 Demand (COD).

3 **2.3.1. Biomethane Potential Test assays (BMP)**

4 BMP tests were carried out in mesophilic conditions (35°C) according to the
5 guidelines proposed by Holliger et al. (2016). Each bottle contained at least 20 g_{VS} of
6 biomass (substrate + inoculum) and a 0.5 substrate/inoculum ratio on Volatile (VS)
7 basis. The inoculum was digested sludge from the wastewater treatment plant of La
8 Feyssine, Lyon, France. Its TS and VS content was 2.7-3%wt and 1.9-2.1%wt
9 respectively. Besides, a mineral solution containing essential elements for microbial
10 growth was added following ISO 117734:1995 standard recommendations (ISO 11734,
11 1995). Finally, glass bottles were purged with 80/20 %_v N₂/CO₂ before incubation. The
12 headspace pressure was measured with a Digitron precision manometer. When the
13 pressure was higher than 1200 hPa, biogas was vented.

14 The BMP tests were stopped when the daily methane production fell below 1% of
15 the total methane production for three consecutive days. The BMP was calculated by
16 subtracting the blank methane production from the inoculum. All tests (including
17 blanks) were carried out in triplicate.

18 The methane production is expressed in standard conditions (0°C, 101325 Pa).
19 We used a 1st-order model to fit the net methane production (*i.e.*, after subtracting the
20 blank methane production) to the following equation:

$$21 \quad V_{CH_4}(t) = B_0(1 - e^{-kt})$$

22

23 - B_0 : the final (or ultimate) maximal biochemical methane potential,

24 - k , the 1st order kinetic parameter.

1 The Biodegradability (*BD*) of the substrates was estimated from *BMP* and *COD*
2 measurements, considering that 1 g *COD* consumed would produce 0.35 L of methane in
3 standard conditions (Buswell and Mueller, 1952).

$$BD (\%) = \frac{BMP [L_{STP}/kg_{VS}]}{COD [g_{COD}/kg_{VS}] \times 0.35 [L_{STP}/g_{COD}]}$$

4 5 6 7 8 9 **2.3.2. Particle size distribution**

10 A wet sieving method with water recirculation was performed. the sieve openings
11 were 0.25, 0.5, 1, 4, 10, 20.0 and 31.5 mm (Coarita Fernandez et al., 2020a; Lindner et
12 al., 2015). Each fraction was dried at 105°C during 24h. The results were expressed in
13 terms of TS distribution. All tests (on raw and pretreated samples) were performed in
14 triplicate.

15 16 17 **2.3.3. Water Retention Capacity (WRC)**

18 The water retention capacity WRC was measured following the procedure
19 described by Coarita Fernandez et al. (2020a). It consists of water contact with substrate
20 in a column for two hours. First, water is pulled out by gravity and then, with a vacuum
21 system during 120seconds. The mass of water retained per mass of total solid was the
22 water retention capacity (Raghavendra et al., 2006). All samples were analyzed in
23 triplicate.

24 **2.3.4. Analytical procedures**

25 The TS and VS contents for all samples were determined through 105°C drying for 24h
26 and calcination at 550°C during 2h. Considering the volatile compounds loss during the
27 drying tests (Kreuger et al., 2011), corrected values were taken into account using the

1 volatilization coefficients at 100°C as suggested by Porter and Murray (2001). For
 2 measuring pH, we used a Consort C3020 device with a SP10B pH-electrode. Particulate
 3 COD was measured following NF U 44-161 and NF ISO 14235 standards.
 4 The biogas composition in the BMP tests was measured by gas chromatography. We
 5 used an Agilent 300 microGC with a thermal conductivity detector (GC-TCD) The
 6 carrier gases were Argon and Helium and the columns as stationary phases were
 7 Molsieve 5A (14 m length; pore size: 5Å) and PoraPlot (10 m length; 0.320 mm ID).

8

9 **3. Results**

10 **3.1. Feedstock characteristics**

11 Incoming substrate characteristics are given in Table 3. The TS (%TW) contrasted
 12 with substrate, it was of 29.9% for M2, 16.0% for S and 23.5% for SM. On the other
 13 hand, VS (%TS) were similar for M2 and SM with 78.5. The VS content of S was
 14 83.9% which is a commonly reported value for this kind of feedstock (Feng et al., 2018;
 15 Gallegos et al., 2017). Values of TS for M2 corresponded to “solid” manure (25-31.9%
 16 TS) (Mönch-Tegeder et al., 2014b; Ruile et al., 2015).

17

18

19

20

21 **Table 3:** Untreated feedstock characteristics. M1 to M4 are cattle manure, S is silage, SM is a
 22 mixture of cattle manure M2 and silage S.
 23

	Unit	M1	M2	M3	M4	S	SM
TS	%(total weight)	15.9±0.2	29.9±0.1	19.6±0.1	23.9±1.1	16.0±0.1	23.5±0.9
VS	% (TS)	78.4±0.2	78.6±0.6	88.4±0.9	83.0±3.4	83.9±0.4	78.5±0.6
COD	mg/g VS _{RS} ⁽¹⁾	1260	1350	1305	1215	1309	1180
BMP_{RS}	mL/gVS _{RS}	210±11	214±8	275±7	199±15	295±15	180±5

$k^{(2)}_{RS}$	d^{-1}	0.055 ± 0.005	0.056 ± 0.001	0.066 ± 0.005	0.055 ± 0.004	0.20 ± 0.01	0.070 ± 0.009
BMP_{WSP}	mL/gVS_{RS}	16 ± 0.3	32 ± 0.4	11.0 ± 0.2	12.0 ± 0.2	81.3 ± 5.7	7.0 ± 0.3
$WSP^{(3)}$ contribution	$\%BMP_{RS}$	7 ± 0.6	15 ± 1	4.0 ± 0.1	6.1 ± 0.5	27 ± 3	4.2 ± 0.3
BD	$\%$	48 ± 3	46 ± 4	60 ± 1	47 ± 4	64 ± 3	43 ± 1
$WRC^{(4)}$	g_{Water}/g_{TS}	6.5 ± 0.1	4.5 ± 0.2	5.6 ± 0.1	5.6 ± 0.3	7.2 ± 0.3	5.3 ± 0.1

(1): RS = Raw sample; (2): $k=$ 1st order kinetic constant. (3): WSP= Water Soluble Phase; (4): WRC= Water Retention Capacity.

The COD/VS ratio was similar for M2 and S with 1350 mg/g VS_{RS} and 1309 mg/g VS_{RS} respectively. A lower ratio was found for SM (1180 mg/g VS_{RS}). However, the fresh SM sample (measured immediately after sampling, not shown in Table 3) had a slightly higher ratio (1203 mg/g VS_{RS}). That means that some oxidizable fraction was probably lost upon storage. In addition, a decrease of VFA contents was found between fresh and stored SM (results not shown) along the time which it is probably due to the slight biological consumption of VFAs during storage at 4°C (Gallegos et al., 2017).

The methane potential for cattle manure was in accordance with commonly reported data (Coarita Fernandez et al., 2020b; Tsapekos et al., 2016). The BMP values were higher for S than previously reported for similar products (Herrmann et al., 2012; Tsapekos et al., 2015). The BMP of the SM sample (180) was much lower than the one measured on the fresh sample (227.1 ± 4.4 mL/ g_{VS} , not shown in Table 3), which confirms that the sample was degraded after 1 week storage at 4°C. Nevertheless other results near to ours were also reported (Wei et al., 2015). In fact, the effect of storage (at 4°C in the dark for 1 week) was obvious. The instability even at low temperature of this kind of mixture was already noticed (Teixeira Franco et al., 2018). Bergland et al. (2014) reported no biochemical changes on for pig manure after storage at 4-6°C. Consequently, even if storage at 4°C is considered sufficient for a short period (Astals et al., 2020), this was obviously not the case for silage +manure.

1 The mechanical pretreatments were all evaluated with the stored products. This is
2 problematic since SM evolved significantly during storage, while the other products did
3 not change. In the following, SM will be considered as a specific product (and not as a
4 combination of the two other substrates).

5 The methane production rate can be compared through the 1st order kinetic
6 constant. The substrate with the highest methane rate was S ($0.20\pm 0.01\text{ d}^{-1}$), while SM
7 and manure (M1 to M4) presented lower values (from 0.055 to 0.070 d^{-1}). Once again,
8 fresh SM had a kinetic constant 25% higher than stored SM, which indicates that readily
9 degradable products were probably degraded upon storage.

10 The anaerobic biodegradability was similar for manure M2 and SM (44%
11 approximately), and higher for silage S (64%). Likewise, this does not sound logical if
12 we consider that SM is a mixture of both, but once again the evolution upon storage is a
13 reasonable explanation for this result. The biodegradation of water-soluble compounds
14 of fresh SM was twice higher than that of stored SM (results not shown) which confirms
15 a significant degradation of organic soluble compounds upon storage. Soluble
16 compounds can be rapidly converted to methane (Shrestha et al., 2017; Teixeira Franco
17 et al., 2019). Indeed, for SM, a low contribution of the water-soluble phase to the total
18 BMP was found.

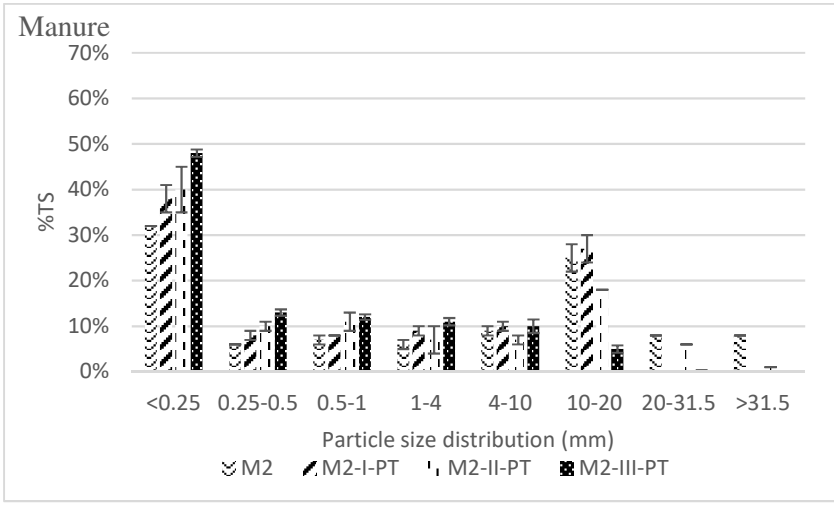
19 20 21 22 23 **3.2. Lab-scale pretreatments**

24 **3.2.1. Particle Size Reduction**

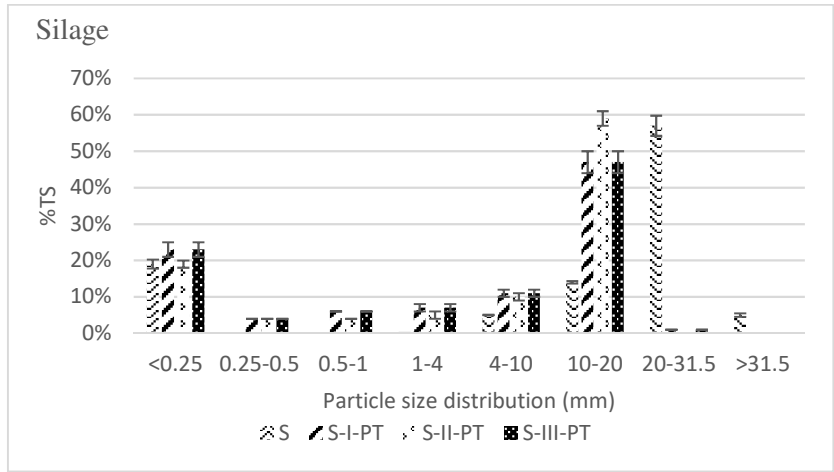
25 The effect of the pretreatments on particle size is shown on Figure 1. For manure
26 (Figure 1a), most of TS was composed by particles shorter than 0.25 mm (30%),

1 followed by particles between 10-20 mm (25%). Other fractions did not reach more than
 2 10% TS. M-I-PT led to a main reduction of particle size, higher than 31.5 mm that
 3 turned into smaller fibers. The proportion of particles shorter than 0.25 mm remarkably
 4 increased from 30% (M2) to 50% for M2-III-PT. Indeed, M2-III-PT was the substrate
 5 with the shortest particles compared to the other treated samples.

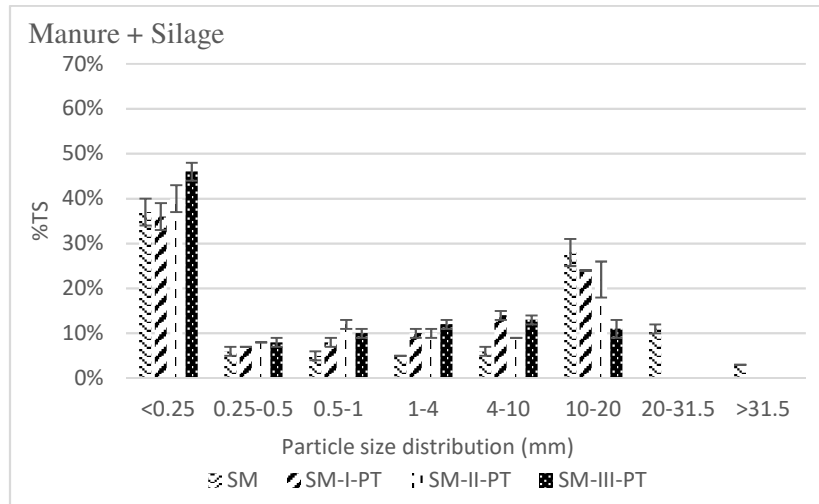
6 (a)



7
 8
 9 (b)



10
 11
 12 (c)



1 **Figure 1.** Particle size distribution a) M2: Cattle manure b) S: Silage c) SM: Mix of silage +
 2 cattle manure. I-PT: Shredding pretreatment, II-PT: Shredding +mixing pretreatment; III-PT:
 3 Shredding +mixing+ blending pretreatment.
 4

5 For silage, more than 50% of particles were 20-31.5 mm sizes and the second
 6 largest proportion was particles shorter than 0.25 mm (20% approximately), other
 7 measured fractions were not higher than 10% TS. S-I-PT showed the most obvious
 8 reduction of particle size, those 20-31.5 mm particles turned into 10-20 mm sizes.
 9 Others measured particles sizes were practically unchanged (Figure 1b); particle size
 10 was mainly impacted by the first pretreatment.

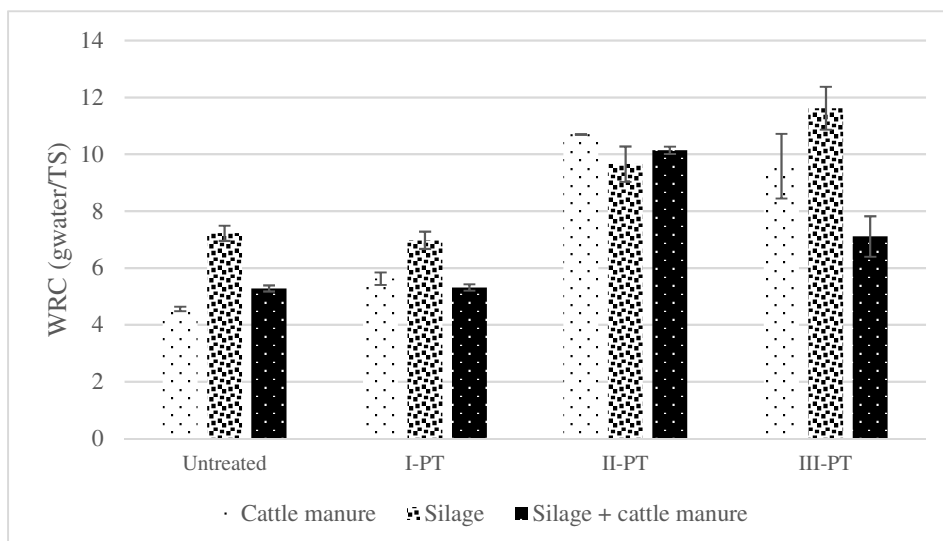
11 For SM, particles shorter than 0.25 mm and between 10-20 mm were the largest
 12 fractions (30% approximately each one). SM-I-PT led the largest particles (20-31.5
 13 mm) turning into shorter particles (4-0.5 mm). In SM-II-PT and SM-III-PT, 10-20 mm
 14 fraction was reduced and it turned into shorter particles (Figure 1c). SM-III-PT
 15 presented the shortest particle size in general.

16 To summarize the results, the shredding operation (I-PT) mostly reduces the size
 17 of the larger particles (>20 mm) into smaller ones, probably in the range 10-20 mm. The
 18 blending operation (III-PT) produces fine particles (<0.25 mm) from higher particles.
 19 However, blending does not change much the overall structure of the distribution. This

1 size particle reduction trend was also found by Herrmann et al. (2012) using laboratory
2 devices. By their part, Tsapekos et al. (2015) reported mostly a coarse reduction of
3 ensiled meadow grass particles applying different mechanical devices; the coarsest
4 particles turned into medium ones. However, the fraction reduction of the thinnest
5 particles was not uniform and it depended on the mechanical equipment.

6 3.2.2. Water Retention Capacity (WRC)

7 The WRC of untreated products ranged from 4.56 to 7.22 $\text{g}_{\text{water}}/\text{g}_{\text{TS}}$. This
8 parameter was positively correlated with the presence of silage: M2 had the lowest
9 value, followed by SM and S. Sanchez et al. (2019) reported WRC values between 4.4
10 and 6.7 $\text{g water}/\text{TS}$ for different lignocellulosic feedstock's. For other manure samples,
11 we obtained values between 5.6 and 6.5 $\text{g water}/\text{TS}$. Raw silage is commonly
12 recognized as an easily biodegradable product and high WRC is one of the parameter
13 that makes it more accessible to micro-organisms (Tsapekos et al., 2015). The reason is
14 that WRC is associated to the internal porosity of the solid. The effect of mechanical
15 pretreatments on the WRC is represented on Figure 2.



16 **Figure 2.** Water retention capacity of untreated and treated samples (cattle manure M2, silage S,
17 mixture SM).
18

1
2 For the 3 products, WRC increased noticeably during the second pretreatment (II-
3 PT). This means that the combination of shredding and mixing enables to favor the
4 penetration of water into the biomass. Interestingly, the blending operation (III-PT) led
5 to a global decrease of the WRC, with the exception of silage. This was probably caused
6 by the combined effect of size reduction and of alteration of the macrostructure of
7 lignocellulosic fibers, as mentioned by other authors (Dumas et al., 2015).

8 **3.2.3. Biochemical Methane Potential (BMP)**

9 Table 4 presents the result of biochemical methane potential of raw and pre-
10 treated samples. The contribution of the soluble fraction to the total BMP is also shown,
11 as well as the 1st order kinetic constant (k). For cattle manure M2, pretreatments did not
12 lead to a significant increase of BMP (+8% after the last pretreatment III-PT compared
13 to untreated M2), in agreement with our previous results (Coarita Fernandez et al.,
14 2020b). In contrast, other authors obtained a more significant enhancement (up to 45%)
15 under a combination of two heavy plates pretreatment with digested fibers manure
16 sieved at 2mm (Tsapekos et al., 2016). The BMP of silage increased by 21% upon
17 blending (III-PT). However, moderate treatments (S-I-PT and S-II-PT) had no
18 significant effect. ANOVA test showed only statistically differences between untreated
19 and blended silage (S and S-III-PT). These results indicate that for the silage, an
20 intensive mechanical pretreatment could be favourable to an increase of the
21 biodegradability. For example, Feng et al. (2018) did not find differences on
22 biodegradability between untreated and chopped plant silage (tall fescue, *Festuca*
23 *arundinacea*). In the same way, Bruni et al. (2010b) reported an enhancement of 10%
24 on methane yield for maize silage with a kitchen blender pretreatment. More generally,
25 the BMP increase was not statistically significant for M2 and SM (test ANOVA at p:

1 0.05). In the same way, the anaerobic biodegradability during mechanical pretreatment
2 operations followed a similar trend than the BMP.
3 In other words, coarse size reduction (like shredding in our case, or chopping in the
4 cited reference by Feng et al.) does not change the biochemical methane potential of the
5 product tested. In contrast, the use of a blending operation may, in some cases, increase
6 the BMP. This result was observed for silage in our case, and in Bruni et al., which
7 means that lignocellulosic structures are probably disrupted upon blending.

8 **Table 4.** Total BMP, BMP distribution and kinetics on untreated and pretreated samples.

Substrate	Parameter	Unit	Untreated	I-PT ¹	II-PT ²	III-PT ³
M2	BMP _{RS} ⁴	mL/gVS _{RS}	214.5±8.3	202.3±5.8	224.0±11.5	231.7±7.3
	k_{RS}^5	d ⁻¹	0.056±0.001	0.061±0.003	0.068± 0.004	0.074±0.003
	BMP _{WSP} ⁶	mL/gVS _{RS}	32.1±0.4	36.9 ±0.5	35.1 ±1.1	43.6 ±0.6
	WSP contribution	%BMP _{RS}	15±0.7	18.2±0.7	15.7±1.2	18.9±0.7
	BD	%	45.4±1.4	43.9±1	48.7±2	47.9±1.2
Silage (S)	BMP _{RS}	mL/gVS _{RS}	295.5±15.4	292.9 ±2.5	314.9 ±18.6	359.7 ±6.0
	k_{RS}^5	d ⁻¹	0.197±0.007	0.248±0.004	0.222±0.008	0.221±0.011
	BMP _{WSP}	mL/gVS _{RS}	81.3±5.7	94.3±1.2	98.7±0.3	103.03 ±1.90
	WSP contribution	%BMP _{RS}	27.6±3.1	32.2±0.6	31.3±2.5	28.7 ±0.9
	BD	%	64.5±2.7	63.7±0.4	64.9±3.2	74.2±1
Silage + cattle manure (SM)	BMP _{RS}	mL/gVS _{RS}	180.1±5.5	177.8 ±3.2	193.3 ±4.1	186.7±6.6
	k_{RS}	d ⁻¹	0.070±0.009	0.129±0.001	0.133±0.006	0.133±0.008
	BMP _{WSP}	mL/gVS _{RS}	7.6±0.3	33.2±	33.2 ±1.1	39.6 ±2.9
	WSP contribution	%BMP _{RS}	4.2±0.3	15.3 ±0.3	17.2 ±0.9	21.2 ±2.2
	BD	%	43.6±1.1	45.6±0.6	45.9±0.8	42.0±1.2

¹I-PT : Shredding pretreatment ; ²II-PT : Shredding + Mixing pretreatment ; ³III : Shredding + Mixing + Blending pretreatment ; ⁴RS : Raw sample ; ⁵k : Kinetics constant ; ⁶WSP : Water soluble phase

9

10 The contribution of the water-soluble fraction to the BMP did not vary much
11 along the successive pretreatments. For cattle manure samples, it increased from 15
12 (raw sample) to 18% (after blending). For silage, this contribution increased upon
13 shredding S-I-PT (32%). Surprisingly, S-II-PT and S-III-PT did not contribute to the

1 release of further soluble biodegradable organic matter. For SM however, the initial
2 contribution was only 4%, and it increased noticeably to around 21% along the
3 pretreatments.

4 **3.2.4. Kinetics**

5 For each product tested (untreated and pre-treated), the BMP tests were run
6 simultaneously with the same inoculum. The differences observed in the methane
7 production rate is thus representative of a modification of the bioavailability of the
8 products upon pretreatments. The net methane production always followed a 1st order
9 kinetics. The kinetic constant k is thus a good indicator of the efficiency of the
10 pretreatment (Table 4). For manure, k increased when the pretreatments became
11 stronger (+ 23% between M2 and M2-II-PT and + 32% between M2 and M2-III-PT). A
12 similar enhancement (+18%) was reported with the same substrate in a previous work
13 (Coarita Fernandez et al., 2020b). For silage, an enhancement of 27% of methane rate
14 was obtained between S and S-I-PT, and 12% between S-II-PT and S-III-PT. Hence, for
15 silage, the coarse shredding operation was more effective than the following successive
16 pretreatments. Some authors reported a decrease in the digestion time between 35.7% –
17 43% with a grinding pretreatment for lignocellulosic substrate (Dumas et al., 2015;
18 Kang et al., 2019). The behaviour of SM was interesting, with a noticeable increase of k
19 with pretreatments; indeed, an enhancement of 84% (SM-I-PT) and 90% (SM-II-PT and
20 SM-III-PT) compared to SM was obtained. ANOVA test on k -values indicated a
21 significant difference between untreated and pre-treated samples for this parameter.

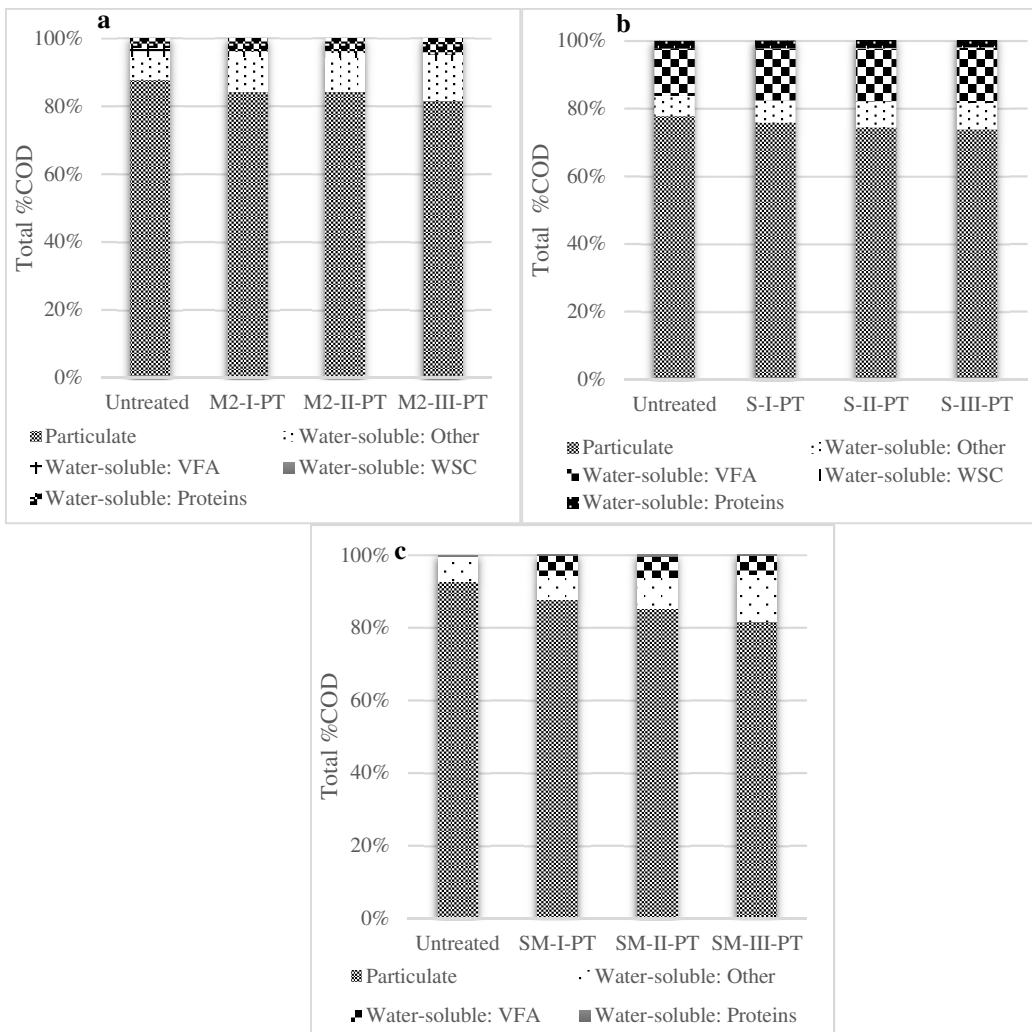
22 For the tested products, the first coarse shredding operation seemed to have the
23 most important effect on methane production rate. This illustrates that particle size
24 reduction may rather affect bioaccessibility (increase of methane production rate) than

1 biodegradability (increase of BMP). This outcome is also in line with other reported
 2 results (Carrere et al., 2016; Palmowski and Müller, 2000; Sharma et al., 1988).

3 3.2.5. COD distribution

4 The COD distribution between the particulate and the water-soluble fractions are
 5 shown on Figure 3a, 3b and 3c for M2, S and SM, respectively.

6



7

8

9

10 **Figure 3.** COD distribution of untreated and treated cattle manure M2 (a), silage S (b), and
 11 mixture of cattle manure and silage SM (c).

12

13

14 For manure, the soluble COD fraction increased from 12 to 18 % with the
 successive pretreatments. This increase was attributed to the release of soluble proteins

1 according to the TKN balance (results not shown). The other soluble compounds (VFA
2 and soluble carbohydrates) remained practically unchanged. Other water-soluble
3 compounds also increased with the pretreatments.

4 For silage, COD solubilisation was more limited (increased from 22 to 27%)
5 (Figure 3b). In general, the concentrations of soluble compounds increased regularly
6 with pretreatments. Both shredding and blending operations contributed to
7 solubilisation. Other organic compounds concentrations was low and their increase was
8 not significant.

9 For the SM mixture, a significant COD solubilisation occurred (Figure 3c). The
10 most important enhancement was between the SM and SM-III-PT, with the decrease of
11 around 10% of particulate COD. A release of VFA was observed with pretreatments,
12 but most organic compound solubilisation occurred during blending(SM-III-PT).

14 **3.3. Effect of pretreatments at full-scale**

15 The values of the most important parameters obtained at full scale are summarized in
16 Table 5. For cattle manure M3 the BMP did not change after the pretreatment with the
17 mobile hammer mill. The fixed hammer mill used for M4 slightly increased the BMP
18 (from 199 to 224 mL/gVS) but this improvement remains close to the confidence interval.
19 These results are in line with those obtained by Herrmann *et al.*, 2012, and by Mönch-
20 Tegeder *et al.* (2014a). Only the chain mill had a significant impact on the BMP of the
21 mixture of silage and manure (SM: +49%).

22 The most striking effect of all mechanical pretreatments is the significant enhancement
23 of the kinetic constant of methane production rate, who is improved by 14, 27 and 140%
24 for HM3, HM4 and CHSM, respectively. The general trend observed is an increase of the

1 soluble compounds (results not shown) and an increase of the contribution of the soluble
 2 fraction to the methane potential.

3 **Table 5:** Biochemical and physical characteristics of evaluated substrates before and after
 4 pretreatment at full-scale.
 5

	Unit	M3	HM3	M4	HM4	SM	CHSM
TS	%(total weight)	19.6±0.1	18.8±0.5	23.9±1.1	25.8±0.4	23.5±0.9	8.63**±0.1
VS	% (TS)	88.4±0.9	87.1±0.1	83.0±3.4	75.6±2	78.5±0.6	69.6±0.3
COD	mg/gVS _{RS}	1305	1317	1215	1219	1180	1215
BMP_{RS}*	mL/gVS _{RS}	275±7	269±10	199±15	224±7	180±5	269±4
k¹_{RS}	d ⁻¹	0.066±0.005	0.077±0.004	0.055±0.004	0.070±0.04	0.070±0.009	0.170±0.008
BMP_{WSP}⁽²⁾	mL/gVS _{RS}	11±0.2	27±1	12±0.2	14±0.1	7±0.3	28±1
WSP⁽²⁾ contribution	%BMP _{RS}	4±0.1	10±0.7	6.1±0.5	6.2±0.4	4.2±0.3	16±1
BD³	%	60±1	58±2	47±4	52±2	43±1	45±1
WRC	g _{water} /TS	5.6±0.1	6.7±0.3	5.6±0.3	5.2±0.1	5.3±0.1	13.3±0.4

¹k: kinetics constant, ²WSP: Water soluble phase, ³ Biodegradability, *RS: Raw sample, ** TS with digested matter addition

6

7 **4. Discussion**

8 **4.1. Major effects of mechanical pretreatments**

9 The first effect of the investigated pretreatments is particle size reduction. The first
 10 shredding operation (I-PT) eliminated the largest particles (> 20 mm) that are generally
 11 responsible for mechanical problems in AD plants (clogging). The fine blending
 12 operation (III-PT) further reduced the number of particles above 10-20 mm. It also
 13 promoted the production of fine particles (< 0.25 mm). In practice, this comminution
 14 effect would also reduce viscosity, as shown by other authors (Coarita Fernandez et al.,
 15 2020a; Ruys, 2017). The second effect was the noticeable increase of the water retention
 16 capacity upon shredding and mixing operation (I-PT and II-PT). This result is in
 17 accordance with the expected effect of increasing the bioaccessibility of the solid matrix
 18 (Karimi and Taherzadeh, 2016). The blending operation (III-PT) however globally
 19 reduced the WRC; this is most probably due to the production of fine particles in

1 suspension in the bulk liquid and to the reduction of the macro-porosity due to fiber
2 destruction. The mixing and the blending operations were the most efficient steps to
3 extract soluble compounds from the solid matrices.

4 Concerning the methane production, two possible effects could be expected: first, the
5 increase of biodegradability, which would have resulted in a higher methane potential
6 (BMP); second, the increased bioaccessibility of organic compounds, which would have
7 resulted in a quicker methane production (increase of the 1st order kinetic constant k).

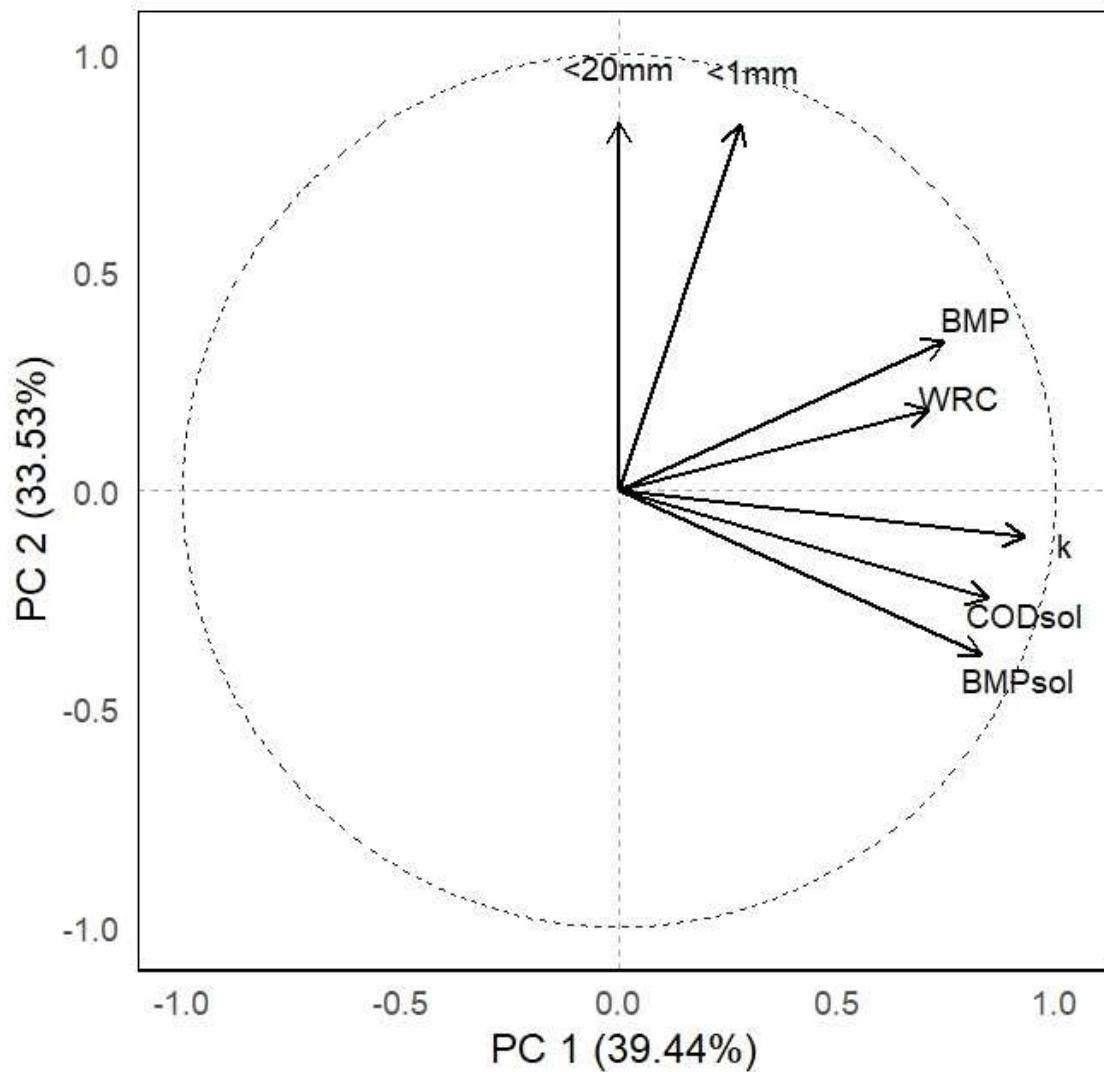
8 From our results, the mechanical pretreatments mostly affected the methane production
9 kinetics. The most surprising result is that the shredding operation (I-PT) was the most
10 efficient step concerning this parameter at the lab-scale, while the blending step only
11 brought a limited input. Indeed, we could expect that the increase of the kinetics would
12 be related to the solubilisation of organic compounds, the latter being rather observed
13 during the following treatments. Another noticeable result is the BMP did not change
14 much during the pretreatment operation, with exception for silage after blending (III-
15 PT). However, pretreatments have impacts on several parameters and this complexity
16 can be better understood by a statistical analysis presented in the following section.

17 **4.2. Assigning functions to full-scale mechanical pretreatment devices**

18 In order to have a better vision of the influence of the effects of the mechanical
19 pretreatments on these properties, a statistical analysis of correlation was carried out. A
20 principal component analysis (PCA) of all the results obtained at the laboratory scale
21 was performed. The first two axes of the analysis explain approximately 73% of the
22 variance. These two axes therefore allow us to describe the variability of the trials in a
23 satisfactory manner. We use these axes to find different correlations among the
24 evaluated physicochemical properties (Figure 4). Besides the parameters listed in Table

1 3, two supplementary parameters concerning particle size have been added: the
2 percentage of particles below 20 mm and the percentage of particles below 1 mm.
3 According to our results, the kinetic constant " k " is directly correlated to the
4 solubilization of COD. It is also correlated to the contribution of the soluble fraction to
5 the methanogenic potential. The solubilization of biodegradable organic compounds is
6 therefore significantly correlated with the rate of methane production, which sounds
7 logical since soluble organic compounds are more rapidly accessible to microorganisms
8 (Zhang et al., 2019). However, the reduction in particle size does not appear to correlate
9 with the increase in kinetics. This can be explained by the fact that the reduction of the
10 size of the largest particles by simple grinding does not allow significant solubilization
11 of the organic matter, unlike the blending step which seems to have a disrupting effect
12 on the fibers. The results suggest a correlation between size reduction (production of
13 particles smaller than 1 mm, i.e, relevant of fiber destruction) and methane production.
14 This correlation confirms the importance of particle size reduction for anaerobic
15 digestion. For example, Gallegos et al, (2017) report a statistically significant
16 correlation between particle size and biodegradability. These authors clearly
17 demonstrated an increase in methane production during particle size reduction on
18 biomasses rich in lignocellulosic compounds such as wheat straw. To fewer extent, the
19 water retention capacity is also correlated to the BMP, as already mentioned by other
20 authors (Karimi and Taherzadeh, 2016). In brief, the increase of small particles and the
21 increase of the water retention capacity could be valuable indicators of fiber destruction
22 and of an increased biodegradability.

23



1
 2 **Figure 4:** PCA correlation circle of biochemical and physical parameters according to the two
 3 principal components.
 4 All different samples (untreated and mechanically pretreated, lab-scale and full-scale)
 5 have been plotted according the two principal component (Figure 5). From this plot, we
 6 have drawn 4 area (in color):
 7 - The red area (bottom left) entails all untreated samples (lab-scale);
 8 - The brown area contains all samples after simple shredding at lab scale (I-PT);
 9 - The green area contains all the samples after mixing at lab-scale (II-PT);
 10 - The blue-green area contains all the samples after blending at lab-scale (III-PT).

1 In addition, the untreated and pretreated full-scale samples have also been added (3
2 points each due to triplicate tests). The purple zone corresponds to the cattle manure M3
3 treated by the mobile hammer mill (HM3), the pink zone to the cattle manure pretreated
4 by the fixed hammer mill (HM4) and the blue zone to the mixed manure silage SM
5 pretreated with the chain mill (CHSM).

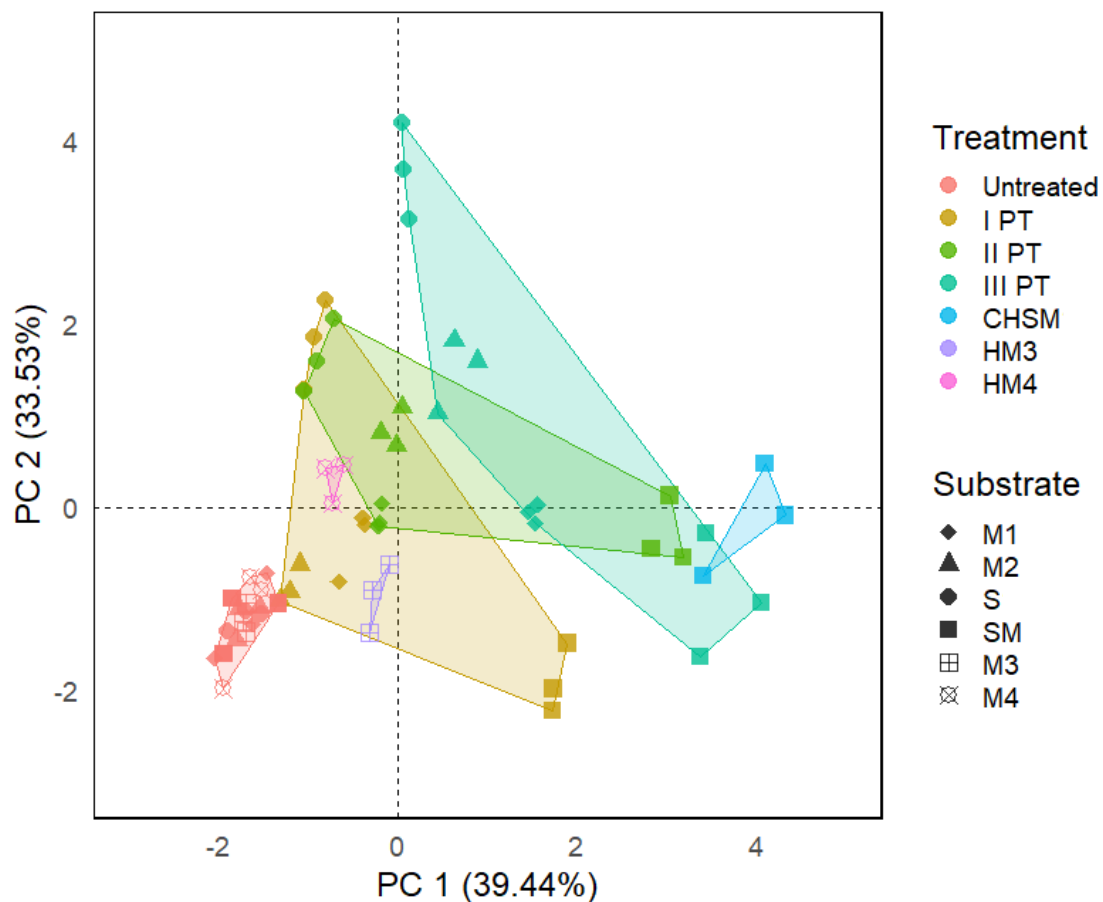
6 This representation allows to see a shift from the bottom left to the top and to the right
7 according to the intensity of the pretreatments. First, all untreated sample are located in
8 the red zone (bottom left). The simple shredding zone (I-PT) and the blending (III-PT)
9 zone are clearly separated, while the mixing (II-PT) is an intermediate.

10 The full-scale treatments with a hammer mill (HM3 and HM4) fall into the shredding
11 region, HM3 being more shifted to the right and HM4 to the top. On the other hand, the
12 chain mill zone (CHSM) falls even further than the blending operation at lab-scale.

13 This enables to assign more precisely the role of each full-scale pretreatment according
14 to their function:

- 15 - The tested hammer mills have a similar impact than a simple shredder: reduction
16 of particle size, slight increase of methane production rate, no increase of
17 biodegradability;
- 18 - The chain mill has an impact that is more pronounced than the blending
19 operation: particle comminution (increase of the amount of small particles), fiber
20 destruction, increase of the biodegradability (BMP).

21



1

2 **Figure 5:** Location of the different feedstocks (untreated and treated, lab-scale and full scale)
 3 according to the principal components.

4

5. Conclusions

6

7 We used sequential mechanical pretreatment at lab-scale in order to reproduce
 8 several supposed effects associated to industrial mechanical pretreatments before
 9 anaerobic digestion. Mechanical pretreatments influenced mainly on kinetics and they led
 10 to a soluble compounds release who enables somehow a faster methane production rate.
 11 Nevertheless, they did not influence significantly the methane yield. According to the
 12 type of substrate used, every mechanical function had a different effect. However, some
 13 common characteristics may be pointed out. The shredding operation mostly reduced the
 14 amount of larger fibers. It increased the water retention capacity and the methane

1 production rates. The mixing pretreatment further increased the WRC and favored
2 solubilisation. The blending pretreatment was stronger, its effects were observed on i) the
3 improvement of the amount of small particles and ii) the BMP improvement for silage,
4 probably because it modified the lignocellulosic structure. The principal component
5 analysis of the results made possible to assign the full-scale pretreatment according to
6 the functions expected from the lab-scale tests. The tested devices were two hammer mills
7 and a chain mill. The PCA reveals that the effect of the tested hammer mills was similar
8 to that of a simple shredding operation, while the effect of chain mill was stronger than
9 that of the blending operation.

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24

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