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# Effects of grinding processes on anaerobic digestion of wheat straw

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Lignocellulosic biomass represents an important part of the agricultural wastes that can be transformed into a renewable energy. The biodegradability and biodegradation kinetics of ultra-finely ground wheat straw was studied under mesophilic anaerobic conditions. Biological methane potential (BMP) tests and batch reactors were performed on samples resulting from successive grinding processes (759–48 µm). The main conclusion is that micronization do not improve methane yield but has a positive effect on the biodegradation kinetics. In addition, the results presented here showed for the first time that no significant increase of kinetics was observed below a size threshold value around 200 µm. This can be explained by the modifications in the lignocellulosic network from 759 to 200 µm but not below 200 µm. Therefore, micronization, increasing significantly the kinetics of anaerobic digestion, can decrease the retention time or the size of the digesters to treat the same quantity of waste.

## 1. Introduction

Because of earth global warming and the reduction of fossil fuels resources, the use of renewable energy sources is becoming increasingly necessary. The conversion of biomass into energy can be achieved in many of ways, for example by producing fuel thanks to various technologies. This study explores the potential of anaerobic digestion (AD) of lignocellulosic biomass. The AD can process biomass and produces a gas rich in methane and digestates rich in mineral elements (Mata Alvarez et al., 2000). Nowadays, processing lignocellulosic biomass thanks to AD has become of much interest. Researchers explore the possibility of transforming some non-food competitive agricultural crops in energy.

However, the hydrolysis step of complex polymeric substances constitutes the rate limiting step (Vavilin et al., 1996), and therefore, it has engaged the most attention from researchers. In order to increase the hydrolysis rate and optimize the biological trans-

formations, physic and/or chemical pretreatments need to be implemented, such as mechanical (grinding), physical (thermal) (Maroušek, 2013), chemical (acid or alkali) (Nieves et al., 2011), or a combination of them (Barakat et al., 2013). These pretreatments aimed at increasing the substrate surface area, decreasing crystallinity and/or disrupting lignin-hemicelluloses cross-linked network. Therefore, biodegradable compounds become accessible to enzymes and microorganisms. Mechanical fractionation is a necessary step in lignocellulosic bioconversion to (i) decrease particle size and increase total accessible specific surface area (ii) increase pore size of particles and the number of contact points for inter-particle bonding in the compaction process and eventually (iii) decrease cellulose crystallinity. All these parameters improve the digestibility and the conversion of saccharides during enzymatic hydrolysis (Khullar et al., 2013; Silva Ghizzi Damasceno et al., 2012; Wang et al., 2013) and bioconversion (Barakat et al., 2013; Galbe and Zacchi, 2012; Lindmark et al., 2012; Palmowski and Müller, 2000a; Taherzadeh and Karimi, 2008). Mechanical fractionation can represent an interesting and efficient pretreatment or raw lignocellulosic matter for bioconversions without water addition and effluents production (Barakat et al., 2013).

Some authors investigated the effect of reducing particle size and solubilizing food wastes. Izumi et al. tested substrates bead milled (food wastes) in batch process experiments at mesophilic temperature (37 °C) for 16 days (Izumi et al., 2010). The reduction in diameter from 0.843 to 0.391 mm involved 40% of total

*Abbreviations:* AD, anaerobic digestion;  $d_{med}$ , median diameter; COD, chemical oxygen demand; VFA, volatile fatty acids.

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chemical oxygen demand (COD) solubilization. In batch experiments, particle size reduction from 0.888 to 0.718 mm improved the methane production by 28%. However, excessive particle reduction resulted in volatile fatty acids (VFA) accumulation, which composition depended of particle size reduction. Acetic acid was accumulating when particle size was reduced, while the concentration in butyric acid was decreasing for particle size inferior to 0.5 mm. No significant effect was observed on propionic acid. The maximum substrate utilization constant was inversely proportional to particle size (reduction from 2.14 to 1.02 mm) in anaerobic thermophilic digestion in food wastes (Kim et al., 2000). Sharma et al. reported the effects of particle size on agricultural and forest residues on biogas generation through anaerobic digestion in batch digesters at 37 °C (Sharma et al., 1988). Out of five particle sizes (0.088, 0.40, 1.0, 6.0, and 30.0 mm), the maximum quantity of biogas was produced with the smallest particles. Palmowski et al. (2000) investigated the effect of comminution (to millimeters or hundreds of micrometers) of several organic solids on the particles biodegradation (Palmowski and Müller, 2000b). The gas production was increased up to 20% during AD in batch digesters, depending on the state of the ground sample and on the characteristics of the substrate. The maximum gas production increase was observed in the case of high fiber content substrates. The generation of active surface area and the reduction of material structure implied that cell compounds from areas, previously difficult to reach for microorganisms and enzymes, were released and therefore became accessible. No improvement for more biodegradable substrates such as a mixture of apples, carrots and potatoes, meat was observed. Considering small particles (specific surface greater than 20 m<sup>2</sup>/kg), a small effect was observed on biogas production, but a significant effect was observed in the case of big particles (specific surface ranged from 3 to 20 m<sup>2</sup>/kg). An increase in gas production rate led to a decrease in digestion time, which allowed the size of the reactor to be reduced without any losses in gas production. Mshandete et al. (2006) studied the degradation and biogas production potential of sisal fiber with fiber sizes ranging from 2 to 100 mm, at 33 °C (Mshandete et al., 2006). The total fiber degradation increased from 31% to 70% for the 2 mm fibers compared to untreated sisal fibers. Moreover, the methane yield was inversely proportional to particle size with an increase of 23% when the fibers were cut at 2 mm size (0.22 m<sup>3</sup>CH<sub>4</sub>/kg organic matter for 2 mm, compared to 0.18 m<sup>3</sup> CH<sub>4</sub>/kg organic matter for untreated fibers). Hu et al. presented the influence of cellulose particle size (50–100 µm) on anaerobic degradation by rumen microbes. Particle size reduction resulted in a decrease of methane production and an increase of soluble products (particularly VFA) (Hu et al., 2005). The majors VFA were acetate and propionate. The maximum substrate utilization rate coefficient doubled with a decrease in the average particle size from 2.14 to 1.02 mm. The influence of grinding pretreatment of cardboard materials was studied on various aspects of the AD (biodegradation rate and methane production) (Pommier et al., 2010). The authors observed no significant differences, neither on the biogas production quantity, nor on the biogas production rate.

These studies demonstrated that the particles size reduction generally allows an increase of the biogas potential and/or the digestion rate. Reducing particle size can also involve VFA accumulation, and therefore, may inhibit methanogens generate a decrease of AD performances. Most of these articles investigated the effect of particle size reduction to some hundreds micrometers. Moreover, the conclusions could be opposite depending on either the size reduction or the type of substrate.

Therefore, the hydrolysis limitation due to substrate specific surface area and accessibility of organic matter to microorganisms is still disputed and needs to be clearly demonstrated.

The novelty of our study was the investigation of the effect of lignocellulosic biomass micronization on the AD performances (Silva Ghizzi Damasceno et al., 2012, 2011). Indeed, most of the previous articles dealt with coarse milling, with a minimum particle size around 100–200 µm. Here, the present work aimed at determining if a significant variation of the median diameter of particles (more than one order of magnitude), obtained by micronization, could increase the maximum biogas production value or/and the kinetics of the AD. This study focuses on one type of mechanical grinding (centrifugal grinding) in order to focus on the influence of the particle size reduction on the AD.

## 2. Material and methods

Wheat straw (*Triticum aestivum* cv. Apache) was grown in Aveyron (FR) and harvested in 2007. The size reduction process was detailed and presented in Silva Ghizzi Damasceno et al. (2011) and constituted 5 steps. Wheat straw was first comminuted using a cutting mill (Retsch SM2000, Haan, Germany) equipped with a 2.0 mm sieve followed by 4 successive steps of centrifugal grinding (Retsch ZM200, Haan, Germany) using 1.0, 0.5, 0.25 and 0.12 mm sieves.

### 2.1. Granulometry and specific surface analysis

Particle size distributions were determined from 0.02 µm to 2000 µm using a laser diffraction granulometer Mastersizer 2000 (Malvern Instruments Ltd., United Kingdom) in ethanol suspensions in duplicates at least. The wheat straw samples were characterized as their median diameter ( $d_{med}$ ) obtained from the particle size distributions. For knowing their median diameter and assuming that particles are spherical, the surface area could be estimated.

$$\text{Surface area} = 4 \times \text{Pi} \times (d_{med}/2)^2 \quad (1)$$

Bulk density was determined as the tapped volume of 1 g of powder in a graduated cylinder.

### 2.2. Biochemical composition of the wheat straw

The dry matter and organic matter (DM and OM) were measured according to standard APHA methods (1992) by drying the biomass at 105 °C (24 h) followed by incineration at 550 °C (2 h).

The cellulose and hemicellulose contents of each class were determined by gas chromatography (GC) after a two-step sulfuric acid hydrolysis (36N H<sub>2</sub>SO<sub>4</sub> for 30 min at 25 °C then 2N H<sub>2</sub>SO<sub>4</sub> for 2 h at 100 °C) and derivatisation as alditol acetates (Blakeney et al., 1983). The alditol acetates obtained were injected on a DB 225 capillary column (J&W Scientific, Folsom, CA), using allose as the internal standard. Cellulose content was calculated as the sum of anhydro-glucose content. Hemicelluloses content was calculated as the sum of anhydro-arabinose, anhydro-xylose, anhydromannose and anhydro-galactose contents.

Klason lignin was determined as the insoluble residue remaining after a two-step sulfuric acid hydrolysis of cell wall polysaccharides (Monties, 1984). Briefly, 200 mg of sample was suspended in 2 mL of 72% H<sub>2</sub>SO<sub>4</sub> for 2 h at 20 °C. These acidic suspensions were then diluted to 5% with deionized water and boiled for 3 h then filtered. The remaining residues were dried at 105 °C and then incinerated at 550 °C for 6 h.

The protein content was determined by the KJELDAHL method (total nitrogen × 6.3).

### 2.3. Structural analysis

The Fourier transform infra-red (FTIR)-spectroscopy was performed on 2 mg of dry sample carefully mixed with 100 mg

dry spectroscopic grade KBr and pressed into a self-supporting pellet. FTIR measurements were performed using a Nicolet spectrometer. For each sample, 32 spectra were accumulated between 4000 and 400  $\text{cm}^{-1}$  and averaged. The resulting spectra were normalized to the highest peak in the fingerprint region between 2000 and 800  $\text{cm}^{-1}$ . The ratio between lignin and cellulose was studied at respectively 1509 and 1160  $\text{cm}^{-1}$ , the ratio lignin/xylans was estimated at 1509 and 1730  $\text{cm}^{-1}$ , and the ratio lignin/(cellulose + hemicelluloses) at 1509 and 1375  $\text{cm}^{-1}$  (Colom et al., 2003; Da Costa Lopes et al., 2013)

X-ray diffraction (XRD) measurement was carried out with an X-ray diffractometry (D/MAX-1200, Rigaku Denki Co. Ltd., Japan). The X-ray radiation used was Ni-filtered Cu-K $\alpha$  with a wavelength of 1.5406 Å. The voltage was set at 40 kV and the current was set at 30 mA. The samples were mounted on a solid circular holder, and the proportional counter detector was set to collect data at a rate of  $2\theta = 1^\circ/\text{min}$  over the  $2\theta$  range from  $4^\circ$  to  $45^\circ$ .

The crystallinity obtained with XRD measurements was calculated by the ratio between the intensity of the crystalline peak ( $I_{2\theta=22} - I_{2\theta=16}$ ) and the total intensity ( $I_{2\theta=22}$ ) (Park et al., 2010).

#### 2.4. Water distribution analysis

The hydration properties and solubility of the samples were determined through various criteria. Apparent volumic mass was determined by introduction, settlement and compaction of wheat straw particles in a graduated flask. The results were obtained in  $\text{g}/\text{cm}^3$ . Solubility in water and capacity of water retention were determined by mixing 0.5 g of sample and 5 mL of distilled water for 2 h (rotative mixer Reax 4, HEIDOLPH, Germany) at  $20^\circ\text{C}$ . After this period, the samples were centrifuged ( $12,000 \times g$ ,  $2 \times 10$  min,  $20^\circ\text{C}$ ) in a Beckman AvantiTM J-30I centrifuge (Beckman, Fullerton, CA, USA), drained and weighed in order to determine the water retention capacity.

The residues were dried ( $105^\circ\text{C}$  until constant mass) and weighed as non-solubilized dry mass. The solubility in water of the samples was expressed as g solubilized dry mass/g initial dry mass.

The bound water (and free water) quantity was measured. The samples, previously humidified with distilled water, were dried in thin layer in 10 cm diameter aluminum pans at constant conditions in a Precisa XM60Halogen moisture analyzer at  $100^\circ\text{C}$ . The system was connected to a PC for the on-line measurements of the weight loss. The critical point  $W_c$ , marking the limit between bound and free water content was determined from the experimental curve of the evaporation rate in dependence of the moisture content ( $g_{\text{water}}/g_{\text{DM}}$ ). Above this critical point, the water is considered as free water.

#### 2.5. Anaerobic biodegradability in flasks

The anaerobic biodegradability of each sample was measured in batch mode in flasks (biochemical methane potential). The method used for biochemical methane potential (BMP) was adapted from Angelidaki and Sanders (2004) and carried out in serum flasks closed with septa. For these experiments the inoculum was a granular sludge treating sugar effluent. The granules were broken thanks to mixing, and diluted to 5 g/L of organic matter (OM). The substrate to degrade was equivalent to 0.5  $g_{\text{OM}}$  of substrate/ $g_{\text{OM}}$  of inoculum. Each flask contained substrate, inoculum (sludge), macroelement solution (for correct C/N ratio i.e., around 30), oligoelement solution, phosphate buffer solution and was placed at  $35^\circ\text{C}$  and hand-mixed once a day. Two control samples were realized: a blank (water), a control sample (ethanol: completely and rapidly biodegradable compound to check methanogens activity). The biogas volume was quantified with liquid displacement (water, pH 2, NaCl 10%) and the biogas composition was determined by gas

**Table 1**

Composition of wheat straw expressed as dry matters.

	Wheat straw
Organic matter (OM/ $g_{\text{DM}}$ )	$0.93 \pm 0.003$
Total C (%)	$45.7 \pm 0.21$
Total N (%)	$0.6 \pm 0.11$
Ratio C:N	$7 \pm 1.3$
Carbohydrates	
Cellulose (%)	$32.0 \pm 0.75$
Hemicelluloses (%)	$20.5 \pm 0.45$
arabinoxylans	
were considered ad total hemicellulose	
Klason lignin (%)	$17.4 \pm 0.30$
Proteins (%)	$3.8 \pm 0.69$
Ash (%)	$6.1 \pm 0.06$

chromatography ( $\mu$ -GC from Varian). Each test was carried out in duplicate. Experiments lasted around 120 days.

#### 2.6. Kinetic study and statistical analysis

##### 2.6.1. First-order kinetic modeling of BMP curves

In order to study the influence of the particle size reduction on the kinetics of the anaerobic biodegradation, a first order kinetic model was fitted to BMP experimental data. The kinetics obtained with BMP tests are used by some researchers as scale up indicators for semi-continuous or continuous systems. Nevertheless, BMP tests are mainly designed to assess the maximum methane potential of a substrate. The rate of reaction in a BMP test is dependent on the nature of the test (high solids or liquid) and the characteristics of the inoculum, including its degree of acclimatization and the ratio of inoculum to substrate at the start of the test.

As in this study, the biodegradation tests of the different substrates were performed simultaneously through the same experimental run using identical conditions of inoculum/substrate ratio, inoculum type and sampling times, the kinetics patterns could be compared.

Consideration of the kinetics can provide some information on the effect of pretreatment on substrate material. To determine the kinetic constants, the specific methane production was modeled using a first order model. The methane production is given by

$$V = V_0 \times (1 - e^{-kt}), \quad (2)$$

with  $V$ : the total volume of methane produced at  $t$ ,  $V_0$  the maximum methane potential and  $k$  the kinetic constant.

Analysis of variance (ANOVA) was performed on modeled values of BMP tests in order to check that the significance of grinding process on BMP and kinetic values. The test assesses the statistical significance of the difference between samples means.

##### 2.6.2. Study of substrates biodegradation in fed-batch reactors

Four anaerobic digesters (2.5 L) were operated in parallel to treat wheat straw samples, and to measure: their ability to be converted into methane and their biodegradation kinetics. A large concentration of sludge (20  $g_{\text{OM}}/\text{L}$  of reactor) was used as inoculum to neglect biomass growth (inoculum to substrate ratio = 20) and successive substrate addition were made to study the acclimatization (accumulation) of the microorganisms to solids substrates. The reactors were operated in batch mode during around two months that corresponded to 12 batches. The reactors were fed with a small quantity of substrate (1  $g_{\text{OM}}/\text{L}/\text{batch}$ ) to ensure complete biodegradation and absence of accumulation. At the end of each batch OM, DM and VFA were measured. VFA measurements were also carried out during the batches in order to investigate the effect of particle size reduction on solubilization. The biogas production was monitored

continuously during the batches in order to quantify the methane yield and kinetics. A batch trial was considered as finished when the biogas production returned to a low level around 0.15 mL/min corresponding to the endogenous production (determined at the beginning of the experiment after several ethanol additions). Batch trials lasted between 70 and 80 h. The methane production rate was quantified for batch runs 8–11 when the sludge was well acclimated to substrate. Therefore, the kinetics measured were only dependent on the characteristics of substrate.

### 3. Results and discussion

#### 3.5. Wheat straw characteristics

The median diameter of particles classes was determined by laser diffraction granulometry. The particles were assimilated as spheres, and the median diameter of each class of particles was estimated. The first class had a  $d_{med}$  of 759  $\mu\text{m}$ , while the next reductions were of 421  $\mu\text{m}$ , 206  $\mu\text{m}$ , 88  $\mu\text{m}$  and 48  $\mu\text{m}$ , respectively.

The surface area of the coarsest and the finest ground sample of wheat straw equaled 25.5 and 282.2  $\text{m}^2/\text{kg}$ , respectively. The ratio of specific surface area between the smaller and the larger class is equal to 11, which could be considered as significantly different.

The dry matter and organic matter (DM and OM), the fiber content (cellulose, hemicellulose and lignin compounds) were determined in the initial substrate and in each class of milled wheat straw. No significant variation of the OM concentration and of lignocellulosic compounds concentrations was observed with particle size reduction. Therefore, only the value of the initial wheat straw was presented. The wheat straw contains  $0.932 \pm 0.003 \text{ g}_{OM}/\text{g}_{DM}$ . Carbohydrates measurements were made on the wheat straw (before grinding). It contains 32.0% of cellulose, 20.5% of hemicelluloses. The other biochemical analysis reveal that the wheat straw was composed of 17.4% of Klason lignin, 6.1% of ash, 3.8% of protein (Table 1).

#### 3.6. Fourier transformed infra-red spectroscopy

In order to link the anaerobic biodegradability to the structural properties of each class of particles, physical characteristics of wheat straw were investigated by infrared spectroscopy. Infrared spectroscopy is an appropriate technique to study the variations of structure introduced by milling process, and particularly the crystallinity of the cellulose in samples. The crystallinity of the cellulose was measured by XRD measurements. The XRD crystallinity decreased from 0.41 to 0.35 (Table 2). No difference of the crystallinity index (XRD) was observed between 206 and 48  $\mu\text{m}$ . Grinding process modifies the internal structure of the cellulose and particularly reduces the cellulose crystallinity till 206  $\mu\text{m}$ . This

result is in accordance with literature. Barakat et al. (2013) showed the positive effect of mechanical size reduction on the cellulose crystallinity. Crystallinity is known to confer some hardly accessible properties to cellulose regarding biodegradability (Barakat et al., 2014). However, our results showed that the excessive reduction above 50  $\mu\text{m}$  was not beneficial to the reduction of cellulose crystallinity.

The lignin/cellulose ratio ( $1509/1160 \text{ cm}^{-1}$ ) increased with particle size reduction, particularly above 421  $\mu\text{m}$  (+16.6% for  $d_{med} = 88 \mu\text{m}$ ), this could be either due to a relative increase of cellulose quantity or a decrease of lignin in the milled wheat straw. If both components were modified, the cellulose would be therefore more affected by grinding than lignin. The ratio lignin/xylans also increased (+13.5% for  $d_{med} = 88 \mu\text{m}$ ) with grinding showing that the decrease of lignin was less important than the decrease of hemicellulose content. Comparing these two ratios evolution (+13.5% for lignin/xylans and +16.6% for lignin/cellulose), it appears that with lignin as a reference, cellulose seems to be more susceptible to be ground than hemicelluloses since a lower quantity of this polymer was found in small particles. Analyzing the particles smaller than 50  $\mu\text{m}$  during successive grinding, Silva Ghizzi Damasceno et al. (2011) showed that there were three different groups of components exhibiting different behaviors face to grinding. In straw, ash + proteins, lignin + hemicelluloses, cellulose could be classified from the least to the most grindable. Our results are coherent with these conclusions. Under  $d_{med}$  of 50  $\mu\text{m}$ , the particles contained a smaller quantity of hemicelluloses and celluloses than the larger particles. These conclusions might be due to difference of tissue hardness.

#### 3.7. Particles behavior in water

Particles behavior in water was investigated thanks to different kinds of experiments (Table 2). Water retention capacity and solubility were studied by mixing particles with water before centrifugation and separation of particulate and soluble fraction. The results presented in Table 2 shows that the lower the  $d_{med}$ , the lower the water retention capacity and the higher the solubility. Grinding process favored the solubility in water of wheat straw. The solubility was increased by 1.7 times from 6.3 to 10.8 ( $\text{g}_{solubles}/100 \text{ g}_{DM}$ ). The decrease in water retention capacity involves a decrease in swelling properties, and therefore, a higher concentration of soluble in water solution.

Further analyses were carried out in order to determine the water distribution for each wheat straw particles class. The results were analyzed in terms of critical water content (Wc), which represents the limit between free and bound water. The bound water and the moisture content are important parameters for optimizing the biodegradation of solids, as only the free water is bioavailable to microorganisms. Wc seemed to be relatively con-

**Table 2**

physical properties of the different ground samples of the wheat straw: granulometry, specific surface area, critical water content - fourier transformed infrared (FTIR) analysis of wheat straw fractions.

$d_{med}(\mu\text{m})$	759	421	206	88	48
Type of grinding	Cutting	Centrifugal			
Specific surface area ( $\text{m}^2/\text{kg}$ ) <sup>a</sup>	25.5	46	88.2	188.8	282.2
Weq crit ( $\text{g}_{water}/\text{g}_{DM}$ )	3.5	3	4	2.8	2.9
Retention capacity ( $\text{g}_{water}/\text{g}_{DM}$ )	$8.0 \pm 0.2$	$6.4 \pm 0.5$	$5.9 \pm 0.4$	$5.1 \pm 0.4$	$4.2 \pm 0.5$
Mass density ( $\text{g}/\text{mL}$ )	0.3	0.3	0.3	0.4	0.4
Swelling ( $\text{mL}_{water}/\text{g}$ )	$11.1 \pm 0.6$	$9.1 \pm 0.4$	$9.5 \pm 0.3$	$8.8 \pm 0.5$	$8.4 \pm 0.5$
Solubility ( $\text{g}_{solubles}/100 \text{ g}_{DM}$ )	$6.3 \pm 1.5$	$9.7 \pm 2.5$	$9.0 \pm 0.6$	$10.0 \pm 0.8$	$10.8 \pm 0.7$
Crystallinity	XRD	$0.409 \pm 0.001$		$0.369 \pm 0.002$	$0.354 \pm 0.005$
FTIR analysis					
Lignin/xylans	1509/1730	0.59	0.62	0.66	0.67
Lignin/cellulose	1509/1158	0.42	0.43	0.48	0.49
Lignin/(cellulose + hemicelluloses)	1509/1375	0.54	0.53	0.55	0.54

<sup>a</sup> Assimilating the particle as spherical particles.



**Table 3**  
BMP and batch reactors performances (experimental and modeled).

Experimental values		In BMP tests ( $S/X = 0.5$ in OM) – inoculum not adapted		In batch reactors tests ( $S/X = 0.05$ in OM) inoculum adapted			
		Modelled parameters		Experimental values		Modelled parameters	
$d_{med}(\mu m)$	$mL_{CH_4}/g_{OM}(\text{average value})$	$V_0 (mL_{CH_4}/g_{OM})$	$k (d^{-1}) \times 10^2$	$mL_{biogas}/g_{OM}/h$	$mL_{biogas}/g_{OM}^a$	$V_0 (mL_{biogas}/g_{OM})$	$k (d^{-1})$
759	$306 \pm 16$	$300 \pm 7$	$3.3 \pm 0.1$	183.4	$531 \pm 50$	$325.7 \pm 1.6$	$10.1 \pm 1.1$
421	$281 \pm 4$	$277 \pm 6$	$3.5 \pm 0.0$	148.4	$525 \pm 43$	$336.2 \pm 0.9$	$22.3 \pm 2.1$
206	$283 \pm 10$	$284 \pm 11$	$3.7 \pm 0.4$	/	/	/	/
88	$291 \pm 26$	$281 \pm 18$	$4.7 \pm 0.6$	252.8	$493 \pm 48$	$425.5 \pm 1.3$	$22.7 \pm 2.3$
48	$282 \pm 16$	$281 \pm 13$	$4.7 \pm 0.6$	168.1	$593 \pm 61$	$336.8 \pm 1.2$	$16.1 \pm 1.5$

<sup>a</sup> Sum of mL produced/sum of OM added.

stant for all the classes which  $d_{med}$  ranging from 759 to 206  $\mu m$  (around  $3.2 g_{water}/g_{DM}$ ) and then decreases (around  $2.4 g_{water}/g_{DM}$ ) for smaller particle classes. These results show that when the particle size is reduced to less than 206  $\mu m$ , the transition between free and bound water seems to be shifted to lower values. It means that bound water is less important in particles having a lower size. These results are coherent with water retention capacity measurements. In presence of the same quantity of water, the water would be more accessible (free water) with smaller particles than with larger ones. These results should be completed by further experiments of water adsorption, which could clearly classify bound, capillary and free water. Garcia-Bernet et al. showed that this water critical content is related to the capillary water fraction of wastes (García-Bernet et al., 2011).

### 3.8. Anaerobic biodegradability

Biochemical methane potential (BMP) tests were performed in order to quantify the maximal biodegradability of the different wheat straw classes. In these experiments, the inoculum was rich in methanogens (a control tests was made with ethanol as the only substrate) but not particularly adapted to the substrate. The ratio between substrate and inoculum equaled  $0.5 g_{OM}$  of substrate/ $g_{OM}$  of sludge. The BMP values, gathered in Table 3, ranged from  $281 \pm 4 mL_{CH_4}/g_{OM}$  to  $306 \pm 16 mL_{CH_4}/g_{OM}$ . These values are quite high for wheat straw, usually ranging from around 150 to  $400 mL_{CH_4}/g_{OM}$  (Brown et al., 2012; Pouech et al., 1998) depending on the origin and the maturity of the wheat straw. The experiments revealed that the maximum methane production is not significantly different when particle size is reduced. These results were confirmed by analysis of variance (ANOVA) ( $p > 0.6$ ). These results are in accordance with some authors who showed that shredding does not improve the BMP of cardboard shred from 10 to 1 mm (Pommier et al., 2010).

### 3.9. Kinetic studies

#### 3.9.1. During BMP tests

The methane production curves were fitted with a first-order kinetics equation (Eq. (2)). The biogas production kinetic constants ( $k$ ) were increased by around 43%, from  $3.3 \cdot 10^{-2}$  to  $4.7 \cdot 10^{-2} d^{-1}$  when the  $d_{med}$  of the particles was reduced from 759  $\mu m$  to 48  $\mu m$ , respectively (Table 2). It seems that there was a diameter threshold below which the kinetic of anaerobic biodegradation was increased significantly (Table 3). Indeed, a significant rise of the first order kinetics was observed when the median diameter was lower than 206  $\mu m$ . Even if, the standard deviation between replicates increase with particle size reduction, the results of ANOVA revealed that the kinetic constant of particles with  $d_{med}$  of 759 and 421  $\mu m$  are different from the ones of 88 and 48  $\mu m$  ( $p$  value  $< 0.3$ ). The particles of 759 and 421  $\mu m$  have quite the same kinetic behavior ( $p$  value  $> 0.9$ ).

#### 3.9.2. During fed-batch reactors

Four reactors were operated simultaneously in batch mode during around two months, fed with 759, 421, 88, 48  $\mu m$  class of particles. The reactors were fed with a small quantity of substrate ( $1 g_{OM}/L/\text{batch}$ ), though the  $S/X$  ratio was ten times smaller than in BMP test and equal to around  $(0.05 g_{OM}$  of substrate/ $g_{OM}$  of sludge), to ensure complete, rapid biodegradation and absence of accumulation. A batch run lasted between 70 and 80 h but only the slowly biodegradable part of the straw was consumed during the last 50–60 h.

The experimental biogas production (per  $g_{OM}$ ) was not significantly improved by reduction of particle size (Table 3 and Fig. 1A). However, the biogas production rate increased from 183.4 to  $252.8 mL_{biogas}/g_{OM}/h$  (+37%) for, respectively, the particles classes of 759–88  $\mu m$  (Table 3). It seems that decreasing particle size increased the biogas production rate till around 88  $\mu m$  and then leveled off to around  $168.1 mL_{biogas}/g_{OM}/h$  (–8%) for the particles of 48  $\mu m$ . Reduction to lower median diameter value did not increase significantly the methane or biogas production rate.

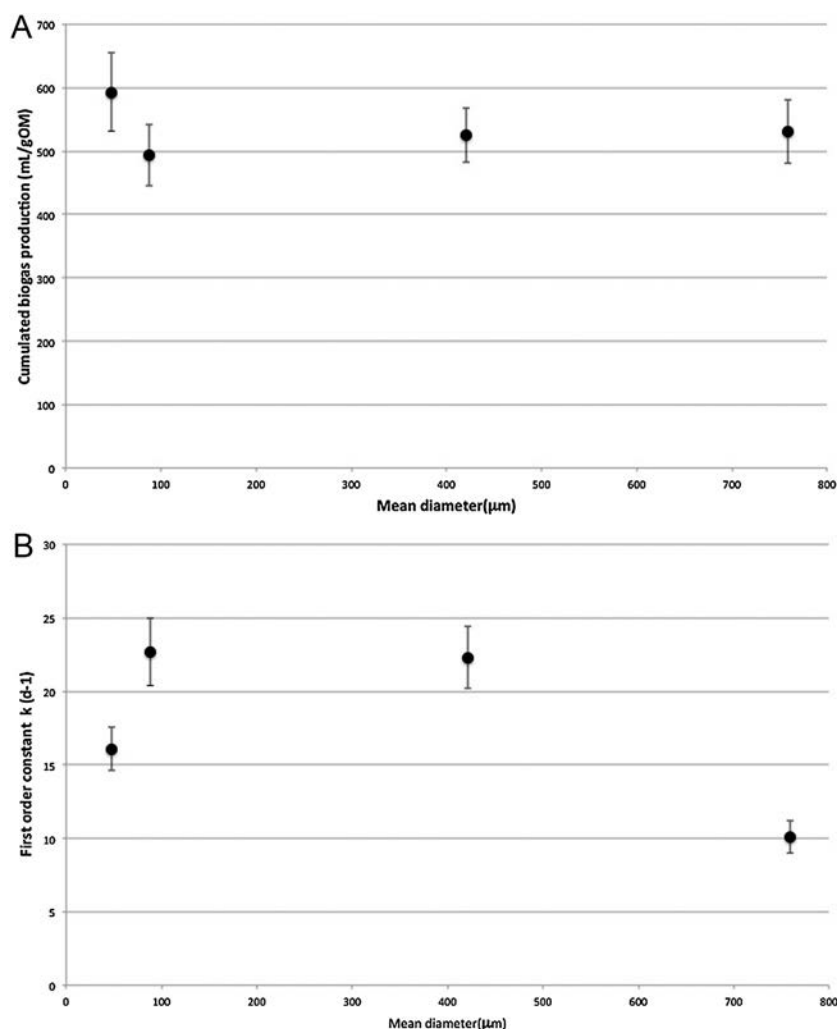
Modeling the experimental values with a first order kinetics. The results revealed the absence of increase of biogas potential but a significant increase of the constant of the first order kinetic from  $10.1$  to  $22.7 d^{-1}$  for particles belonging to the class 759 and 88  $\mu m$ , respectively (Fig. 1B). The kinetics in batch reactor were more rapid than the one in BMP because of the ratio  $S/X$  which was ten times higher in BMP than in reactor and because of the adaptation of the inoculum in batch reactors which accelerated the hydrolysis and methanogenesis steps.

The total VFA concentration and their composition were followed during one batch run for all the particles classes. The total concentration remained low (total concentration of  $0.05 g/L$ ) during the batch run and stable for the particles classes of 759–88  $\mu m$ . For the 48  $\mu m$  class, the concentration was doubled at 21 h i.e., during the first third of the batch duration, before going back to the initial level (data not shown). The higher concentration of VFA was due to the accumulation of propionic acid ( $0.095 g/L$ ). Some specific studies observed that the particle size reduction could be detrimental for anaerobic digestion process. Indeed, excessive particle size reduction results in volatile fatty acids accumulation, and therefore, in methane production decrease. The major acids produced were acetic and propionic acids (Hu et al., 2005; Izumi et al., 2010).

### 3.10. General discussion about the effect of reducing particle size on anaerobic digestion

The results presented in this article about the reduction of particle size showed clearly the presence of a threshold median diameter (around 200  $\mu m$ ) above the one some various phenomena were encountered.

Reducing the median diameter of particles from mm to hundred  $\mu m$ , reduced the crystallinity of the cellulose which impacted the methane production rate when inoculum was not adapted to the



**Fig. 1.** (A) Cumulated biogas production (mL biogas/gOM) during batch reactors tests; (B) first order kinetic constant from model ( $d^{-1}$ ).

substrate (as for BMP tests) and the biogas production rate in batch reactors when inoculum was adapted to a complex substrate. However, the total amounts of methane that is produced during BMP and batch runs were not affected by this size reduction. The fact that a higher quantity of inoculum adapted to the substrate was placed in the reactors increased significantly the kinetic constant. For diameters lower than 200  $\mu m$ , the crystallinity of the cellulose (XRD measurements) was no more impacted. The presence of such a threshold was also observed on the kinetic constant of methane production in BMP tests and on batch reactors. Moreover, the degradation study of wheat straw in batch reactors suggested that an important reduction of particles size could be detrimental to biodegradation. Indeed, the release of soluble molecules could be too fast compared to their assimilation by methanogens and therefore generate accumulation of VFA.

A positive effect was also suggested concerning the interaction of particles with water. Indeed, particle size reduction increased the solubility and decreased the water retention capacity and the bound water in particles. On the one hand, these consequences may facilitate the solubilization of small molecules in saturated media and therefore increase the accessibility of such molecules to microorganisms. On the other hand, all these phenomena could become problematic in systems where the water can be limiting such as high solid fermentations or dry anaerobic digestion.

Therefore, a compromise between increasing the solubility and preventing the accumulation of VFA due to particles size reduction

needs to be found. This compromise depends on the characteristics of the substrate and particularly its soluble part. In water limiting conditions, some mixing or separation strategies need to be implemented.

#### 4. Conclusion

The effect of ultra-fine milling of wheat straw was investigated on biogas production. Particles classes from 759 to 48  $\mu m$  were obtained. Anaerobic biodegradability tests revealed that grinding has no effect on the total methane potential values but influences the methane production kinetics, particularly for particle sizes lower than 200  $\mu m$ . The physical characterization (cellulose crystallinity, critical water content) showed the existence of a median diameter optimum around 200  $\mu m$ .

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