

# Effect of ultrasonic post-treatment on anaerobic digestion of lignocellulosic waste

Waste Management & Research  
2021, Vol. 39(2) 221–232  
© The Author(s) 2020  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/0734242X20931940  
journals.sagepub.com/home/wmr  
SAGE

Maria Rosaria Boni, Alessandra Poletti, Raffaella Pomi and Andreina Rossi 

## Abstract

This paper evaluates the effects of ultrasonication (US) applied, individually or in combination with a mechanical treatment, to the effluent of anaerobic digestion (AD) of lignocellulosic waste, on methane (CH<sub>4</sub>) production. US of the substrate downstream of AD is a relatively novel concept aimed at improving the degradation of recalcitrant components in order to enhance the overall energy efficiency of the process. US tests were carried out on real digestate samples at different energies (500–50,000 kJ/kg total solids (TS), corresponding to sonication densities of 0.08–0.45 W/ml). AD tests were performed on mixtures of sonicated (S<sub>us</sub>) and untreated (S) substrate at two different S<sub>us</sub>: S ratios (25:75 and 75:25 w/w), simulating post-sonicated material recycling to the biological process. The US effect was estimated through the solubilization degree of organic matter, as well as the CH<sub>4</sub> production yield and kinetics, which were all found to be enhanced by the treatment. At S<sub>us</sub>: S = 75:25 and E<sub>s</sub> ≥ 20,000 kJ/kg TS (0.25 W/ml), CH<sub>4</sub> production improved by 20% and the values of the kinetic parameters increased by 64–82%.

## Keywords

Sonication, lignocellulosic waste, anaerobic digestion, post-treatment, biomethane, hydrolysis

Received 15th March 2020, accepted 12th May 2020 by Associate Editor Nemanja Stanisavljevic.

## Introduction

Anaerobic digestion (AD) of organic waste has been looked at with renewed interest over the last decade, taking into account the principles of the circular economy in waste management. These principles involve achieving biological stabilization of the residues and at the same time pursuing materials and energy recovery targets. Interest in AD of organic waste is further fostered by the potential to integrate the AD process into decentralized energy production systems as well as into the biorefinery approach.

Despite having been applied for more than a century, AD requires further research efforts to address unresolved issues including the poor efficiency of the conversion of lignocellulosic materials into methane (CH<sub>4</sub>) (Sawatdeenarunat et al., 2014). These materials are typical components of municipal solid waste, including agro-industrial as well as forest residues, which represent interesting streams for bioenergy generation that do not compete with food production. The complex structure of lignocellulosic components, mainly formed by lignin, hemicellulose and cellulose, makes them highly resistant and biologically recalcitrant (Hendriks and Zeeman, 2009; Molinuevo-Salces et al., 2013; Zheng et al., 2014). Consequently, the hydrolysis of lignocellulosic materials often becomes the rate-limiting step during AD and complex organic molecules may be found undegraded in the final digestate (Mbaye et al., 2014; Wojnowska-Baryła et al., 2018). Efforts to tackle the recalcitrance of lignocellulosic materials to biological degradation have been oriented towards the

application of pre/post-treatments to promote the hydrolysis of complex molecules. These rely on various mechanisms, including physico-mechanical (milling, grinding, and ultrasonication (US)), physico-chemical (steam explosion and wet oxidation), chemical (alkaline or acidic treatment, chemical oxidation, and organic solvents treatment) or biological processes (Carlsson et al., 2012).

Such processes have been widely investigated mainly as a pre-treatment stage of lignocellulosic substrates prior to AD. More recently, the application of the mentioned processes downstream of AD has been suggested as a novel concept to improve selectivity towards recalcitrant components and the overall energy efficiency of the process (Boni et al., 2016; Cesaro et al., 2014; Garoma and Pappaterra, 2018; Lindner et al., 2015; Menardo et al., 2011; Sambusiti et al., 2015; Somers et al., 2018).

Several studies (Braeutigam et al., 2014; Cesaro et al., 2012; Elbeshbishy and Nakhla, 2011; Elbeshbishy et al., 2011; Khanal et al., 2007; Pilli et al., 2011; Rasapoor et al., 2016, 2018; Somers et al., 2018; Zeynali et al., 2017; Zou et al., 2016) have suggested

Department of Civil and Environmental Engineering, Sapienza University of Rome, Rome, Italy

## Corresponding author:

Andreina Rossi, Department of Civil and Environmental Engineering, Sapienza University of Rome, Via Eudossiana 18-00184, Rome, Italy.  
Email: andreina.rossi@uniroma1.it

that US can positively affect the anaerobic biodegradability of organic residues including wastewater sludge, manure, food waste, agricultural wastes, distillery residues, and by-products from bio-ethanol production. The cavitation effects caused by the application of ultrasound promote the disintegration of particulate matter, the disruption of complex molecules, and the liberation of more easily degradable monomers, thus enhancing biodegradability (Iskalieva et al., 2012).

The effect of US downstream of AD on the biodegradability/bioavailability of AD digestate has received only limited investigation (Boni et al., 2016; Cesaro et al., 2014; Garoma and Pappaterra, 2018; Somers et al., 2018). In this downstream case, the sonicated digestate can either be directed to a second AD stage or be recycled back to the digester. Application of US as a post-treatment would allow improved selectivity towards the poorly biodegradable and recalcitrant components, thus optimizing energy efficiency and costs.

The performance of US depends on several factors, acting either synergistically or antagonistically. These factors include the operating conditions (treatment duration, temperature, US frequency, and power), the physico-chemical properties of the substrate (solids and lignin content and viscosity of the slurry) and the design parameters (reactor configuration and diameter and position of the transducer) (Gogate et al., 2011).

It must also be emphasized that the factors affecting US may be interdependent, which also complicates the prediction of their overall effect on anaerobic biodegradability. The different experimental conditions adopted (e.g., substrate properties, power input, and transducer design) also explain why a comparison of the US effects on CH<sub>4</sub> production reported in the literature often shows inconsistencies and is difficult to generalize.

Although some studies on US as a pre-treatment method for wastewater sludge (Grönroos et al., 2005; Show et al., 2007) have demonstrated that the US density ( $D_s$ ) represents a key operating parameter for process optimization, many authors (Boni et al., 2016; Cesaro et al., 2014; Elbeshbishy et al., 2011; Gadhe et al., 2014; Zeynali et al., 2017; Zou et al., 2016) have mainly focused on irradiation time and specific energy ( $E_s$ ).

A systematic investigation of the individual and joint influence of the relevant parameters of the US process, including  $E_s$  and  $D_s$ , on the anaerobic biodegradability of digestate is still missing in the literature. This study makes an attempt to fill in the gaps by means of the application of US, individually and in combination with a mechanical treatment, as a post-treatment downstream of AD. We expand the perspective of our previous research, focusing on a more complex residual matrix, investigating the combined influence of the relevant sonication parameters, and combining sonication with enhanced particle size reduction. The influence of the operating variables of the US treatment on the evolution of the AD process was assessed through detailed investigation of the solubilization degree of organic matter upon US, the CH<sub>4</sub> production yield and kinetics of the AD stage, as well as the related carbon mass balance.

**Table 1.** Digestate properties.

| Parameter                              | Unit of measure                        | Value*      |
|--|--|-------------|
| pH                                     | -                                      | 7.7 ± 0.20  |
| Total solids, TS                       | g/l                                    | 83 ± 0.9    |
| Volatile solids, VS                    | g/l                                    | 59 ± 1.1    |
| Total organic carbon, TOC              | g/l                                    | 29 ± 0.9    |
| Dissolved organic carbon, DOC          | g/l                                    | 4.9 ± 0.02  |
| Chemical oxygen demand, COD            | g/l                                    | 85 ± 1.9    |
| Soluble COD, sCOD                      | g/l                                    | 12.1 ± 0.1  |
| Carbohydrates                          | g/l                                    | 13.0 ± 1.2  |
| Soluble carbohydrates                  | mg/l                                   | 1095 ± 112  |
| Kjeldahl nitrogen, TKN                 | mg N-NH <sub>4</sub> <sup>+</sup> /l   | 3404 ± 16   |
| Soluble Kjeldahl nitrogen, sTKN (tot)  | mg N-NH <sub>4</sub> <sup>+</sup> /l   | 1436 ± 18   |
| Soluble N-NH <sub>4</sub> <sup>+</sup> | mg/l N-NH <sub>4</sub> <sup>+</sup> /l | 1088 ± 17   |
| Acetate                                | mg/l                                   | 403 ± 93    |
| Propionate                             | mg/l                                   | 30.5 ± 0.75 |
| Iron                                   | mg/l                                   | 213 ± 1     |
| Manganese                              | mg/l                                   | 15.3 ± 0.6  |
| Cobalt                                 | mg/l                                   | 0.91 ± 0.09 |
| Nicckel                                | mg/l                                   | 2.54 ± 0.90 |

Notes: \* average values and related standard deviations of three replicates; N-NH<sub>4</sub><sup>+</sup>: ammoniacal nitrogen.

## Materials and methods

### Materials

The digestate of an Italian full-scale AD plant, fed with a mixture of organic residues from a food production plant, silage energy crops, olive husks and manure, was collected and stored at 4°C and characterized (see Table 1) according to the methods reported in Boni et al. (2016).

Fourier-transform infrared spectroscopy (FT-IR) was also adopted using an Agilent 640 IR spectrometer operated in the mid-IR range with a 4 cm<sup>-1</sup> resolution and 4000 scans/sample in the absorbance mode with attenuated total reflectance correction.

### Experimental set-up

A number of US tests were performed by applying pre-set  $E_s$  values to the digestate. Each sonicated sample was then characterized to assess changes in composition of the substrate and to evaluate the solubilization degree of the relevant compositional parameters (see 'US and mechanical treatments' subsection). Mixtures of non-sonicated (S) and sonicated (S<sub>us</sub>) digestate were then prepared (see 'AD tests' subsection) to evaluate the effect of US on the CH<sub>4</sub> production rate and yield in a dedicated AD stage. For control purposes, a 100% non-sonicated digestate (S) sample was also included in the AD test campaign.

The effect of digestate milling prior to US was also estimated by using the milled and sonicated material in AD experiments under the conditions mentioned above (see 'AD tests' subsection).

*US and mechanical treatments.* The US treatment was performed by means of a laboratory-scale ultrasound generator

described elsewhere (Boni et al., 2016). The  $E_s$ , that is, the amount of energy supplied per unit of initial mass of total solids (TS), was varied in the range 500–50,000 kJ/kg TS, adopting  $D_s$  values of 0.08, 0.25 and 0.45 W/ml, which in turn corresponded to US intensities of 2.3, 7.7 and 13.4 W/cm<sup>2</sup>.

In order to limit the treatment duration, at a  $D_s$  of 0.08 W/ml the US tests were performed at  $E_s$  values < 20,000 kJ/kg TS.

The mechanical treatment was performed by a knife mill operated for three minutes at 22,000 revolutions per minute; the sample was then sonicated at an  $E_s$  of 20,000 kJ/kg TS at a  $D_s$  of 0.25 W/ml.

The effects of US and the combined milling and US treatment were assessed using equation (1) (see Boni et al., 2016):

$$I_p(\%) = \frac{sX_{us} - sX_0}{X_0 - sX_0} \times 100 \quad I_s(\%) = \frac{sX_{us} - sX_0}{sX_0} \times 100 \quad (1)$$

where  $X$  is the concentration of the parameter of concern (total organic carbon (TOC), chemical oxygen demand (COD), carbohydrates or proteins) before ( $X_0$ ) and after ( $X_{us}$ ) the US process and the prefix  $s$  indicates the corresponding soluble fraction.

The numerical values of  $I_p$  and  $I_s$  are affected by the initial composition of the material, more specifically by the initial partitioning of organic matter between the particulate ( $X_0 - sX_0$ ) and soluble ( $sX_0$ ) forms. The relevant content of the particulate fraction of the initial material makes  $I_p$  more meaningful than  $I_s$  for assessing the effect of US on the disintegration of organic matter.

**AD tests.** The AD tests were arranged to investigate the effect of  $E_s$  applied during the US treatment on the yield and kinetics of CH<sub>4</sub> production at a fixed value of  $D_s$  (0.25 W/ml). A subset of the treated samples (see ‘US and mechanical treatments’ subsection) was selected for the AD tests. In particular, the digestate sonicated at  $E_s$  values of 500 and 30,000 kJ/kg TS were not considered for the AD tests, since the former yielded a very low solubilization degree of organic matter and the behavior of the latter was similar to the sample treated at  $E_s = 20,000$  kJ/kg TS.

The AD experiments were carried out on mixtures of  $S_{us}$  and S at different ratios, namely  $S_{us}: S = 25:75$  and  $75:25$  w/w in order to simulate two different recycling ratios of the post-sonicated material to the biological process. Moreover, on the basis of the results obtained in the AD tests, for the sample treated at  $E_s = 20,000$  kJ/kg TS an additional AD test was performed on the milled and sonicated digestate at a 75% recycling ratio ( $S_{m+us}: S = 75:25$ ).

A control mixture containing the untreated digestate only (100% S) was also tested.

A summary of the experiments is reported in Table 2. Each test was run in duplicate.

The experiments were carried out in stirred batch glass reactors (working volume = 0.5 l) at  $T = 37 \pm 2^\circ\text{C}$ . Before the onset of the experiments, the reactors were flushed with nitrogen gas for a few minutes to drive away air from the reactor headspace. The volumetric production and composition of the produced biogas were periodically analyzed. The AD tests were operated until no further appreciable increase in CH<sub>4</sub> production was observed,

**Table 2.** Summary of the anaerobic digestion tests performed.

| Run                 | Mixture composition<br>% by weight<br>(w/w) | $E_s$<br>(kJ/kgTS) |
|---------------------|---|--------------------|
| Control             | 100% S                                      | -                  |
| 3000_25 $S_{us}$    | 25% $S_{us}$ + 75% S                        | 3000               |
| 3000_75 $S_{us}$    | 75% $S_{us}$ + 25% S                        | 3000               |
| 6000_75 $S_{us}$    | 75% $S_{us}$ + 25% S                        | 6000               |
| 10000_25 $S_{us}$   | 25% $S_{us}$ + 75% S                        | 10,000             |
| 10000_75 $S_{us}$   | 75% $S_{us}$ + 25% S                        | 10,000             |
| 20000_25 $S_{us}$   | 25% $S_{us}$ + 75% S                        | 20,000             |
| 20000_75 $S_{us}$   | 75% $S_{us}$ + 25% S                        | 20,000             |
| 50000_25 $S_{us}$   | 25% $S_{us}$ + 75% S                        | 50,000             |
| 50000_75 $S_{us}$   | 75% $S_{us}$ + 25% S                        | 50,000             |
| 20000_75 $S_{m+us}$ | 75% $S_{m+us}$ + 25% S                      | 20,000             |

which typically occurred within 56 days from the start of the experiment. In order to assess the metabolic pathways of the biochemical process, individual volatile fatty acids (VFAs), namely acetate, propionate, butyrate, valerate, hexanoate, and heptanoate, were determined at selected time intervals (see Boni et al. (2016) for details on the analytical procedure).

### Kinetic model

The CH<sub>4</sub> production over time was modeled through the modified Gompertz equation or the Richards bacterial growth model (Zwietering et al., 1990). Interpolation was performed using TableCurve 2D<sup>®</sup> v. 5.01, which showed for all experiments that the best degree of fitting was obtained using equation (2):

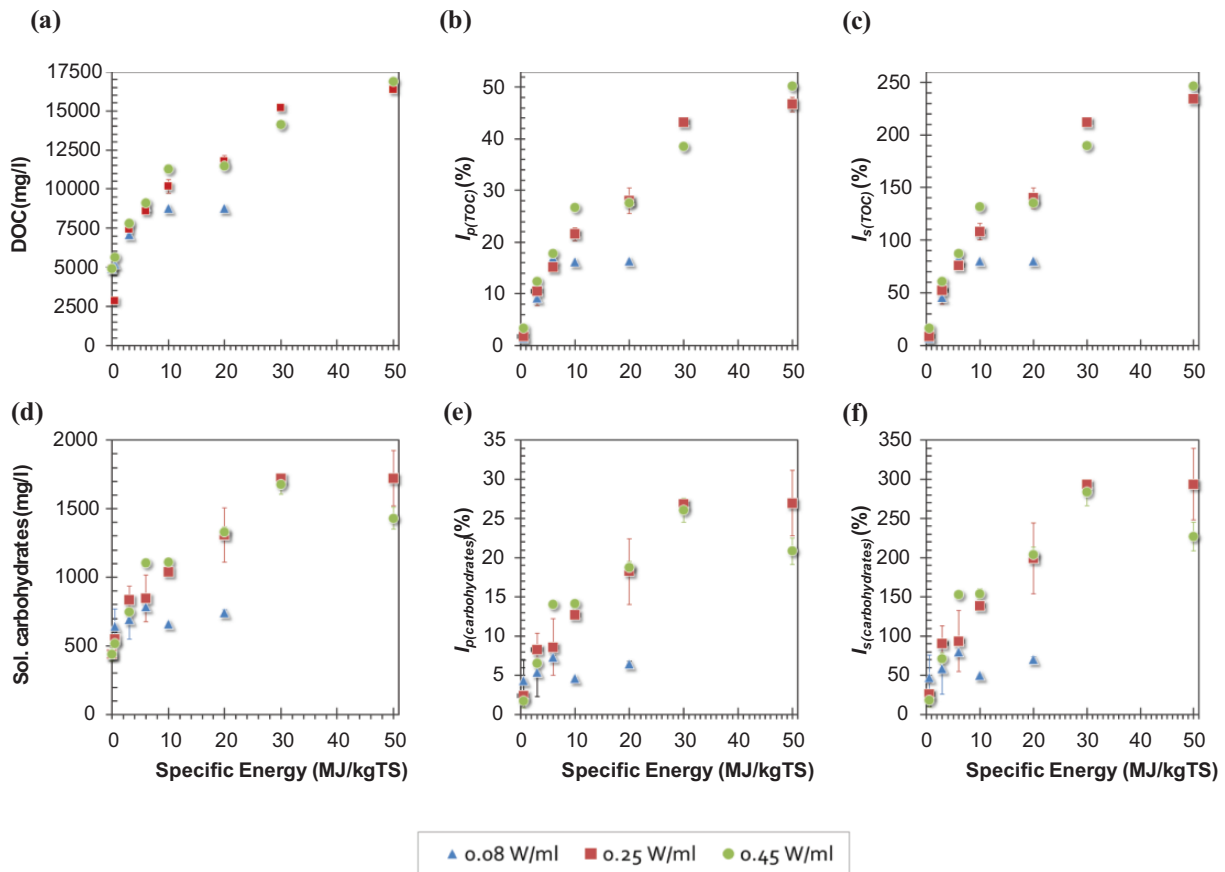
$$M = P \left\{ 1 + \exp(1 + v) \exp \left[ \frac{R_m}{P} (1 + v) \left( 1 + \frac{1}{v} \right) (\lambda - t) \right] \right\}^{(-1/v)} \quad (2)$$

where  $M$  (NI CH<sub>4</sub>/kg volatile solids (VS)) is the CH<sub>4</sub> production at time  $t$  (d),  $P$  (NI CH<sub>4</sub>/kg VS) is the maximum CH<sub>4</sub> production yield,  $R_m$  (NI CH<sub>4</sub>/kg VS·d) is the maximum CH<sub>4</sub> production rate,  $\lambda$  is the lag phase duration (d) and  $v$  is a curve shape parameter. The time required to attain 95% of  $P$  ( $t_{95}$ ) was also estimated.

## Results and discussion

### Effect of US energy on digestate properties

Within the range of the tested US conditions, no significant changes in both the TS and VS concentrations were observed. This implied that no relevant organic matter volatilization nor mineralization effects were produced by US, as also observed in previous studies (Boni et al., 2016; Cesaro et al., 2014). Other authors (Elbeshbishy et al., 2011, 2012) found that US produced a slight decrease in the solids content when applied to organic wastes of different origin before AD. In particular, in food waste samples sonicated at an  $E_s$  of 5000 kJ/kg TS, the total suspended solids and volatile suspended solids content was



**Figure 1.** Effect of specific energy and ultrasonication density on: (a–c) organic carbon; and (d–f) soluble carbohydrates solubilization.

found to decrease by 9% and 7%, respectively. A more pronounced reduction in VS concentration ( $\sim 24\%$  or higher) was observed in agricultural waste sonicated at  $E_s$  values in excess of 2500 kJ/kg TS.

To assess the solubilization degree of the substrate upon US, Figure 1 reports the evolution of dissolved organic carbon and soluble carbohydrates as a function of  $E_s$  and  $D_s$ . At the highest values of  $D_s$  (0.25 and 0.45 W/ml), the  $I_p$  values varied in the ranges 3.3–50.0% for TOC and 1.7–27% for carbohydrates. The corresponding ranges for  $I_s$  were 16.3–250% and 18–290%.

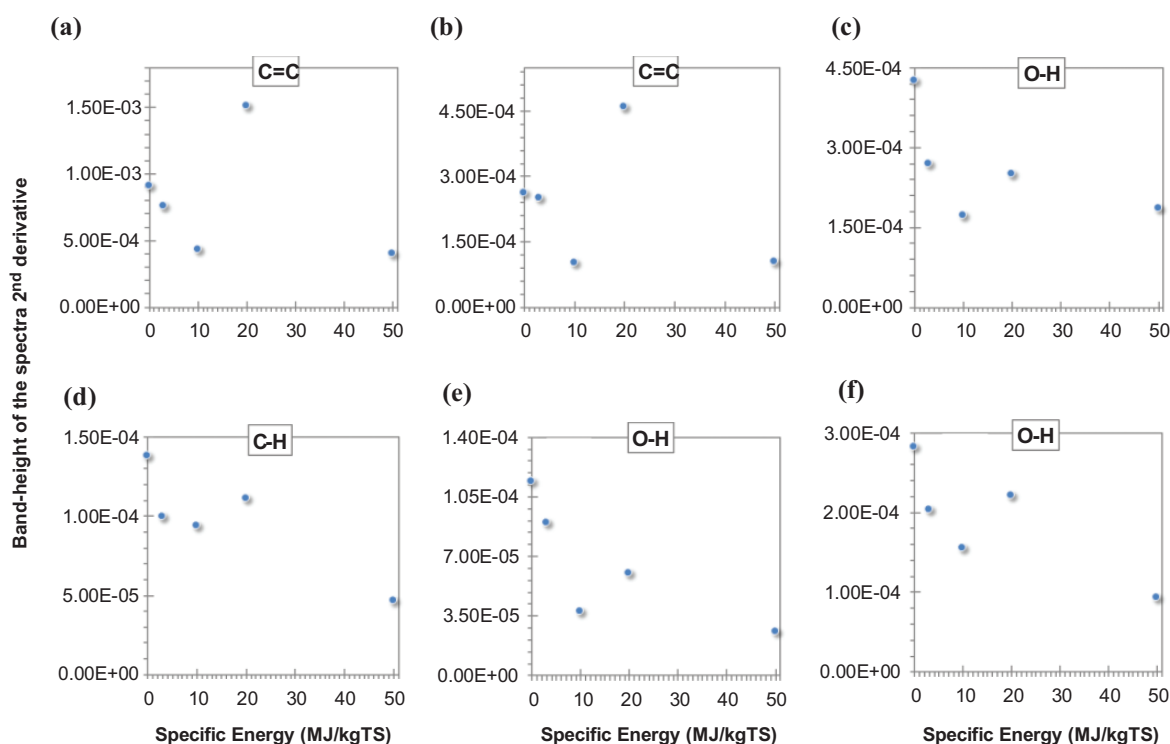
At the lowest  $D_s$  value (0.08 W/ml), the solubilization effect observed at increasing  $E_s$  values was less pronounced, with  $I_p$  varying in the range 1.4–16.3% for TOC and 4.3–7.3% for carbohydrates. Accordingly, the maximum values attained by  $I_s$  (83% for TOC and 80% for carbohydrates) were lower than those observed at 0.25 W/ml and 0.45 W/ml. At all  $D_s$  values, the relationship between the solubilization degree and  $E_s$  was roughly linear up to a threshold beyond which a plateau was reached (see Figure 1). This suggests that further increasing  $E_s$  is ineffective at promoting the disintegration of complex organic molecules. The mentioned threshold value for  $E_s$  was found to be affected by  $D_s$ . In particular, at a  $D_s$  value of 0.08 W/ml, the  $E_s$  threshold was 6000 kJ/kg TS, which increased to 30,000 kJ/kg TS at  $D_s = 0.25$  and 0.45 W/ml.

Moreover, the effect of  $D_s$  on the solubilization yield was less pronounced at lower  $E_s$  values ( $< 6000$  kJ/kg TS), as depicted in

Figure 1. This can be explained considering that, at the greatest specific energies, higher densities produce a larger number of transient bubbles, which could exert stronger cavitation forces, in turn fostering the breakdown of complex molecules. This has also been reported in previous studies on sonicated sewage sludge (Grönroos et al., 2005; Show et al., 2007).

At the highest  $D_s$  investigated (30,000 kJ/kg TS), an  $I_{p(TOC)}$  of 38.6% was observed, corresponding to an  $I_{p(COD)}$  of 37.0, which was comparable with that observed by Elbeshbishy et al. (2011), equal to 27%. Under such conditions,  $E_s = 3192$  kJ/kg TS and  $D_s$  of 0.6 W/ml, the  $I_{p(COD)}$  value reported by Gianico et al. (2013) ( $\sim 2\%$ ) was significantly lower than the value (5.6%) obtained in our study at  $E_s = 3000$  kJ/kg TS. This is related to the initial composition of the substrate, which plays a key role in dictating the degree of solubilization. Stemming from this, the comparison among  $I_p$  values obtained by different authors should be carried out carefully, as the results depend on the specific experimental conditions adopted as well as the substrate characteristics.

An increase in the dissolved organic nitrogen from approximately 350 mg ammonium ( $\text{N-NH}_4^+$ )/l (raw digestate) up to  $\sim 1200$  mg  $\text{N-NH}_4^+$ /l (samples sonicated at 0.25 W/ml and  $E_s$  in the range 500–50000 kJ/kg TS) was observed. Since the ammonia content was not significantly modified by US, it can be argued that, under the tested operating conditions, US was able to produce only a partial disintegration of the complex



**Figure 2.** Spectra band heights in the second-derivative mode as a function of ultrasonication energies.

N-containing molecules (i.e., proteins), while the liberation of simple monomers was negligible.

### Changes in fibers structure of US substrates

The FT-IR spectroscopy was used to identify the molecular changes that occurred in the digestate as a result of US. Digestate samples sonicated at 3000, 10,000, 20,000, and 50,000 kJ/kg TS at a  $D_s$  of 0.25 W/ml were analyzed and compared to the unsonicated digestate. The complex mixture of different chemical components in the digestate causes peak overlapping and suppression in the FT-IR spectra. Thus, a mathematical approach based on the calculation of the second derivative of transmittance was used to enhance the resolution of bands that were not clearly identifiable in the original spectra. The relationships between the band heights of the spectra in the second-derivative mode and the US energies are reported in Figure 2. The decrease in the band heights observed in the 1558 and 1569  $\text{cm}^{-1}$  peaks, which is due to the aromatic ring vibration, was related to the modification of the lignin aromatic structure induced by US (Corredor et al., 2009; Koutsianitis et al., 2015; Sun et al., 2005). In particular, for such wave numbers the band heights decreased as  $E_s$  increased, with the exception of the substrate sonicated at  $E_s = 20,000$  kJ/kg TS. These findings show that a partial delignification of the original substrate occurred as a result of US (see Figure 2(a) and Figure 2(b)).

The band at 1637  $\text{cm}^{-1}$  was associated with the H–O–H stretching vibration of adsorbed water in carbohydrates (Chen et al., 2011). The decrease in band heights (see Figure 2(c)) was particularly significant for  $E_s$  values between 3000 and

10,000 kJ/kg TS, suggesting that US produced a modification of the holocellulose structure.

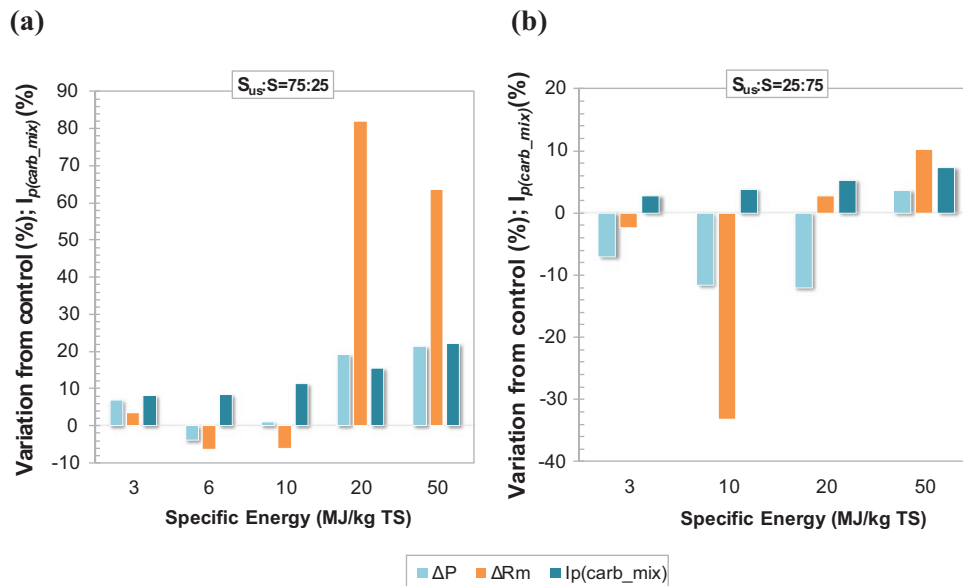
The band at 2985  $\text{cm}^{-1}$  in the FT-IR spectra was associated with the C–H vibration of  $\text{CH}_2$  and  $\text{CH}_3$  groups of cellulose and lignin components (Gastaldi et al., 1998; Koutsianitis et al., 2015). The data reported in Figure 2(d) show that a significant reduction in the content of such groups occurred for the substrate sonicated at 50,000 kJ/kg TS.

Finally, the results reported in Figure 2(e) and Figure 2(f) related to the broad band in the 3600–3000  $\text{cm}^{-1}$  provide information about the existence of hydrogen bonds in O–H groups (Taherdanak and Zilouei, 2014), which are commonly associated to the crystalline structure and the degree of intermolecular regularity (or the crystallinity) of cellulose (Harmsen and Huijgen, 2010; Koutsianitis et al., 2015; Oh et al., 2005). For the analyzed samples, a remarkable decrease in the intensity of the peak associated to O–H groups was observed as  $E_s$  increased, suggesting that US affected the proportion between crystalline and amorphous forms of cellulosic materials.

### Effect of US on $\text{CH}_4$ yields and kinetics

The results of the different AD experiments are summarized in terms of percentage variation from the control sample of the  $\text{CH}_4$  production yield ( $\Delta P$ ) and kinetic parameters ( $\Delta R_m$ ,  $\Delta t_{95}$ ) as well as carbohydrates solubilization yield ( $I_{p(\text{carb\_mix})}$ ) calculated according to equation (3):

$$I_{p(\text{carb\_mix})} (\%) = \frac{sX_{\text{mix}} - sX_0}{X_0 - sX_0} \times 100 \quad (3)$$



**Figure 3.** Percentage variation of methane production yield and kinetics and  $I_{p(\text{carb\_mix})}$  between the tested mixtures and the control samples ( $D_s = 0.25$  W/ml).

where  $X$  is the concentration of carbohydrates for the control ( $X_0$ ) and the  $S_{us}+S$  mixture ( $X_{mix}$ ), and the prefix  $s$  indicates the corresponding soluble fraction.

The results in Figure 3(a) and Figure 3(b) emphasize the combined effect of  $E_s$  and  $S_{us}:S$  ratio on the degree of substrate solubilization as well as on the yield ( $P$ ) and kinetics ( $R_m$ ) of  $\text{CH}_4$  production. The inspection of the parameters of the Richards' bacterial growth model (equation (2)) indicated, for  $S_{us}:S = 75:25$  (see Figure 3(a)), an appreciable increase in  $\text{CH}_4$  production compared to the control run (19.3% at  $E_s = 20,000$  kJ/kg TS and 21.3% at  $E_s = 50,000$  kJ/kg TS). For lower  $E_s$  values the observed change in  $\text{CH}_4$  production was either less significant or not appreciable, with  $\Delta P$  values of between -3.9 and 6.8%. Similar results were also obtained for the rate of  $\text{CH}_4$  production (Figure 3(a)), with  $R_m$  increasing by 82.0 and 63.7% compared to the control at  $E_s \geq 20,000$  kJ/kg TS.

It is also interesting to note that the overall duration of  $\text{CH}_4$  production (as conventionally represented by  $t_{95}$ ) was not significantly affected by US. In fact, at higher US energies, despite  $R_m$  being larger than for the control run,  $t_{95}$  remained virtually unchanged. The results obtained for the kinetic parameters may be interpreted in the sense that US produced to some extent simpler structures from the original substrate, allowing a faster initial hydrolysis phase as mirrored by the increased  $R_m$  values. However, the final stages of the digestion process were likely to be dominated by the degradation of more complex components that had evidently been poorly affected by the US pre-treatment. This conclusion may also be supported by the evidence gained from the FT-IR analyses, which indicated that cellulose and lignin structures, although reduced as a consequence of US, were still present in the sonicated samples.

The results reported in Figure 3(b) show that at a  $S_{us}:S$  ratio of 25:75, in agreement with previous results (Boni et al., 2016), no gain in the maximum  $\text{CH}_4$  production compared to the control test

was evident. Conversely, a slight reduction was observed for the mixtures containing the substrate sonicated at  $E_s$  of 3000–20,000 kJ/kg TS. Such a reduction may tentatively be ascribed to the abovementioned effect of biomass inactivation upon US coupled with an only moderate degree of carbohydrates solubilization (2.7–5.2%) produced by the treatment. At  $E_s = 50,000$  kJ/kg TS, the  $\text{CH}_4$  production yield was slightly increased ( $\sim 3.7\%$ ) from that of the control run, possibly as a result of the fact that the higher degree of substrate solubilization obtained at high US energies was capable of counterbalancing the biomass inactivation effect caused by US. Notwithstanding this, for  $S_{us}:S = 25:75$  no appreciable effect of US on either the  $\text{CH}_4$  production yield or the degradation kinetics could be identified (Figure 3(b)). On the basis of the results obtained, the hydrolysis stage of the AD process appeared to be positively affected by the US treatment applied when the content of soluble carbohydrates was at least 12% higher than that in the original substrate (see Figure 3(a)).

Table 3 provides a summary of the performance reported in previous studies on anaerobic degradability of solid waste as a result of US operated at low frequencies ( $< 50$  kHz). However, the comparison of the results from different studies is limited by the different substrate characteristics, the experimental methods adopted (in some cases not fully detailed), as well as the way the results are reported. Often, the operating parameters of US are used with no normalization, but their influence on the process performance may be lower than that exerted by other factors. In the studies focusing on the role played by the substrate properties on the effect of US on the AD process, only TS concentrations  $> 50$  g/l were considered.

It is evident from Table 3 that a limited number of studies has been carried out to evaluate the ability of US to increase the biodegradability/bioavailability of recalcitrant organic compounds remaining in the digestate downstream of AD, as it has been more widely regarded as a method for substrate pre-treatment.

**Table 3.** Summary of the performance of anaerobic digestion (AD) of solid waste as result of ultrasonication (US), as reported in previous studies.

| Substrate  | Operating conditions |          |                       |                                  |                                   |                        |                        |  |   |               | Reference |                       |                     |
|--|----------------------|----------|-----------------------|----------------------------------|-----------------------------------|------------------------|------------------------|--|---|---------------|-----------|-----------------------|---------------------|
|  | US treatment         |          |                       |                                  |                                   | AD                     |                        |  |   |               |           |                       |                     |
|  | T (°C)               | TS (g/l) | D <sub>s</sub> (W/ml) | E <sub>s</sub> (kJ/kgTS)         | AD                                | i <sub>p,COO</sub> (%) | i <sub>p,TKN</sub> (%) | i <sub>p,carb.</sub> (%)                           | Variation from control (%)                          | AD yield      |           | AD kinetics           |                     |
| <b>Post-treatment</b>  |                      |          |                       |                                  |                                   |                        |                        |  |   |               |           |                       |                     |
| Mixture of organic wastes of a food industry, silage energy crops, olive husks, manure   | < 30°C               | 83       | 0.08                  | 500                              | Batch reactor<br>T = 37 ± 2°C     | —                      | —                      | 4.3  | —   | —             | —         | This study            |                     |
|  |                      |          | 0.25                  | 500                              |                                   | 5.5                    | 6.9                    | 2.4  | —   | —             | —         |                       |                     |
|  |                      |          | 0.45                  | 500                              |                                   | 4.7                    | —                      | 1.7  | —   | —             | —         |                       |                     |
|  |                      |          | 0.08                  | 3000                             |                                   | —                      | —                      | 5.4  | —   | —             | —         |                       |                     |
|  |                      |          | 0.25                  | 3000                             | *Sus: S = 25:75<br>Sus: S = 75:25 | 5.6                    | 18.1                   | 8.3  | -7.0 (CH <sub>4</sub> )<br>6.8 (CH <sub>4</sub> )   | -2.4<br>3.4   | —         | —                     |                     |
|  |                      |          | 0.45                  | 3000                             |                                   | 11.1                   | —                      | 6.5  | —   | —             | —         |                       |                     |
|  |                      |          | 0.08                  | 6000                             |                                   | —                      | —                      | 7.3  | —   | —             | —         |                       |                     |
|  |                      |          | 0.25                  | 6000                             | Sus: S = 75:25                    | 10.4                   | 25.5                   | 8.6  | -3.9 (CH <sub>4</sub> )                             | -6.3          | —         | —                     |                     |
|  |                      |          | 0.45                  | 6000                             |                                   | 12.5                   | —                      | 14.0   | —   | —             | —         | —                     |                     |
|  |                      |          | 0.08                  | 10,000                           |                                   | —                      | —                      | 4.6  | —   | —             | —         | —                     |                     |
|  |                      |          | 0.25                  | 10,000                           | Sus: S = 25:75<br>Sus: S = 75:25  | 19.1                   | 24.1                   | 12.7   | -11.6 (CH <sub>4</sub> )<br>1.0 (CH <sub>4</sub> )  | -33.1<br>-6.2 | —         | —                     |                     |
|  |                      |          | 0.45                  | 10,000                           |                                   | 26.9                   | —                      | 14.1   | —   | —             | —         | —                     |                     |
|  |                      |          | 0.08                  | 20,000                           |                                   | —                      | —                      | 6.4  | —   | —             | —         | —                     |                     |
|  |                      |          | 0.25                  | 20,000                           | Sus: S = 25:75<br>Sus: S = 75:25  | 32.8                   | 26.3                   | 18.3   | -12.1 (CH <sub>4</sub> )<br>19.3 (CH <sub>4</sub> ) | 2.8<br>82.0   | —         | —                     |                     |
| Organic wastes of a food industry  |                      |          | 0.45                  | 20,000                           |                                   | 27.5                   | —                      | 18.7   | —   | —             | —         |                       |                     |
|  |                      |          | 0.08                  | 30,000                           |                                   | —                      | —                      | —  | —   | —             | —         |                       |                     |
|  |                      |          | 0.25                  | 30,000                           |                                   | 34.5                   | —                      | 26.9   | —   | —             | —         |                       |                     |
|  |                      |          | 0.45                  | 30,000                           |                                   | 37.0                   | —                      | 26.0   | —   | —             | —         |                       |                     |
|  |                      |          | 0.08                  | 50,000                           |                                   | —                      | —                      | —  | —   | —             | —         |                       |                     |
|  |                      |          | 0.25                  | 50,000                           | Sus: S = 25:75<br>Sus: S = 75:25  | 43.1                   | 47.9                   | 26.9   | 3.7 (CH <sub>4</sub> )<br>21.3 (CH <sub>4</sub> )   | 10.2<br>63.7  | —         | —                     | (Boni et al., 2016) |
|  |                      |          | 0.45                  | 50,000                           |                                   | 33.6                   | —                      | 20.8   | —   | —             | —         | —                     |                     |
|  |                      |          | 0.05                  | 500                              | Batch reactor<br>T = 37 ± 2°C     | 2.8                    | —                      | 3.0  | —   | —             | —         | —                     |                     |
|  |                      |          | 0.05                  | 1000                             |                                   | 3.7                    | —                      | 4.0  | —   | —             | —         | —                     |                     |
|  |                      |          | 0.05                  | 6000                             |                                   | 9.1                    | —                      | 8.9  | 8.6 (CH <sub>4</sub> )<br>6.6 (CH <sub>4</sub> )    | 23.0<br>2.5   | —         | —                     |                     |
|  |                      |          | 0.05                  | 10,000                           | *Sus: S = 25:75<br>Sus: S = 75:25 | 12.5                   | —                      | 10.8   | 18.5 (CH <sub>4</sub> )                             | 56.0          | —         | —                     |                     |
|  |                      |          | 0.05                  | 15,000                           |                                   | 12.5                   | —                      | 12.0   | —   | —             | —         | —                     |                     |
|  |                      |          | 0.05                  | 20,000                           | Sus: S = 25:75<br>Sus: S = 75:25  | 16.3                   | —                      | 13.7   | -1.4 (CH <sub>4</sub> )<br>19.1 (CH <sub>4</sub> )  | 10.2<br>52.7  | —         | —                     |                     |
|  |                      |          | 0.05                  | 30,000                           |                                   | 16.9                   | —                      | 14.7   | —   | —             | —         | —                     |                     |
|  |                      | 0.05     | 50,000                | Sus: S = 25:75<br>Sus: S = 75:25 | 22.1                              | —                      | 21.3                   | -6.5 (CH <sub>4</sub> )<br>25.4 (CH <sub>4</sub> ) | 4.4<br>48.1   | —             | —         | (Cesaro et al., 2014) |                     |
| Organic Fraction of Municipal Solid Waste digestate (TS=25 g/l)<br>Lignocellulosic material and dried distilled grain with solubles digestate<br>TS=16.9 g/l | None                 | 50–100   | 0.372                 | 2102                             | Unspecified                       | —                      | —                      | —  | 46 (biogas)<br>60 (biogas)<br>71 (biogas)           | —             | —         |                       |                     |
|  |                      |          |                       | 4219                             |                                   | —                      | —                      | —  | —   | —             | —         |                       |                     |
|  |                      |          |                       | 6291                             |                                   | —                      | —                      | —  | —   | —             | —         |                       |                     |
|  |                      |          |                       | 2264                             |                                   | —                      | —                      | —  | —   | —             | —         |                       |                     |
|  |                      |          | 4581                  |                                  | —                                 | —                      | —                      | —  | —   | —             |           |                       |                     |
|  |                      |          | 6858                  |                                  | —                                 | —                      | —                      | —  | —   | —             |           |                       |                     |

(continued)

Table 3. (continued)

| Substrate  | Operating conditions |          |                       |                          |   |               |                       |                       |   |                            | Biodegradability enhancement (for substrate solubilization) |                       |                         | AD kinetics                   |          |                            |           |                         |
|--|----------------------|----------|-----------------------|--------------------------|---|---------------|-----------------------|-----------------------|---|----------------------------|---|-----------------------|-------------------------|-------------------------------|----------|----------------------------|-----------|-------------------------|
|  | US treatment         |          |                       |                          |   | AD            |                       |                       |   |                            | I <sub>pCOD</sub> (%)                                       | I <sub>pTKN</sub> (%) | I <sub>pcarb.</sub> (%) | Variation from control (%)    | AD yield | Variation from control (%) | Reference |                         |
|  | T (°C)               | TS (g/l) | D <sub>s</sub> (W/ml) | E <sub>s</sub> (kJ/kgTS) | Batch reactor   | Recycle ratio | I <sub>pCOD</sub> (%) | I <sub>pTKN</sub> (%) | I <sub>pcarb.</sub> (%)   | Variation from control (%) |   |                       |                         |                               |          |                            |           |                         |
| Digestate from a domestic wastewater treatment plant<br>TS=12.6 g/l            | Ambient              | 12.6     | 0.60                  | 14,286                   | Batch reactor<br>T = 37°C                                     | —             | —                     | —                     | 60 (CH <sub>4</sub> )<br>74 (CH <sub>4</sub> )<br>78 (CH <sub>4</sub> ) | —                          | —   | —                     | —                       | (Garoma and Pappaterra, 2018) |          |                            |           |                         |
| Manure digestate (MAN)<br>TS=98.5 g/l  | < 20°C               | 64.3     | 0.13                  | 3000                     | Batch reactor<br>T = 37 ± 1°C<br>F: M (on VS-basis) = 0.8     | 3.9           | —                     | —                     | —   | —                          | —   | —                     | —                       | (Somers et al., 2018)         |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.2      | —                          | —         | -0.7 (biogas)           |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.5      | —                          | —         | 1.6 (biogas)            |
| Potato waste digestate (POT)<br>TS=140 g/l                                     | None                 | 33.5     | 0.4                   | 9000                     | Unspecified   | 10.8          | —                     | —                     | —   | —                          | —   | —                     | —                       | —                             |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.2      | —                          | —         | 3.0 (biogas)            |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.5      | —                          | —         | -10.3 (biogas)          |
| Mixed organic waste digestate (OW)<br>TS=193.8 g/l                             | None                 | 83.9     | 0.2                   | 15,000                   | Unspecified   | 16.2          | —                     | —                     | —   | —                          | —   | —                     | —                       | —                             |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.2      | —                          | —         | 4.4 (biogas)            |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.5      | —                          | —         | 8.6 (biogas)            |
| Pre-treatment Organic Fraction of Municipal Solid Waste and sewage sludge      | None                 | 90 ± 30  | 0.4                   | 90,692 ± 4,534           | Semi-continuous reactor<br>T = 35 ± 40°C                      | 13.6          | —                     | —                     | —   | —                          | —   | —                     | —                       | (Cesaro et al., 2012)         |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.2      | —                          | —         | —                       |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.5      | —                          | —         | —                       |
| Lignocellulosic material and dried distilled grain with solubles<br>TS=930 g/l | None                 | 50       | 0.372                 | 2835                     | Unspecified   | 19.5          | —                     | —                     | —   | —                          | —   | —                     | —                       | (Cesaro et al., 2014)         |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.2      | —                          | —         | 3 (biogas)              |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 0.5      | —                          | —         | 11 (biogas)             |
| Hog manure<br>TS: 93 g/l<br>sCOD/TCOD=0.38                                     | < 30°C               | 93       | Unspecified           | 5000                     | Batch reactor<br>T = 37°C<br>F/M = 4 gCOD/gVSS                | 7.0           | 0.7                   | —                     | —   | —                          | —   | —                     | —                       | (Elbeshbishy et al., 2011)    |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 250      | —                          | —         | 11.7 (CH <sub>4</sub> ) |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 500      | —                          | —         | 28.0 (CH <sub>4</sub> ) |
| Chicken manure<br>TS: 50 g/l   | Room temperature     | 50       | 0.22                  | 288                      | Batch reactor<br>T = 38°C<br>F/M = 0.1 (w/w)                  | 11.0          | 16.7                  | —                     | —   | —                          | —   | —                     | —                       | (Braeutigam et al., 2014)     |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 2500     | —                          | —         | 26.9 (CH <sub>4</sub> ) |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 5000     | —                          | —         | 30.6 (CH <sub>4</sub> ) |
| Food wastes<br>TS: 60 g/l  | None                 | 60       | 0.2                   | 6000                     | Hybrid plug flow digester<br>T=27 ÷ 30°C<br>OLR = 500 gVS/m3d | 16.0          | 12.1                  | —                     | —   | —                          | —   | —                     | —                       | (Rasapoor et al., 2016)       |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 10,000   | —                          | —         | 10.9 (CH <sub>4</sub> ) |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 21,000   | —                          | —         | 16.3 (CH <sub>4</sub> ) |
| Organic fraction of municipal solid waste                                      | None                 | 60       | 0.2                   | 6000                     | Batch reactor<br>T = 37 ± 1°C<br>F/M = 0.78 (VS basis)        | 24.0          | 33.5                  | —                     | —   | —                          | —   | —                     | —                       | (Rasapoor et al., 2016)       |          |                            |           |                         |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 900      | —                          | —         | 19.9 (CH <sub>4</sub> ) |
|  |                      |          |                       |                          |   |               |                       |                       |   |                            |   |                       |                         |                               | 6000     | —                          | —         | 18.7 (CH <sub>4</sub> ) |

(continued)



Table 3. (continued)

| Substrate  | Operating conditions |                      |  |  |   |   |   |  |                            |   | AD yield | AD kinetics | Reference              |                            |
|--|----------------------|----------------------|--|--|---|---|---|--|----------------------------|---|----------|-------------|------------------------|----------------------------|
|  | US treatment         |                      |  |  |   | AD  |   |  |                            |   |          |             |                        | Variation from control (%) |
|  | T (°C)               | TS (g/l)             | D <sub>5</sub> (W/ml)                  | E <sub>s</sub> (kJ/kgTS)   | Batch reactor<br>T = 35°C   | i <sub>pCOD</sub> (%)                             | i <sub>pTKN</sub> (%)                             | i <sub>pcarb</sub> (%)                     | Variation from control (%) |   |          |             |                        |                            |
| Fruit and vegetable wastes   | None                 | 50                   | 0.6<br>0.110                           | 13,500<br>1175<br>2380<br>3560   | Batch reactor<br>T = 35°C   | —   | —   | —  | —                          | 13 (biogas)<br>29 (biogas)<br>80 (biogas)<br>63 (biogas)  | —        | —           | (Zeynali et al., 2017) |                            |
| Food waste<br>TS: 72.5 g/l   | < 30°C               | 50<br>50<br>80<br>80 | 1.5                                    | 18,000<br>27,000<br>5625<br>11,250<br>16,875   | Unspecified   | 5.05<br>10.76<br>15.02<br>13.75<br>28.00<br>42.37 | 5.12<br>11.57<br>17.88<br>21.95<br>20.79<br>46.86 | —  | —                          | —   | —        | —           | —                      | (Gadhe et al., 2014)       |
| Food waste<br>TS: 65.5 g/l   | < 30°C               | 65.5                 | 0.8<br>0.5<br>0.7<br>0.6<br>0.8<br>0.8 | 350<br>1200<br>3000<br>5500<br>15,000<br>23,000  | Unspecified   | 0.2<br>5.1<br>8.2<br>22.1<br>24.2<br>26.3         | —<br>—<br>—<br>—<br>—<br>—                        | 5.7<br>9.1<br>18.0<br>22.0<br>22.4<br>22.9 | —                          | —   | —        | —           | —                      | (Elbeshbishy et al., 2012) |
| Maize straw (MS) and dairy manure (DM)<br>TS <sub>MS</sub> : 893 g/l<br>TS <sub>MS</sub> : 222 g/l | None                 | 220                  | 0.25                                   | 189.39 (DM)<br>284.09 (DM)<br>378.79 (DM)<br>189.39 (MS)<br>284.09 (MS)<br>378.79 (MS) | Batch reactor<br>T = 37 ± 2°C<br>DMus: MS = 1:1 (w/w)<br>DMus: MS = 1:1 (w/w)<br>DMus: MS = 1:1 (w/w)<br>MSus: DM = 1:1 (w/w)<br>MSus: DM = 1:1 (w/w)<br>MSus: DM = 1:1 (w/w) | —   | —   | —  | —                          | 43.9 (biogas)<br>15.1 (biogas)<br>-38.7 (biogas)<br>51.5 (biogas)<br>69.7 (biogas)<br>27.8 (biogas) | —        | —           | (Zou et al., 2016)     |                            |

The observed gain in  $\text{CH}_4$  production is in agreement with the results of our previous study on a different substrate (Boni et al., 2016), while a larger increase in biogas production was attained by Cesaro et al. (2014) and Garoma and Pappaterra (2018), who used digestate as the substrate. The results here are comparable with those observed by other authors (Elbeshbishy et al., 2011; Rasapoor et al., 2016) when applying US as a pre-treatment, at approximately the same  $E_s$ .

In order to evaluate the fate of the original substrate during the process, the carbon mass balance at the end of the experiments was calculated. To this aim, the contributions of the following forms of carbon were accounted for (see Figure 4): (a) carbon (C) in the form of the analyzed metabolic products (C2–C7 VFAs); (b) residual C in both the soluble and the particulate forms (including C present as non-degraded organic compounds and/or additional metabolic products, as well as microbial cells); and (c) gasified C, in the form of  $\text{CH}_4$  and carbon dioxide ( $\text{CO}_2$ ) (for  $\text{CO}_2$ , the

amount dissolved in the liquid was also included). The term “balance” in Figure 4 represents the C mass that was apparently lost due either to inaccuracies in the analytical measurements or sample inhomogeneity and was thus required to close the materials balance. All contributions to the mass balance were calculated from direct measurements in the liquid and gaseous phases, with the exception of dissolved inorganic C. This was indirectly estimated using the chemical equilibrium model Visual Minteq based on  $\text{CO}_2$  solubility as a function of pH and temperature.

It was evident that most of the initial C mass was retained in the digestate as residual C (44–60% in particulate forms and 17–33% as soluble species). The amount of C gasified as  $\text{CH}_4$  was always found to account for a low fraction of the initial TOC (7–9%), corresponding to 138–174 NI  $\text{CH}_4/\text{kg TOC}$ . It is worth mentioning that 3–4% of initial TOC was associated with the measured metabolic products, which clearly suggests that the VFAs in the system were completely degraded at the end of the process; in other words, although a high organic load was adopted, no inhibitory phenomena related to the potential accumulation of VFAs were found to occur during the experiments.

### Effect of milling and US on anaerobic degradability of digestate

In order to further exploit US as a feasible treatment option for complex substrates to enhance their anaerobic degradability, a combined treatment based on milling and US was investigated. This stemmed from the consideration that the size of complex organic materials is expected to influence US efficiency. On the basis of the results discussed in the previous subsection that indicated that only  $E_s$  values in excess of 10,000 kJ/kg TS positively affected the AD process, the combined treatment was performed at an  $E_s$  of 20,000 kJ/kg TS.

The milled and subsequently sonicated substrate displayed a higher degree of TOC dissolution compared with the sonicated-only substrate under the same US conditions. Conversely, the proteins and carbohydrates contents were not found to be affected by the treatment to any statistically significant degree (Figure 5(a)).

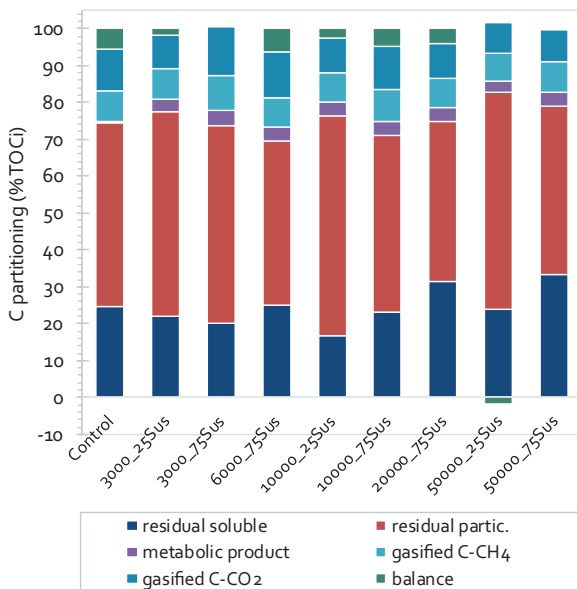


Figure 4. Carbon mass balance for the experimental runs.

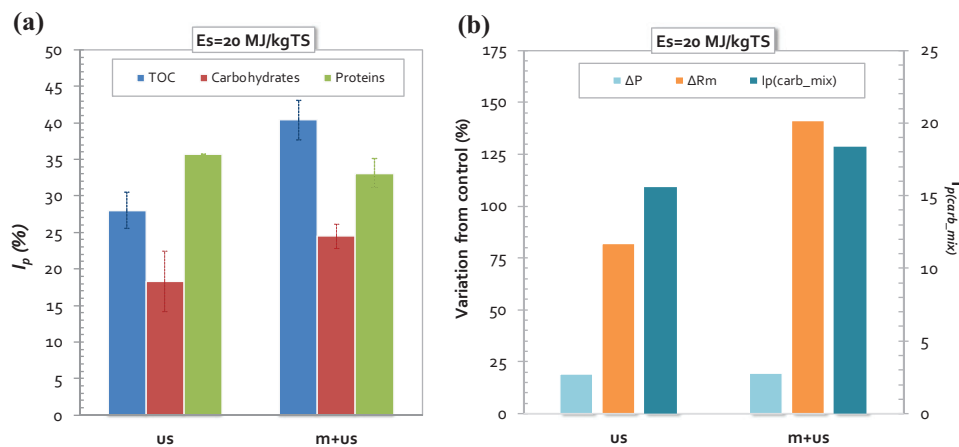


Figure 5. Influence of milling and ultrasonication combination on: (a)  $I_p(\text{TOC})$ ,  $I_p(\text{Carbohydrates})$  and  $I_p(\text{proteins})$  (a) and  $I_p(\text{carb\_mix})$  as well as percentage variation of methane  $\text{CH}_4$  production yield; and (b) kinetics between the tested mixtures and the control samples at  $E_s = 20,000 \text{ kJ/kgTS}$  and  $D_s = 0.25 \text{ W/ml}$ .

Starting from the results shown so far, the combined treatment appeared unable to produce the full disintegration of the lignocellulosic structure, since the solubilization yield of the mixture 20000\_75S<sub>(m+us)</sub> (expressed as  $I_{p(\text{carbohydrates})}$ ) was similar to that obtained for the corresponding mixture 20000\_75S<sub>us</sub>. At the same time (results not reported graphically), the change in the total process duration ( $t_{95}$ ) upon milling and US was not found to be statistically significant. This finding may explain why no significant gain in CH<sub>4</sub> yields is produced when US is applied on particles of smaller size. However, from Figure 5(b), we note that the application of milling prior to US positively affected the process kinetics. This result suggests that additional efforts should be devoted to improve the mechanical processing method prior to US, with a view to improving the overall degree of substrate solubilization.

## Conclusions

The application of post-US of AD digestate produced a significant increase in CH<sub>4</sub> yield (~20%) and maximum production rate (64–82%) at  $E_s \geq 20,000$  kJ/kgTS and  $D_s = 0.25$  W/ml. Although the outcomes of the study indicated an improvement in the digestibility of the substrate, the processing conditions adopted suggested significant energy requirements that would in fact impair the full-scale applicability of the process. The results appear to indicate that, in order to improve the energetic profile of the combined process and therefore the associated overall environmental sustainability, efforts should be directed to reducing the US energy applied. Thus, further insights into the effects of different combinations of  $D_s$  and  $E_s$  should be obtained, more specifically by evaluating the effect of increasing  $D_s$  as a strategy for reducing  $E_s$ .


## Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

## ORCID iD

Andreina Rossi  <https://orcid.org/0000-0001-7448-611X>

## References

- Boni MR, D'Amato E, Poletini A, et al. (2016) Effect of ultrasonication on anaerobic degradability of solid waste digestate. *Waste Management* 48: 209–217.
- Braeutigam P, Franke M and Ondruschka B (2014) Effect of ultrasound amplitude and reaction time on the anaerobic fermentation of chicken manure for biogas production. *Biomass and Bioenergy* 63: 109–113.
- Carlsson M, Lagerkvist A and Morgan-Sagastume F (2012) The effects of substrate pre-treatment on anaerobic digestion systems: A review. *Waste Management* 32: 1634–1650.
- Cesaro A, Naddeo V, Amodio V, et al. (2012) Enhanced biogas production from anaerobic codigestion of solid waste by sonolysis. *Ultrasonics Sonochemistry* 19: 596–600.
- Cesaro A, Velten S, Belgiorno V, et al. (2014) Enhanced anaerobic digestion by ultrasonic pretreatment of organic residues for energy production. *Journal of Cleaner Production* 74: 119–124.
- Chen W, Yu H, Liu Y, et al. (2011) Individualization of cellulose nanofibers from wood using high-intensity ultrasonication combined with chemical pretreatments. *Carbohydrate Polymers* 83: 1804–1811.
- Corredor DY, Salazar JM, Hohn KL, et al. (2009) Evaluation and characterization of forage Sorghum as feedstock for fermentable sugar production. *Biotechnology and Applied Biochemistry* 158: 164–79.
- Elbeshbishy E and Nakhla G (2011) Comparative study of the effect of ultrasonication on the anaerobic biodegradability of food waste in single and two-stage systems. *Bioresource Technology* 102: 6449–6457.
- Elbeshbishy E, Aldin S, Hafez H, et al. (2011) Impact of ultrasonication of hog manure on anaerobic digestability. *Ultrasonics Sonochemistry* 18: 164–171.
- Elbeshbishy E, Hafez H and Nakhla G (2012) Viability of ultrasonication of food waste for hydrogen production. *International Journal of Hydrogen Energy* 37: 2960–2964.
- Gadhe A, Sonawane SS and Varma MN (2014) Ultrasonic pretreatment for an enhancement of biohydrogen production from complex food waste. *International Journal of Hydrogen Energy* 39: 7721–7729.
- Garoma T and Pappaterra D (2018) An investigation of ultrasound effect on digestate solubilization and methane yield. *Waste Management* 71: 728–733.
- Gastaldi G, Capretti G, Focher B, et al. (1998) Characterization and properties of cellulose isolated from the Crambe abyssinica hull. *Industrial Crops and Products* 8: 205–218.
- Gianico A, Braguglia CM, Mescia D, et al. (2013) Ultrasonic and thermal pretreatments to enhance the anaerobic bioconversion of olive husks. *Bioresource Technology* 147: 623–626.
- Gogate PR, Sutkar VS and Pandit AB (2011) Sonochemical reactors: Important design and scale up considerations with a special emphasis on heterogeneous systems. *Journal of Chemical & Engineering Data* 166: 1066–1082.
- Grönroos A, Kyllönen H, Korpijärvi K, et al. (2005) Ultrasound assisted method to increase soluble chemical oxygen demand (SCOD) of sewage sludge for digestion. *Ultrasonics Sonochemistry* 12: 115–120.
- Harmen P and Huijgen W (2010) Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. *Energy* 1–49.
- Hendriks TWM and Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology* 100: 10–18.
- Iskhalieva A, Yimmou BM, Gogate PR, et al. (2012) Cavitation assisted delignification of wheat straw: A review. *Ultrasonics Sonochemistry* 19: 984–993.
- Khanal SK, Grewell D, Sung S, et al. (2007) Ultrasound applications in wastewater sludge pretreatment: A review. *Critical Reviews in Environmental Science and Technology* 37: 277–313.
- Koutsianitis D, Mitani C, Giagli K, et al. (2015) Properties of ultrasound extracted bicomponent lignocellulose thin films. *Ultrasonics Sonochemistry* 23: 148–155.
- Lindner J, Zielonka S, Oechsner H, et al. (2015) Effects of mechanical treatment of digestate after anaerobic digestion on the degree of degradation. *Bioresource Technology* 178: 194–200.
- Mbaye S, Dieudé-Fauvel E and Baudez JC (2014) Comparative analysis of anaerobically digested wastes flow properties. *Waste Management* 34: 2057–2062.
- Menardo S, Gioelli F and Balsari P (2011) The methane yield of digestate: Effect of organic loading rate, hydraulic retention time, and plant feeding. *Bioresource Technology* 102: 2348–2351.
- Molinuevo-Salces B, Gómez X, Morán A, et al. (2013) Anaerobic codigestion of livestock and vegetable processing wastes: Fibre degradation and digestate stability. *Waste Management* 33: 1332–1338.
- Oh SY, Yoo I, Shin Y, et al. (2005) FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydrate Research* 340: 417–428.
- Pilli S, Bhunia P, Yan S, et al. (2011) Ultrasonic pretreatment of sludge: a review. *Ultrasonics Sonochemistry* 18: 1–18.
- Rasapoor M, Adl M, Baroutian S, et al. (2018) Energy performance evaluation of ultrasonic pretreatment of organic solid waste in a pilot-scale digester. *Ultrasonics Sonochemistry* 51: 517–525.

- Rasapoor M, Ajabshirchi Y, Adl M, et al. (2016) The effect of ultrasonic pretreatment on biogas generation yield from organic fraction of municipal solid waste under medium solids concentration circumstance. *Energy Conversion and Management* 119: 444–452.
- Sambusiti C, Monlau F, Ficara E, et al. (2015) Comparison of various post-treatments for recovering methane from agricultural digestate. *Fuel Processing Technology* 137: 359–365.
- Sawatdeenarunat C, Surendra KC, Takara D, et al. (2014) Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Biore-source Technology* 178: 178–186.
- Show KY, Mao T and Lee DJ (2007) Optimisation of sludge disruption by sonication. *Water Research* 41: 4741–4747.
- Somers MH, Azman S, Sigurnjak I, et al. (2018) Effect of digestate disintegration on anaerobic digestion of organic waste. *Bioresource Technology* 268: 568–576.
- Sun X, Xu F, Sun R, et al. (2005) Characteristics of degraded cellulose obtained from steam-exploded wheat straw. *Carbohydrate Research* 340: 97–106.
- Taherdanak M and Zilouei H (2014) Improving biogas production from wheat plant using alkaline pretreatment. *Fuel* 115: 714–719.
- Wojnowska-Baryła I, Bernat K and Sartowska S (2018) Biological stability of multi-component agri-food digestates and post-digestates. *Waste Management* 77: 140–146.
- Zeynali R, Khojastehpour M and Ebrahimi-Nik M (2017) Effect of ultrasonic pre-treatment on biogas yield and specific energy in anaerobic digestion of fruit and vegetable wholesale market wastes. *Sustainable Environment Research* 27: 259–264.
- Zheng Y, Zhao J, Xu F, et al. (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science* 42: 35–53.
- Zou S, Wang X, Chen Y, et al. (2016) Enhancement of biogas production in anaerobic co-digestion by ultrasonic pretreatment. *Energy Conversion and Management* 112: 226–235.
- Zwietering MH, Jongenburger I, Rombouts FM, et al. (1990) Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* 56: 1875–1881.