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## Research Paper

# Bio-physical pre-treatments in anaerobic digestion of organic fraction of municipal solid waste to optimize biogas production and digestate quality for agricultural use

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## ABSTRACT

This study optimized the anaerobic digestion (AD) of separated collected organic fractions of municipal solid waste (OFMSW) to produce energy and digestate as biofertilizer. Due to OFMSW's partial recalcitrance to degradation, enzymatic (UPP2, MCPS, USC4, USE2, *A. niger*) and physical (mechanical blending, heating, hydrodynamic cavitation) pre-treatments were tested. Experimental and modeling approaches were used to compare AD performance regarding energy sustainability and digestate quality. Digestate was separated into solid and liquid fractions, and then chemically and physically characterized by investigating the nutrient release mechanisms. Principal Component Analysis was applied, equally weighing energy and digestate productions. Unlike previous studies focusing only on biogas, this study evaluated the effects of pre-treatments on both biogas and digestate production, viewing AD as a biorefinery process for urban waste valorization. Results showed that all pre-treatments were energetically sustainable, but enzymatic pre-treatments yielded digestates richer in nutrients (increase of 80% N, 200% P and 150% K as compared to OFMSW) and with greater organic matter degradation compared to physical pre-treatments. The liquid fraction of digestate from enzymatic pre-treatments had higher nutrient concentrations, while those from physical pre-treatments had more balanced nutrient content, making them more suitable for fertigation.

## 1. Introduction

The world population growth is increasing food and energy demands as well as waste production, causing global warming, energy safety issues, and consumption of non-renewable resources. Circular bioeconomy plays a strategic role, providing a new platform for more sustainable chemicals and energy production, aiming to replace fossil fuel consumption (Perišić et al. 2022; Feng et al. 2023). Anaerobic digestion (AD) is a key process to proceed toward this aim, producing

renewable energies and promoting the adoption of a sustainable agricultural system (Millati et al. 2023). AD is a well-established biochemical process performed under anaerobic conditions, able to stabilize and convert organic wastes originating from agriculture, industry residues, water treatment, or the organic fraction of municipal solid waste (OFMSW), into high-added value products such as biogas and digestate (Panigrahi and Dubey 2019; Naresh Kumar et al. 2022; Feng et al. 2023). Raw biogas mainly consists of methane (50–75 %), carbon dioxide (25–50 %), and smaller amounts of nitrogen (2–8 %) (Gao et al. 2020),

**Abbreviations:** AD, anaerobic digestion; CAS, mesophilic digestate of cow-agricultural sludge; WAS, mesophilic digestate of wastewater activated sludge; HC, hydrodynamic-cavitation; COD, Chemical Oxygen Demand; OFMSW, organic fraction municipal solid waste; TOC, total organic carbon; TN, total nitrogen; TS, total solids; VS, volatile solids; ICP-OES, inductively coupled plasma optical emission spectrometry; <sup>13</sup>C-CPMAS NMR, <sup>13</sup>C solid-state cross-polarization magic-angle-spinning nuclear magnetic resonance; FTIR, Fourier Transform Infrared; PCA, Principal Component Analysis.

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and volatile organic compounds (VOC) with a variable and relative abundance of functional groups according to AD conditions and feedstock (Randazzo et al. 2022). In the last decade, in the European Union (EU) biogas was partially employed as fuel for heat and electricity generation producing 7.38 TWh and 61 TWh, respectively, and partially upgraded to produce biomethane, injected into the natural gas network or used in transport vehicles (Eurostat, 2023). The other product of AD is digestate, which is rich in plant nutrients and low-degradable organic compounds, and can be employed as a biofertilizer (Alburquerque et al. 2012; Monlau et al. 2015b). Aside from the normative requirements for its use in agriculture, digestate may present a high potential for the large content of macronutrients, such as nitrogen (N) and phosphorus (P) in both organic and inorganic forms, offering a range of compounds that cover plant needs during a longer time compared to mineral fertilizers (Baştabak and Koçar 2020). In addition to macronutrients, digestate may contain meso and micronutrients that can improve soil fertility and functionality, compensating for the progressive loss of soil nutrients, exacerbated by climate change (Vaneckhaute et al. 2019). The suitability of digestate application in agriculture depends on its physical and chemical characteristics, strongly influenced by the type of feedstock feed in the AD reactor (Möller and Müller 2012; Nkoa 2014). Among the organic wastes employed for AD, the organic fraction of municipal solid waste represents an important secondary renewable feedstock, being constantly available during the whole year (Tyagi et al. 2018). In the EU, over 58 million tons of OFMSW (131 kg/inhabitant) are annually generated with an associated market value estimated at 132 billion euros (Eurostat, 2022). OFMSW management is hence a tough challenge. The most frequently adopted technologies are landfilling and incineration, which are not suitable to address the current energy and environmental requirements (Kusch-Brandt 2019), because they produce GHG emissions that must be captured and stored. Both the waste and GHG emissions from these processes pose management challenges. In contrast, AD, according to the circular economy principles, treats waste as secondary raw materials, producing valuable products: energy from biogas and digestate for use as fertilizer. OFMSW-based biorefinery represents a new waste recovery and valorization strategy, aimed at promoting a circular economy and environmental benefits (Panigrahi and Dubey 2019). OFMSW is a heterogeneous material with components ranging from labile to recalcitrant to degradation. Therefore, the application of pre-treatment processes to help organic matter hydrolysis and conversion into methane can improve AD performance (Tyagi et al. 2018; Atelge et al. 2020; Ebrahimian et al. 2023). Pre-treatments can be classified as physical, biological, chemical, and combinations of all of them (Atelge et al. 2020). Pre-treatments break down organic matter by enhancing microbial interactions and improving hydrolysis rates and nutrient accessibility (Deepanraj et al. 2017).

Lately, the most studied physical pre-treatments are mechanical, thermal and microwaves (Mancini and Raggi 2021). Here, enzymatic pre-treatments are considered since they can improve microbial activity in subsequent biological treatments, preventing inhibitor accumulation in the substrate (El Gnaoui et al. 2022) and decreasing energy costs.

According to a previous study, the most efficient physical pre-treatments for OFMSW are mechanical, thermal, and hydrodynamic cavitation (Demichelis et al. 2023), while among the enzymatic pre-treatments the best options are the commercial enzymes UPP2, MPC5, USC4, USE2 and *A. niger* (Demichelis et al. Under revisions). Although several publications have reported the application of pretreatments on OFMSW to promote anaerobic digestion, most of them focus on the quantity and quality of produced biogas, while, to the best of our knowledge, there is no available study focusing simultaneously on biogas and digestate production (Agarwal et al. 2022). Herein, the aim of this study is to assess the effect of pre-treatments on OFMSW for AD, considering both the biogas and the digestate quality and composition, to boost their application as energy vectors and soil biofertilizers. Particular attention was also given to the liquid fraction of the digestate for its potential employment in fertigation cropping systems. To reach

this aim, pre-treated OFMSW was subjected to AD and the produced biogas and digestate or its separated liquid fraction were chemically and physically characterized to identify the best AD configuration for both energy and bi.

ofertilizer production, through an experimental and modelling analysis approach.

## 2. Materials and methods

### 2.1. OFMSW characteristics and pre-treatments

The organic fraction of municipal solid waste (OFMSW) was provided by San Carlo S.p.A (Fossano, Italy), which is a biological organic waste treatment plant performing wet thermophilic anaerobic digestion. The OFMSW is derived from a separate collection of MSW. The representativeness of the sample of OFMSW tested in the experimental campaign was proved by a chemical and physical characterization of OFMSW over two years (2020–2021) reported in Table S1.

Physical and enzymatic pre-treatments on OFMSW were selected according to the most promising configurations investigated by Demichelis et al. (2023). The physical pre-treatments included mechanical, thermal, and hydrodynamic cavitation. The enzymatic pre-treatments were performed with four commercial enzymes (UPP2, MPC5, USC4, USE2, Biopract ABT GmbH, Berlin, Germany) and with *Aspergillus niger* cellulase. A detailed description of the pre-treatments is reported in the Supporting Information. The sample names corresponding to each pre-treatment are summarized in Table 1.

### 2.2. Anaerobic digestion

Anaerobic digestion was carried out in duplicate on physically and enzymatically pre-treated OFMSW in batch feed conditions at 37 °C in a 55 L thermostatic water-bath (Julabo-Corrio-C). AD was performed at 6 % TS of OFMSW in 0.5 L Pyrex glass bottles, with a working volume of 0.4 L, according to Valero et al. (2016). Each digester was manually shaken four times per day and AD tests interrupted when the daily biogas rate was below 1 % of the total volume of biogas produced up to that time (Angelidaki et al. 2009). Each digester was connected by 6 mm Teflon tubes (PTFE, Germany) to 1 L Tedlar gas bag in which the biogas was collected. The significance was evaluated with one-way ANOVA considering  $p < 0.5$ .

The tested AD configurations combining pre-treatments and inoculum effects were 22 (tested in duplicate), comprising 12 physically and 10 enzymatically pre-treated OFMSW. AD tests were performed on each type of pre-treated OFMSW, using both digestates from waste active sludge (WAS) and cow agricultural sludge (CAS) as inocula. Then, to evaluate the efficiency of the pre-treatments, 4 configurations of AD on non-pre-treated OFMSW were performed (in duplicate) with inoculum CAS and WAS at two S:I ratios (1:1 and 2:1) based on volatile solids (VS). Furthermore, 2 configurations of AD tests of only inoculum WAS and CAS (without OFMSW) were performed (in duplicate) to evaluate the contribution of the inocula. A detailed description of the anaerobic digestion procedure and biogas analysis is reported in the Supporting Information.

To evaluate the energetic sustainability of the AD configuration, the energy sustainable index (ESI) was calculated according to the Eq. (1). ESI provides an initial assessment of the energetic sustainability of a technology, and should be higher than 1 to be associated with a sustainable process.

$$ESI = \frac{Q_{pro}}{Q_s} \quad (1)$$

where  $Q_{pro}$  is the energy produced from AD tests considering that methane content equals to 7.2 kWh/m<sup>3</sup> according to Rillo et al. (2017).  $Q_s$  is the system thermal energy measured in kWh and corresponds to the

**Table 1**

Analyses of total solids (TS) determined on the fresh matter (FM), volatile solids (VS), total C content ( $C_{\text{tot}}$ ), volatile solids/ $C_{\text{tot}}$  ratio and pH performed on OFMSW and digestates achieved after different pre-treatments and AD with WAS or CAS inoculum. The results are reported as average  $\pm$  standard deviation.

Pre-treatment	Sample	TS (% FM)	VS (% TS)	$C_{\text{tot}}$ (%)	VS/ $C_{\text{tot}}$	pH
–	OFMSW	11.0 $\pm$ 0.12	83.90 $\pm$ 0.10	42.61 $\pm$ 0.82	1.97	6.7
<b>Digestate after enzymatic pre-treatment</b>						
UPP2, 35 °C, 2 h, S:I=2:1	WAS_UPP2	3.10 $\pm$ 0.01	71.80 $\pm$ 0.80	43.850 $\pm$ 0.060	1.64	8.3
	CAS_UPP2	4.20 $\pm$ 0.06	71.00 $\pm$ 0.40	45.97 $\pm$ 0.42	1.54	7.9
MPCS, 35 °C, 2 h, S:I=2:1	WAS_MPCS	4.00 $\pm$ 0.05	69.70 $\pm$ 0.10	49.50 $\pm$ 0.11	1.41	5.8
	CAS_MPCS	5.50 $\pm$ 0.20	73.0 $\pm$ 1.7	51.9 $\pm$ 2.7	1.41	5.9
USC4, 25 °C, 2 h, S:I=2:1	WAS_USC4	4.30 $\pm$ 0.03	70.20 $\pm$ 0.70	51.8 $\pm$ 1.4	1.35	5.7
	CAS_USC4	4.50 $\pm$ 0.06	70.30 $\pm$ 0.40	46.7 $\pm$ 1.7	1.50	5.7
USE2, 25 °C, 2 h, S:I=2:1	WAS_USE2	4.10 $\pm$ 0.01	69.3 $\pm$ 1.0	52.6 $\pm$ 1.5	1.32	5.7
	CAS_USE2	4.30 $\pm$ 0.01	71.4 $\pm$ 2.0	50.37 $\pm$ 0.73	1.42	5.7
A. niger, 35 °C, 2 h, S:I=2:1	WAS_Niger	6.10 $\pm$ 0.20	75.90 $\pm$ 0.10	49.6 $\pm$ 1.3	1.53	5.7
	CAS_Niger	5.10 $\pm$ 0.10	74.2 $\pm$ 1.2	48.5 $\pm$ 1.1	1.53	5.7
<b>Digestate after physical pre-treatment</b>						
Comminution, 25 °C, 15 min, S:I=2:1	WAS_MEC15	1.80 $\pm$ 0.98	83.7 $\pm$ 2.8	48.74 $\pm$ 0.23	1.72	7.3
	CAS_MEC15	1.67 $\pm$ 0.34	82.50 $\pm$ 0.10	48.60 $\pm$ 0.47	1.70	7.7
Cavitation, 50 °C, 10 min, S:I=1:1	WAS11_CAV50	1.36 $\pm$ 0.47	83.7 $\pm$ 1.2	46.6 $\pm$ 1.41	1.80	7.0
	CAS11_CAV50	1.09 $\pm$ 0.44	83.9 $\pm$ 1.8	48.04 $\pm$ 0.45	1.75	6.8
Cavitation, 50 °C, 10 min, S:I=2:1	WAS21_CAV50	2.53 $\pm$ 0.18	79.20 $\pm$ 0.10	44.800 $\pm$ 0.090	1.77	7.3
	CAS21_CAV50	2.25 $\pm$ 0.66	81.2 $\pm$ 1.7	44.35 $\pm$ 0.25	1.83	7.2
Cavitation, 25 °C, 10 min, S:I=2:1	WAS_CAV25	2.32 $\pm$ 2.0	81.10 $\pm$ 0.30	47.45 $\pm$ 0.11	1.71	6.8
	CAS_CAV25	2.86 $\pm$ 0.15	82.10 $\pm$ 0.10	47.23 $\pm$ 0.25	1.74	6.7
Heating, 120 °C, 15 min, S:I=2:1	WAS_TERM15	2.02 $\pm$ 0.800	80.60 $\pm$ 0.20	46.90 $\pm$ 0.14	1.72	7.0
	CAS_TERM15	2.11 $\pm$ 0.11	80.9 $\pm$ 1.4	47.56 $\pm$ 0.47	1.70	7.1
Heating, 120 °C, 45 min, S:I=2:1	WAS_TERM45	2.34 $\pm$ 0.070	81.00 $\pm$ 0.80	46.23 $\pm$ 0.21	1.75	7.3
	CAS_TERM45	2.03 $\pm$ 0.32	82.7 $\pm$ 1.1	48.05 $\pm$ 0.30	1.72	7.0

sum of the thermal energy required for heating the substrate ( $Q_{\text{sub}}$ ) according to Mehr et al. (2017) and the energy consumed to carry out the pre-treatments ( $Q_{\text{pre-treatment}}$ ). The detailed description of ESI calculation is reported in the Supporting Information.

### 2.3. Chemical analyses of OFMSW and digestates

The digestates were separated into solid and liquid fractions by centrifugation at 3500 rpm for 10 min. The two fractions were characterized separately. The data reported for the whole digestate were achieved by combining the results of the analyses performed on the liquid and solid fractions, considering their percentage in the starting material. The chemical analyses performed are: total solids (TS) and volatile solids (VS), determined by drying the samples at 105 °C overnight and at 650 °C for 4 h, respectively; pH; carbon and sulfur content, quantified by elemental analyses; total and ammoniacal nitrogen, determined by the Kjeldahl method; nitrate content, total and soluble phosphorus (P), quantified spectrophotometrically, calcium (Ca), magnesium (Mg) and potassium (K), determined by inductively coupled plasma optical emission spectrometry. In addition,  $^{13}\text{C}$ -CPMAS NMR and Fourier Transform Infrared (FTIR) spectroscopy were performed. Complete methods are described in detail in the Supporting Information.

### 2.4. Data analysis

PCA was carried out by MATLAB R2014a (TheMathworks, Natick, MA, USA) using in-house-developed routines; graphical representations were carried out by Statistica v.7 (Statsoft Inc., Tulsa, OK, USA), and Excel 2016 (Microsoft Corporation, Redmond, WA, USA). PCA provides two tools for data analysis: the scores and the loading (both are detailed in the Supporting Information). To identify the best configurations, data were first pre-processed to calculate, for each configuration, its distance from the “optimal target result” for each variable separately. The “optimal target result” correspond to a condition showing the best possible values for all the variables characterizing the digestate and its liquid fraction, the values considered as the best ones are reported in Table S2. Therefore, for each configuration, the distance from the “optimal target result” was calculated, for each variable independently. For the variables showing, as the “optimal target result”, a range of values, the distance was calculated as the lowest between those calculated from both the extremes of the optimal target range. All distances were considered as absolute values. For both the digestate and its liquid fraction, the dataset consisted of each configuration characterized by the absolute values of the distances from the “best” condition that was also added to the dataset and characterized by distances all equal to zero. Both datasets were auto-scaled before performing PCA.

## 3. Results and discussion

### 3.1. OFMSW characteristics

As OFMSW can widely vary depending on the sampling period and location, the substrate utilized for the anaerobic digestion experiments was sampled and analyzed monthly for two years, as reported in Table S1. The pH, always comprised between 5.5 and 6.5, results suitable for the microorganism population involved in anaerobic digestion (Zamri et al. 2021). Bacteria require high humidity to propagate the AD, hence TS value is another important factor for biogas conversion. The biomass herein utilized presents slightly lower TS% content (9–11 %) as compared to the range of 15–50 TS% generally found in OFMSW (Zamri et al. 2021), thus being a promising substrate for AD. Also, the C/N ratio, ranging between 19 and 20, is suitable for AD (Tyagi et al. 2018).

### 3.2. Methane production and energetic sustainable index of anaerobic digestion

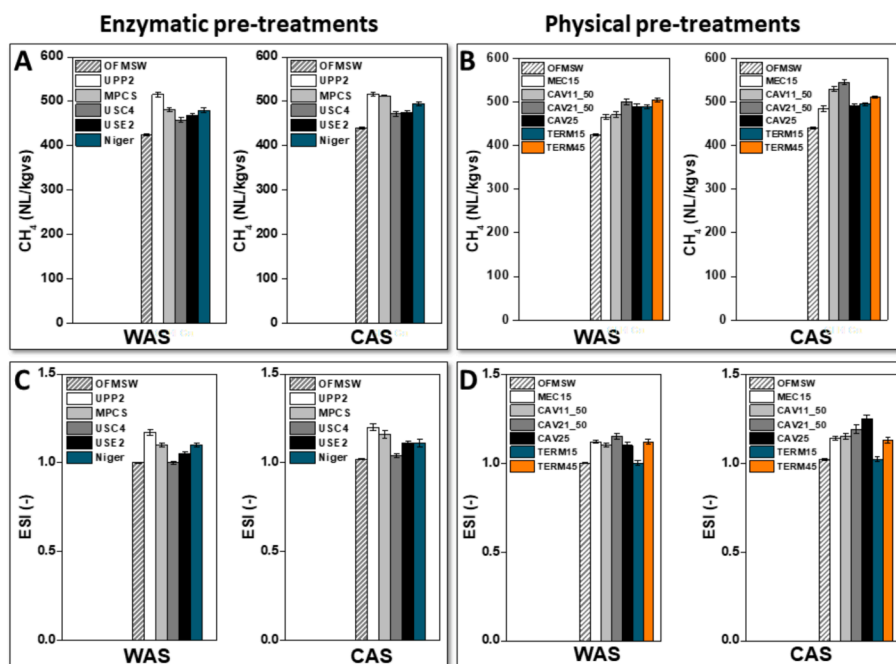
Anaerobic digestion was performed on (enzymatically and physically) pre-treated OFMSW, and non-pre-treated OFMSW as a reference to evaluate the efficiency of the pre-treatments. The energetic yields and performances are summarized in [Table S3](#), while [Table S4](#) reports the statistical difference between the achieved results. The methane production and energetic sustainable index are highlighted in [Fig. 1](#) to identify the most energetically sustainable configurations, e.g. the ones where the produced energy is higher than the consumed one ( $ESI > 1$  considering both pre-treatment and AD process). In this section, the performances of enzymatic and physical pre-treatments are discussed separately and then compared each other and with the non-pre-treated OFMSW.

Scrutinizing the statistical analysis, no significant differences have been detected between USC4 and USE2 with both inocula WAS and CAS, as well as between UPP2 and MPCS with inoculum CAS, and between UPP2 with WAS and CAS. The non-significant difference between USC4 and USE2 could be attributable to their similar composition (the only difference being the presence of protease in USE2), same dose (2 mL/100 g TS), and hydrolysis temperature and time. Also, the comparable results for UPP2 and MPC with inoculum CAS can be explained by their similar composition (differing only for the presence of protease in UPP2), same doses (1 mL/100 g TS), and hydrolysis temperature and time. Interestingly, UPP2 and USE2, which contain proteases, exhibited slightly higher  $CH_4$  than MPCS and USC4, respectively. This suggests that protease may contribute to the hydrolysis of proteins in OFMSW, thereby enhancing AD performance. However, UPP2 and MPCS achieved higher overall performance than USE2 and USC4, possibly due to the inhibitory effects of protein surfactants present in the latter. This finding agrees with [Chen et al. \(2008\)](#). AD performed with *A. niger* with inoculum WAS and CAS reached lower  $CH_4$  production than MPCS and UPP2. These results could suggest that a mix of enzymes could synergically cooperate to degrade complex substrates better than only one type of enzyme. For *A. niger*, the  $CH_4$  productions achieved with inocula WAS and CAS were not significantly different. In the enzymatic pre-treatment, the  $CH_4$  production and ESI values were consistent, as the

hydrolysis time was the same (2 h) for all enzymes and the temperature ranged between 25 °C (USC4 and USE4) and 35 °C (UPP2, MPCS, and *A. niger*).

Concerning the AD carried out after physical pre-treatments, no significant difference was detected. The slightly highest  $CH_4$  production was reached after hydrodynamic cavitation at 50 °C (CAS21\_CAV50 and CAS11\_CAV50), while CAS\_CAV25 resulted in the slightly most sustainable energetic configuration according to the ESI values. In physically pre-treated AD, the configurations achieving the highest  $CH_4$  production were not the same as those achieving the highest ESI value due to the energy costs associated with the pre-treatment. This contrasts with enzymatic pre-treatment, where these discrepancies were not observed due to low variation of temperature for the five enzymes. The  $CH_4$  surplus produced with hydrodynamic cavitation at 50 °C was employed to cover the pre-treatment energy need. The hydrodynamic cavitation at 50 °C achieves higher  $CH_4$  production than at 25 °C because, in the collapse phase, the highest temperature generates a more reactive environment, promoting a diffuse turbulence and heat exchange, both at macro and micro scales ([Calcio Gaudino et al. 2018](#)). However, the energy cost of working at 25 °C is lower than working at 50 °C. Similar considerations can be made about hydrodynamic cavitation at 50 °C and thermal pre-treatments at 120 °C performed for 15 and 45 min. The energy cost of keeping 120 °C for 15 and 45 min is higher than performing hydrodynamic cavitation at 50 °C for 10 min. It is of interest underling that the  $CH_4$  productions achieved by CAV25 and TERM15 were not significantly different (both with inocula WAS and CAS), but the ESI values were, due to the highest energy cost of thermal pre-treatment at 120 °C compared to hydrodynamic cavitation performed at 25 °C.

Regarding the thermal pre-treatment, the application time (15 vs 45 min) did not affect the final performance, with  $CH_4$  production in the range of 489–510 NL/kg VS. Both enzymatic and physical pre-treatments reached significantly ( $p < 0.05$ ) higher biogas and methane productions than AD performed with non-pre-treated OFMSW. In detail, all the AD performed with the five investigated enzymes increased the  $CH_4$  production and content by + 10–19 % compared to the AD carried out on the non-pretreated OFMSW, following the study of [Mutschlechner et al. \(2015\)](#). For the physical pre-treatments, the biogas



**Fig. 1.** Methane production (A and B), and energetic sustainable index (C and D) of anaerobic digestion performed on enzymatically (A and C) and physically (B and D) pre-treated OFMSW with WAS and CAS inoculum.

production of mechanically pre-treated OFMSW agrees with Coarita et al. (2020), and thermal pre-treatments with Chen et al. (2020).

The increase in biogas and methane production after pre-treatments is due to their capacity to degrade complex molecules thereby increasing the exposure of the biodegradable matter to microorganisms (Zhen et al. 2017).

Comparing enzymatic and physical pre-treatments no significant difference could be detected considering the CH<sub>4</sub> production and ESI values. However, the benefit of performing pre-treatments is calculated through the ESI, for which the energy produced must be higher than the energy consumed. Fig. 1 depicts that the slightly highest ESI values were obtained by CAS\_CAV25, followed by CAS21\_CAV50, and at the same rank position WAS\_CAV25 and then UPP2 with WAS and CAS and MPCs with CAS.

The ESI values achieved in the present study agree with other studies of AD carried out on pre-treated biomasses at the laboratory scale. In detail, the study of Ruggeri et al. (2015) obtained ESI between 0.5–1.0, proving that the increase of methane production after salts and ultrasound pre-treatments on olive oil and food waste cannot always energetically sustainable due to the high energy costs. The ESI values of the present study align with the review study of Gómez-Camacho et al. (2021), who obtained slightly higher ESI values for AD of OFMSW, as they considered also the energy saved due to the avoided end-of-life consumption of OFMSW. In the present study, the attention was only focused on the energy obtained and consumed by AD and pre-treatments.

In both physical and enzymatic pre-treatments, the origin of the inoculum had no significant effect ( $p < 0.05$ ).

### 3.3. Digestate characteristics

Biomass pre-treatment influences digestate composition and, consequently, its potential for the application as soil amendment or fertilizer (Al Seadi et al. 2013; Monlau et al. 2015a). The digestates produced after different OFMSW pre-treatments were thus physically and chemically analyzed. The content of total solids, volatile solids, and carbon in the OFMSW and in the digestates, as well as the pH values, are summarized in Table 1. Compared to the starting substrate, the TS in the digestate were generally halved by the enzymatic pre-treatments. The volatile solids decrease in the digestates from enzymatic pre-treatments and remained almost the same in the digestates from physical pre-treatments. This suggests that the enzymatic pre-treatments fostered a deeper organic matter degradation during anaerobic digestion. Both TS and VS values fall within the ranges reported in literature for OFMSW digestate: 1.7–12.7 % TS and 48–81 % VS (Cesaro 2021). The TS remaining after AD presented an increased carbon (C) content, as compared to OFMSW, with higher values in enzymatic pre-treatments. This can be attributed to the degradation of simple sugars and other monomers with the selective accumulation of lignin- and wax-derived compounds, which are recalcitrant to degradation and present a high carbon content. The volatile solids/C<sub>tot</sub> ratio of all digestates is lower than for the OFMSW, indicating a degradation of the organic biomass during AD. The lowest ratios were found in the digestates from enzymatic pre-treatments. Overall, the data in Table 1 point out that enzymatic pre-treatments promoted a more pronounced transformation of the substrate during AD, as compared to the physical pre-treatments. On the other hand, the inoculum (WAS or CAS) did not particularly influence the digestate characteristics.

The pH of OFMSW was neutral. At the beginning of anaerobic digestion, the pH rapidly drops due to the quick hydrolysis of polymers into sugars, amino acids and fatty acids. Later, when the monomers are consumed by methanogens to generate methane, the pH rises again to neutral (Macias-Corral et al. 2008; Anitha et al. 2015). The pH of the digestates achieved by physical pre-treatment results neutral, as expected according to literature (Cesaro 2021), indicating that the anaerobic digestion was complete. Considering the digestates from

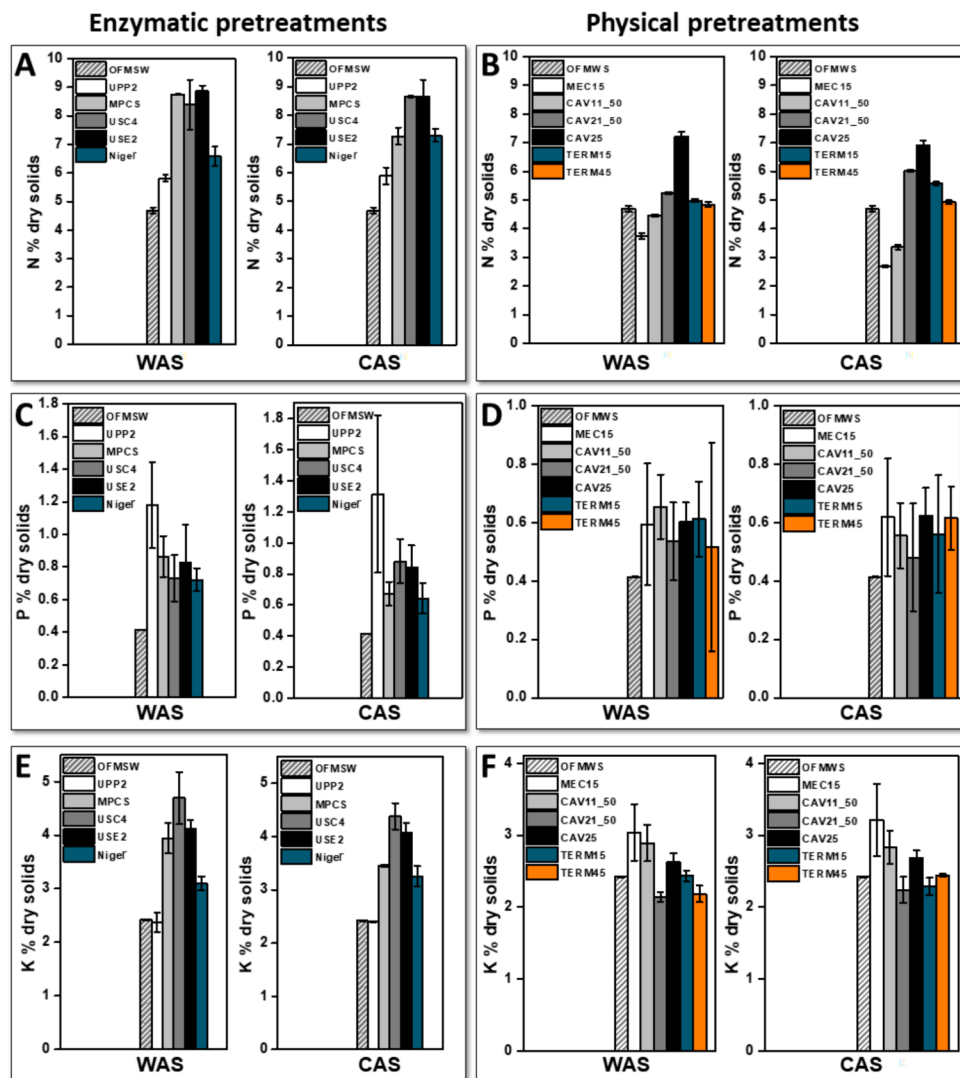
enzymatic pre-treatment, only UPP2 led to complete digestion, and the pH rose again after the first acidification processes. In the other cases, the pH remained low, indicating incomplete digestion. This result agrees with the highest CH<sub>4</sub> production measured with the enzyme mixture UPP2 and can be attributed to the presence of surfactants in the other enzyme mixture, which can inhibit the AD process.

The content of plant macronutrients N, P and K in the OFMSW and all the digestates was quantified and reported in Fig. 2. The quantity of these three elements is fundamental for the potential use of the products as fertilizers in agriculture (Li et al. 2016; Baştabak and Koçar 2020). As compared to the feedstock, N increases in all digestates obtained from enzymatic pre-treatment, and the MPCs, USC4 and USE2 enzyme mixtures allowed a more pronounced N increase than the others. In digestates obtained with physical pre-treatments, N increased significantly after cavitation at 50 and 25 °C and S:I=2:1, while it remained stable with S:I=1:1. The two thermal pre-treatments slightly increased the N content in the digestate, while the mechanical pre-treatment led to a slight decrease. The inorganic N contents (N-NH<sub>4</sub> and N-NO<sub>3</sub>) is specified in Figure S1. Ammonium is normally the prevailing N form in digestates (Czekala 2022), and strongly increased as compared to the starting feedstock. This could reduce its application in soil because high N-NH<sub>4</sub> amounts can be toxic for the plant and impact air quality due to ammonia emission from soil (Esteban et al. 2016; Czekala 2022). To avoid these two unwanted effects, the ammonium fraction in the digestate (normally comprised between 2 and 9.5 %) should be limited (Monlau et al. 2015b). Enzymatic pre-treatments generated digestates with N-NH<sub>4</sub> content < 6 %, with UPP2 treatment giving the lowest values (2.5 %, Fig. S1A), while all physical pre-treatments led to a very low N-NH<sub>4</sub> content, below 1.5 % (Fig. S1B). The nitrate content, representing the N form readily available for plants, is reported in Fig. S1C and D. Enzymatic pre-treatments did not bring major changes in the N-NO<sub>3</sub> content, except for MPCs, which led to an increase from 50 mg/kg in the OFMSW to 70–80 mg/kg in the digestate. Mechanical and cavitation pre-treatments at 50 °C with ratio S:I=1:1 decreased the nitrate content, while the other cavitations and the thermal treatment for 15 min increased the concentration as compared to OFMSW. The thermal pre-treatment for 45 min led to different results depending on the utilized inoculum: nitrate increased with WAS and decreased with CAS inoculum.

The P content (Fig. 2C and D) increased in all digestates, as compared to OFMSW (0.4 % P). Enzymatic pre-treatments allowed a more pronounced P increase in the digestate than physical ones. In particular, the UPP2 pre-treated digestate had a P content three times higher than OFMSW (1.2 % P). The different physical pre-treatments did not seem to influence the P content in the digestate, which remained similar in all samples (0.5–0.6 % P). Phosphorous in digestate can be present in various forms and have different accessibility for crops (Grigatti et al. 2015). The soluble inorganic P, e.g. in the form of H<sub>2</sub>PO<sub>4</sub> directly available for plants, was quantified and presented in Figure S2. Among the enzymatic pre-treatments, MPCs, USC4, USE2 and *A. niger* generated digestates with a higher amount of soluble P, as compared to OFMSW, while UPP2 led to a lower soluble P content. Considering the physical pre-treatments, most of them reduced the soluble inorganic P, except the mechanical treatment with WAS inoculum and the thermal treatment for 45 min with CAS inoculum, which slightly increased the P solubilization.

Enzymatic digestates resulted richer in K than OFMSW (K content increased from 2.5 to 3–5 %), except the ones obtained with UPP2, where the K content did not change (Fig. 2E and F). A smaller rise in K content can also be seen in the mechanically pre-treated digestates (3.2 % K), with cavitation at 50 °C with S:I=1:1 (2.9 % K) and cavitation at 25 °C with S:I=2:1 (2.8 % K). The inoculum type did not influence the K content in the digestates. All digestates present macronutrient contents within the range previously reported in literature (Cesaro 2021).

The content of mesonutrients S, Ca and Mg is summarized in Fig. 3. All digestates resulted richer in S than OFMSW (0.3 % S), especially the



**Fig. 2.** Content of primary macronutrients N (A and B), P (C and D) and K (E and F) of OFMSW and digestates obtained after enzymatic pre-treatments (A, C and E) and physical pre-treatments (B, D and F) with WAS and CAS inoculums. Results and error bars correspond to the average and standard deviation of measurements performed in triplicate.

ones obtained from the UPP2 pre-treatment (0.8–0.9 % S). As compared to the feedstock (1.8 % Ca), the Ca concentration increased in all enzymatic digestates (3–5 % Ca), while a slight Ca content decrease was observed in the digestates from physical pre-treatments (0.5–1.5 % Ca). WAS\_TERM45 was the only exception, where the Ca concentration increased to 2.1 %. The Mg content increased in all digestates, as compared with OFMSW, from 0.2 to 0.3–0.4 %.

The C/N ratio, indicator of organic stability for soil application (Guilayn et al. 2019), can widely vary in OFMSW digestates (Cesaro 2021). The C/N ratio of the herein produced digestates falls within the ranges reported in literature and is reported in Table S5. In comparison with OFMSW, the C/N ratio of the digestates from enzymatic pre-treatments decreased from 9 to 5–8. This is due to the significant selective accumulation of N and corresponding loss of C in these digestates, which makes them good candidates to provide N to the plant. A similar trend can be noticed for the digestates achieved after cavitation at 25 °C (C/N=6.5 and 6.8 with WAS and CAS, respectively), while in the other digestates from physical pre-treatment the C/N ratio resulted the same as OFMSW or even increased.

The mass ratio between macronutrients in the digestates is of primary importance to evaluate the potential of these materials for the use as soil amendments or fertilizers (Table S5). The N/P ratio was quite

well balanced in all digestates (4–12). The lowest N/P values were found in WAS\_UPP2 and CAS\_UPP2, where a high P content was determined (4.9 and 4.5, respectively), as well as in CAS\_MEC15 (4.3), presenting a low N content. On the other hand, the highest N/P values were relative to the digestates that were richest in N, e.g. WAS\_USC4, CAS\_Niger, CAS21\_CAV50, WAS\_CAV25 and CAS\_CAV25.

Digestates achieved after enzymatic pre-treatments presented an equilibrated Ca/Mg ratio, mainly ranging between 8 and 11. WAS\_Niger and CAS\_Niger had a higher Ca/Mg ratio (14.4 and 12.8, respectively), due to the lower Mg content. Digestates from physical pre-treatments showed, instead, a lower Ca/Mg ratio (1–7), due to the lower Ca content.

The Mg/K ratio, which is well balanced in fertilizers when ranging between 2 and 5, was very low in all digestates (0.08–0.22) due to the high content of K.

To investigate the structural composition of the feedstock and the produced digestates, all samples generated with CAS inoculum were analyzed by <sup>13</sup>C solid state NMR spectroscopy (Fig. 4). This analysis allows us to evaluate and compare the efficiency of the pre-treatments on the substrate degradation, and define which ones are the most impactful. In the OFMSW spectrum we can distinguish the typical signal pattern of cellulose, with a strong signal at 105 ppm, relative to the anomeric C, and the weaker signals between 60 and 90 ppm due to C2-

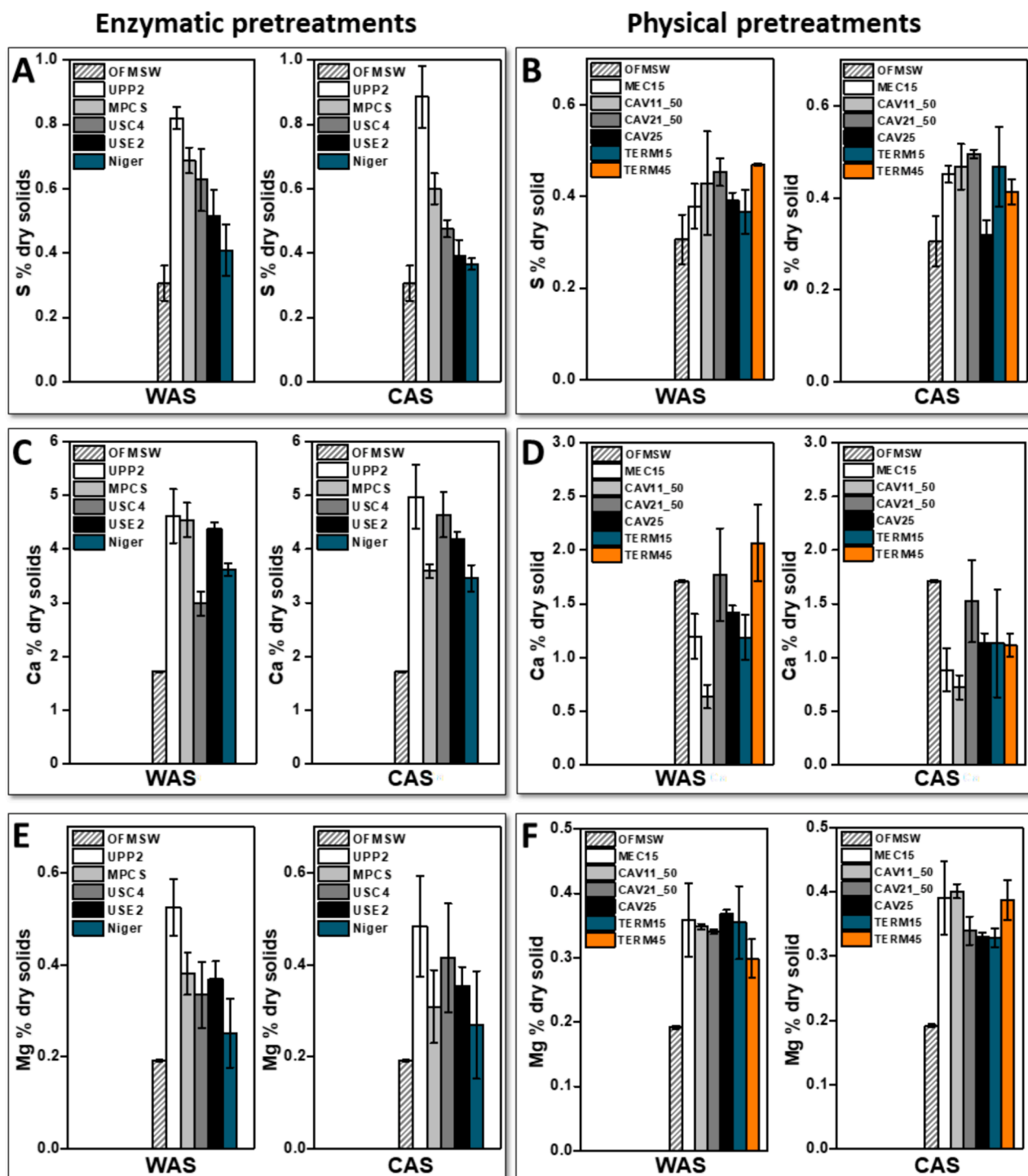


Fig. 3. Content % of mesonutrients S (A and B), Ca (C and D) and Mg (E and F) in OFMSW and digestates obtained after enzymatic pre-treatments (A, C and E) and physical pre-treatments (B, D and F) with WAS and CAS inoculums. Results and error bars correspond to the average and standard deviation of measurements performed in triplicate.

C5 of glucose monomers. OFMSW presented a small proportion of lignin, as deduced by the signal at 55 ppm due to methoxyl groups and by broader signals at 100–150 ppm, typical of aromatic C. Low intensity signals around 30 ppm, due to the hydrocarbon chain of fatty acids in lipids and waxes, indicated the low presence of this recalcitrant component in the original material (Adani 2009; Stutzenstein et al. 2018). After digestion, the spectra show a general increase of this region (0–46 ppm), as reported by the integration areas (Table S6). The same applies to the signals corresponding to the aromatic C (111–166 ppm).

This was followed by the concomitant decrease of cellulose (46–111 ppm). In all cases, except for the pre-treatment with the UPP2 enzyme, the signal corresponding to carboxyl groups (190–166 ppm) did not change with respect to the OFMSW. This confirms that the degradation process took place under anaerobic conditions, with consumption of sugars without an evident increase of oxidative degree. Among the various types of digestate from enzymatic pre-treatment, CAS\_MPCS shows the most marked degradation. Considering the digestates from physical pre-treatment, CAS\_CAV25 was poorly degraded, while the



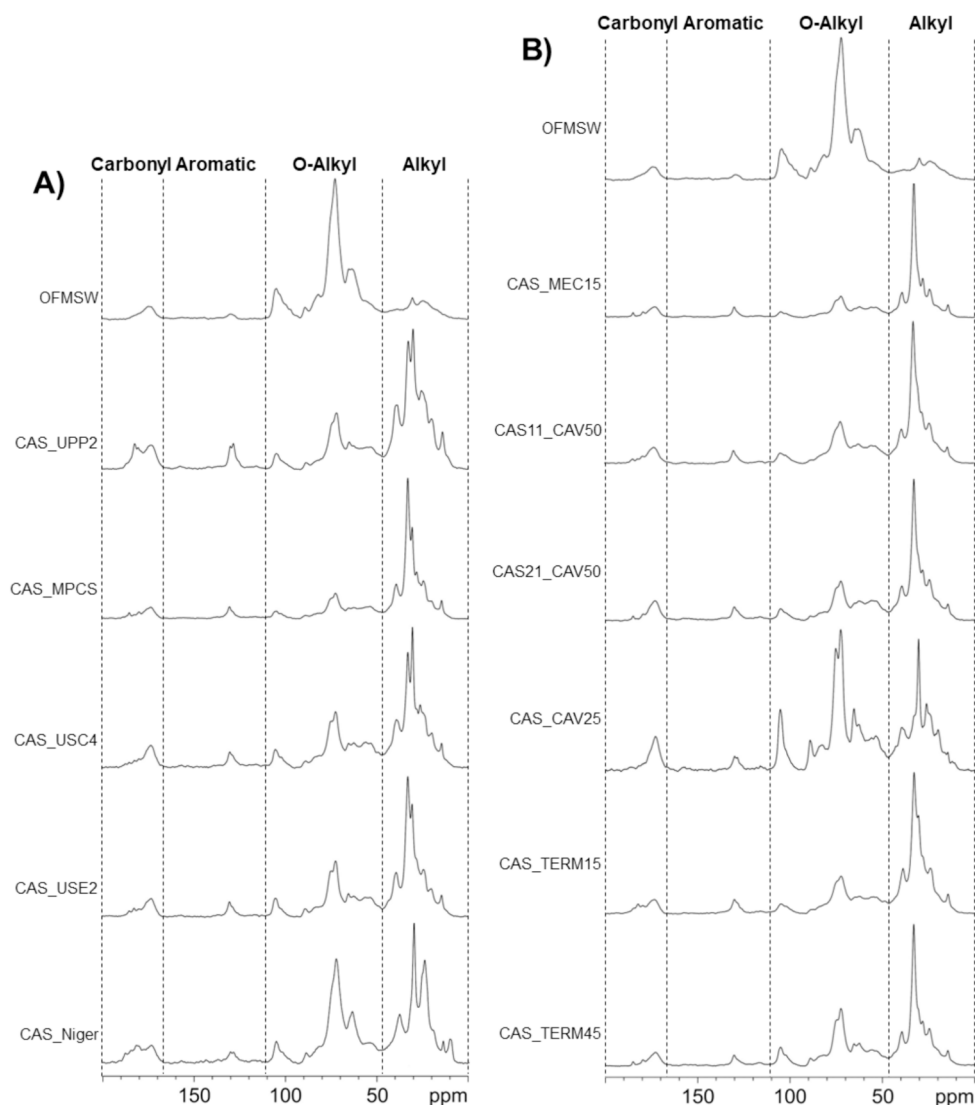


Fig. 4. Solid state  $^{13}\text{C}$  NMR spectra of OFMSW and digestates from A) enzymatic pre-treatments and B) physical pre-treatments using CAS inoculum.

most pronounced degradation is observed for CAS\_MEC15.

The differences in nutrient release can be attributed to the different mechanism of OFMSW modification by the pretreatments. Physical pre-treatments such as comminution and hydrodynamic cavitation can increase the physical fragmentation of cellulose and polymeric structures. Thermal treatment can modify the molecular conformation and increase water penetration, forming a gel that results more accessible to microbial degradation during AD. Depending on the time, temperature and process applied with the treatment, different amount of N, P, K, as well as S, Ca and Mg, can be released.

Enzymatic pre-treatments, on the other hand, promote the hydrolysis of cellulose, hemicellulose and proteins. The quantity and ratio of nutrients released by these processes depend on the combination of enzymes forming the commercial mixture.

The feedstock structural changes after different pre-treatments and AD were further monitored by FTIR spectroscopy, as shown in Figures S3 and S4. The FTIR spectra clearly show cellulose degradation, confirming what has already been observed with  $^{13}\text{C}$  NMR. The broad band at  $3430\text{--}3300\text{ cm}^{-1}$ , relative to the O–H bond stretching, presents an asymmetry due to the overlapping with the N–H stretching band, found at  $3300\text{ cm}^{-1}$ . The O–H signal, mainly due to cellulose, decreased in most of the digestate spectra, while the N–H signal remained constant. The cellulose degradation in the digestates is also proven by the decrease

in C–OH signal at  $1000\text{ cm}^{-1}$  (Rodríguez-Abalde et al. 2013). Contrarily, the lipids and waxes remained unaltered during AD, as shown by the increase in the digestates spectra of aliphatic  $-\text{CH}_3$  and  $-\text{CH}_2-$  signals at  $2900$  and  $2850\text{ cm}^{-1}$  (C–H stretching) and in the range  $1350\text{--}1450\text{ cm}^{-1}$  (C–H bending), as compared to the starting OFMSW. The aromatic C=C signal, found at  $1570\text{ cm}^{-1}$  and corresponding mainly to lignin, was also rising in the digestates as compared to the feedstock, again in agreement with the  $^{13}\text{C}$  NMR results.

### 3.4. Digestate liquid fraction characteristics

To facilitate the digestate handling and storage after anaerobic digestion, companies normally separate the solid from the liquid fraction (Monlau et al. 2015b; Czekala 2022). The solid fraction is generally stabilized by composting and utilized for soil amendment (Teglia et al. 2011), while the liquid fraction is normally disposed of or sent to wastewater treatment plants (Akhlar et al. 2017). Not only this is a waste of plant nutrients, but it also can drive to environmental pollution. Herein, conversely, the digestate liquid fraction was analyzed to evaluate its possible use for fertigation, a smarter management method in line with the principles of the circular economy.

Overall, enzymatic pre-treatments generated a liquid fraction richer in organic C ( $18\text{--}25\text{ g/L}$ ) than physical pre-treatments ( $2\text{--}8\text{ g/L}$ ), except

for the UPP2 enzyme, which produced a liquid fraction with three times less organic C (2–4 g/L) (Fig. 5A). The liquid fraction of digestate pre-treated with UPP2 also contained less N (0.9–1 g/L) than digestates generated by other enzymatic pre-treatments (2.4 g/L) (Fig. 5C).

Physically pre-treated samples showed organic C values lower than enzymatic samples, with the lowest ones obtained by mechanical treatment and cavitation with S:I=1:1 (2–4 g/L) (Fig. 5B). Nitrogen concentrations resulted similar for all samples (1–2 g/L), with the liquid fractions from mechanical treatment and cavitation with S:I=1:1 being the poorest in N (Fig. 5D).

Concentrations of inorganic N forms (ammonium and nitrate) are reported in Figure S5. As already seen in digestates before phase separation, N-NH<sub>4</sub> was the dominant form of N in all samples. Overall, the liquid fractions from physical pre-treatments had higher nitrate and lower ammonium contents than the ones obtained from enzymatic pre-treatments. Among the enzymatic pre-treatments, the most interesting for the application as fertilizer are UPP2, which generated a liquid fraction with similar nitrate content (0.8 mg/L), but less ammonium than the others, and MPCS, presenting the highest nitrate content. Considering the liquid fractions from physical pre-treatments, the ones generated by mechanical treatment and cavitation at 50 °C with S:I=1:1 are the poorest in both ammonium (0.5–1 g/L) and nitrate (3 g/L). Contrarily, cavitation at 50 °C with S:I=2:1 and at 25 °C generated the richest liquid fractions, with 1–1.5 g/L of ammonium and 8–10 g/L of nitrate.

Overall, the enzymatic pre-treatments generated digestate liquid fractions that are richer in P, K, Ca and Mg (Fig. 6). This is because, as previously mentioned, enzymatic pre-treatments led to more pronounced organic matter degradation and hence better solubilization of the nutrients, which end up in the liquid fraction.

Among the enzymatic pre-treatments, UPP2 derived liquid fraction contained the lowest P concentration (40–44 mg/L), while the other samples presented 200–300 mg/L. The P content in the liquid fractions

achieved after physical pre-treatments is less than half (maximum 60–80 mg/L), with mechanical treatment and cavitation at 50 °C with S:I=1:1 leading to the lowest P content (40–60 mg/L). The soluble inorganic fraction over the total P content (Figure S6) was higher in the digestates from enzymatic pre-treatment (almost reaching 100 % in some cases) as compared to the ones achieved by physical pre-treatments (reaching a maximum of 30 %). To evaluate the potential of the digestate liquid fraction for the use as fertilizers, we can compare it with the Hoagland solution, a well-known hydroponic nutrient solution (Hoagland 1933). The Hoagland solution contains 31 mg/L of inorganic P, and all the liquid fractions from enzymatic pre-treatment could be diluted to reach the same value. The samples achieved after physical pre-treatment, instead, would need to be integrated with inorganic P.

The K concentration in UPP2 derived samples (0.5–0.7 g/L) was lower than the other enzymatically achieved liquid fractions (1.7–2 g/L) (Fig. 6C and D). In the samples from physical pre-treatments, the K concentration reached a maximum of 500 mg/L for WAS\_CAV21\_50 and WAS\_TERM45, and a minimum of 100 mg/L for WAS\_CAV11\_50 and CAS\_CAV11\_50. Considering that the Hoagland solution contains 234 mg/L of K, all the liquid fractions achieved after enzymatic pre-treatments, as well as some of the ones obtained with physical pre-treatment, could be diluted to reach the same K concentration.

As previously seen for the K content, the Ca and Mg concentration was lower in the UPP2 derived sample than in the other liquid fractions from enzymatic pre-treatment. A similar Ca content was found in the liquid fractions from physical pre-treatment (200–300 mg/L) and corresponded to the concentration in the Hoagland solution (200 mg/L Ca). For Mg, among the physically pre-treated liquid fractions, the ones prepared via cavitation at 50 °C with S:I=2:1 and at 25 °C contain 47–56 mg/L, analogously to the Hoagland solution (48 mg/L Mg), while in the other samples Mg is present in lower concentrations and would need to be integrated (20–40 mg/L).

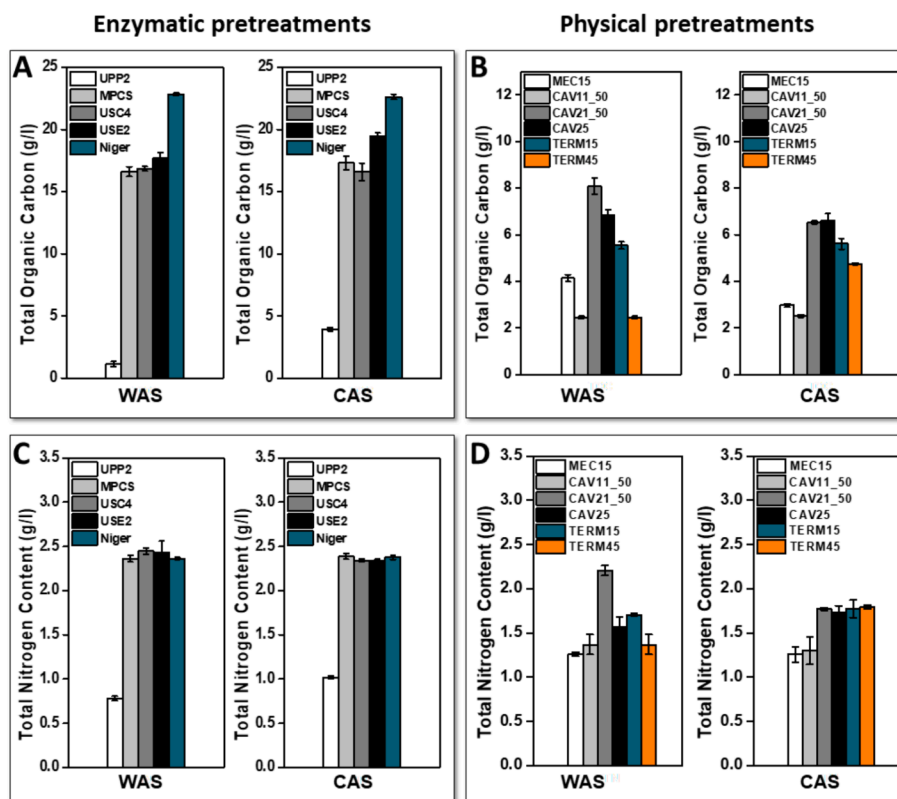


Fig. 5. Organic C (A and B) and total nitrogen (C and D) in the liquid fraction of digestates obtained after enzymatic pre-treatments (A, C) and physical pre-treatments (B, D) with WAS and CAS inoculums. Results and error bars correspond to the average and standard deviation of measurements performed in triplicate.

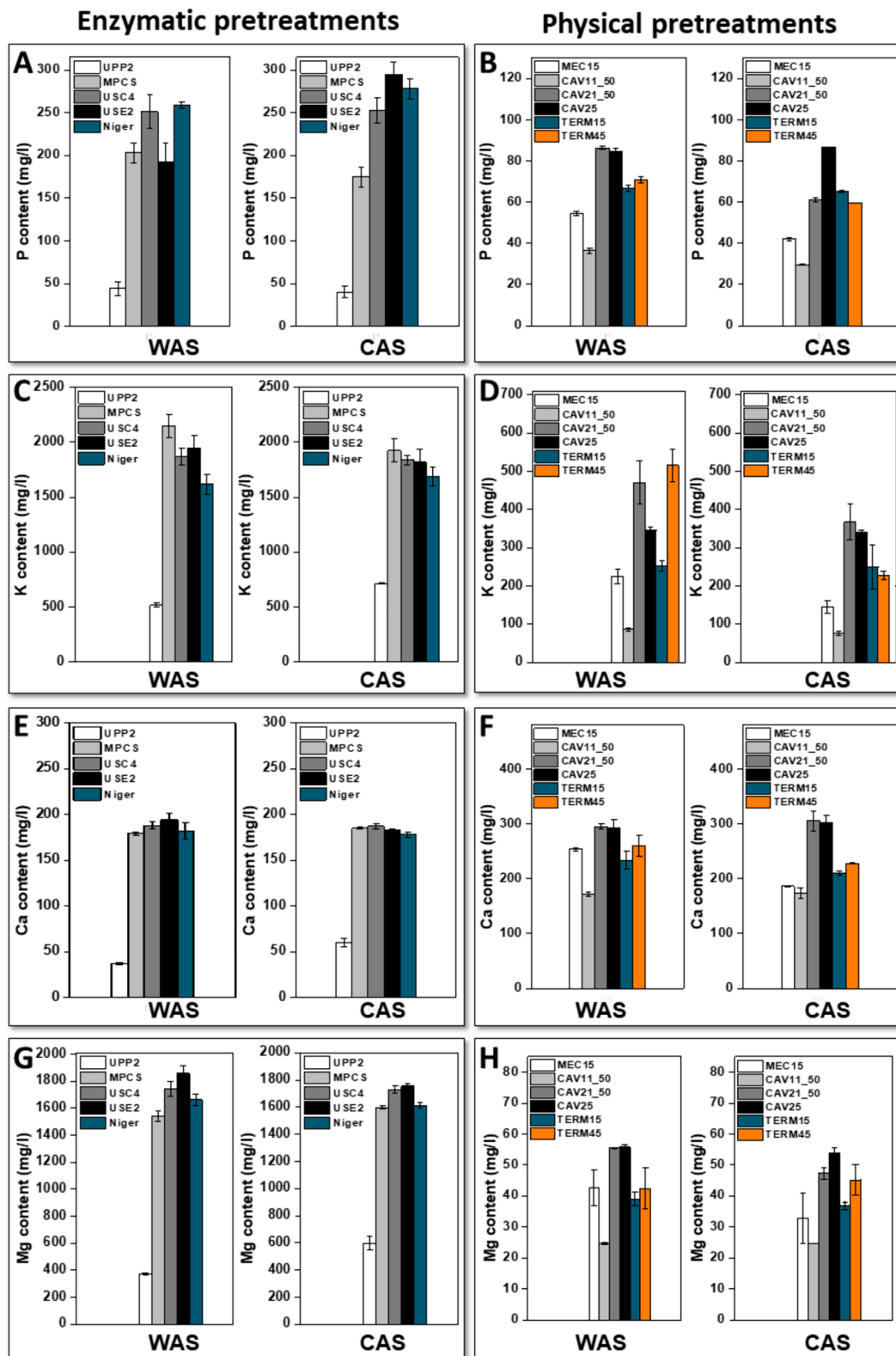


Fig. 6. Content % of P (A and B), K (C and D), Ca (E and F) and Mg (G and H) in digestate liquid fraction obtained after enzymatic pre-treatments (A, C, E and G) and physical pre-treatments (B, D, F and H) with WAS and CAS inoculums. Results and error bars correspond to the average and standard deviation of measurements performed in triplicate.

The mass ratio between nutrients in the digestate liquid fractions (Table S7) is of primary importance to define the sample applicability for fertilization, because it cannot be corrected by simple dilution but requires the addition of one or more nutrients to be balanced. The N/P

ratio, optimal between 2 and 7, results extremely low in all liquid fractions from enzymatic pre-treatment, while it rises in CAS\_TERM15 and all the samples obtained via cavitation at 50 °C. This unbalanced ratio is due to the very high content of P in the liquid fraction.

Considering the Ca/Mg ratio, which ideally ranges between 2 and 5 and is still acceptable between 1.5 and 6, the liquid fraction from physical pre-treatments are well-balanced solutions. Regarding the enzymatically pre-treated samples, the ones derived from UPP2 should be enriched in Ca, and the other ones should be integrated with Mg. Finally, the Mg/K ratio, ideally 0.1–0.8, is balanced in all the physically pre-treated liquid fractions, as well as in the samples achieved with UPP2 and MPCs. The other samples generated via enzymatic pre-treatment should instead be enriched in K.

### 3.5. Multivariate pattern recognition

Multivariate pattern recognition was applied to the collected data, separately for digestate and its liquid fraction, exploiting Principal Component Analysis (PCA) after autoscaling. In both cases, each sample is described in terms of its distance from the “optimal target result”, as described in section 2.4. Fig. 7A reports the loading plot and Fig. S7A the score plot of the first two PCs calculated for the digestate dataset; the first two PCs proved to be the most significant, explaining about the 60 % of the total variance ( $PC_1 = 37.98\%$ ;  $PC_2 = 27.85\%$ ). The score plot reports the “optimal target result” condition (indicated as “best” in the graphic) at positive scores on both  $PC_1$  and  $PC_2$ , therefore, the samples closest to the “optimal target result” sample in the score plot are those characterized by the best experimental conditions. The enzymatic digestates are present at positive scores on the first PC and negative ones on the second, while all the physical pre-treatments presented negative scores on  $PC_1$ . Since each variable is represented by the distance of each object from the “optimal target result”, all the enzymatic pre-treatments showed a higher contribution to the distance given by VS removal,  $H_2S$  and  $CH_4$  production,  $NH_4^+$  content and pH. In the case of the physical

pre-treatments, instead, the highest contribution to the distance from the “optimal target result” was given by the  $CO_2$  production, the content of K, Ca and N and, secondly, Ca/Mg ratio and process time. The loading plot in Fig. 7A reports in red the unit circle: variables close to the unit circle are completely described by the first two PCs present in the graph. As it can be seen, variables belonging to both the process performances and the digestate characterization are close to the unit circle, showing that the first two PC account for information related to both these aspects. Fig. 7B shows the distance of each sample from the “optimal target result”: the samples are reported on the x-axis and the distances on the y-axis. The treatment with UPP2 showed the best result, with the lowest distance (slightly lower for CAS than for WAS). In general, enzymatic pre-treatments showed better results in particular with CAS inoculum, while a strong difference between CAS and WAS appeared for most of the enzymes, except UPP2 and MPCs, this last one showing the second-best result. Regarding the physical pre-treatments, cavitation at  $25^\circ C$  proved to be the best, followed by cavitation at  $50^\circ C$ , even if in this last case there was a poor repeatability when the inoculum source changes. Cavitation at  $25^\circ C$  also showed a good agreement when both CAS and WAS are considered, while the good results obtained by the thermal treatment for 45 min with WAS are not reachable with CAS.

Fig. 7C reports the loading plot and Fig. S7B the score plot of the first two PCs calculated for the liquid fraction of the digestate dataset; the first two most significant PCs account for about 70 % of the total variance ( $PC_1 = 45.39\%$ ;  $PC_2 = 24.29\%$ ). As for the previous case, the “optimal target result” sample had positive scores on both  $PC_1$  and  $PC_2$ . The physical pre-treatments were closer to the “optimal target result” than the enzymatic digestions: physical pre-treatments are in fact located at positive scores on  $PC_1$ , while the enzymatic ones are at negative scores on the same PC. Comparing this result with the loading

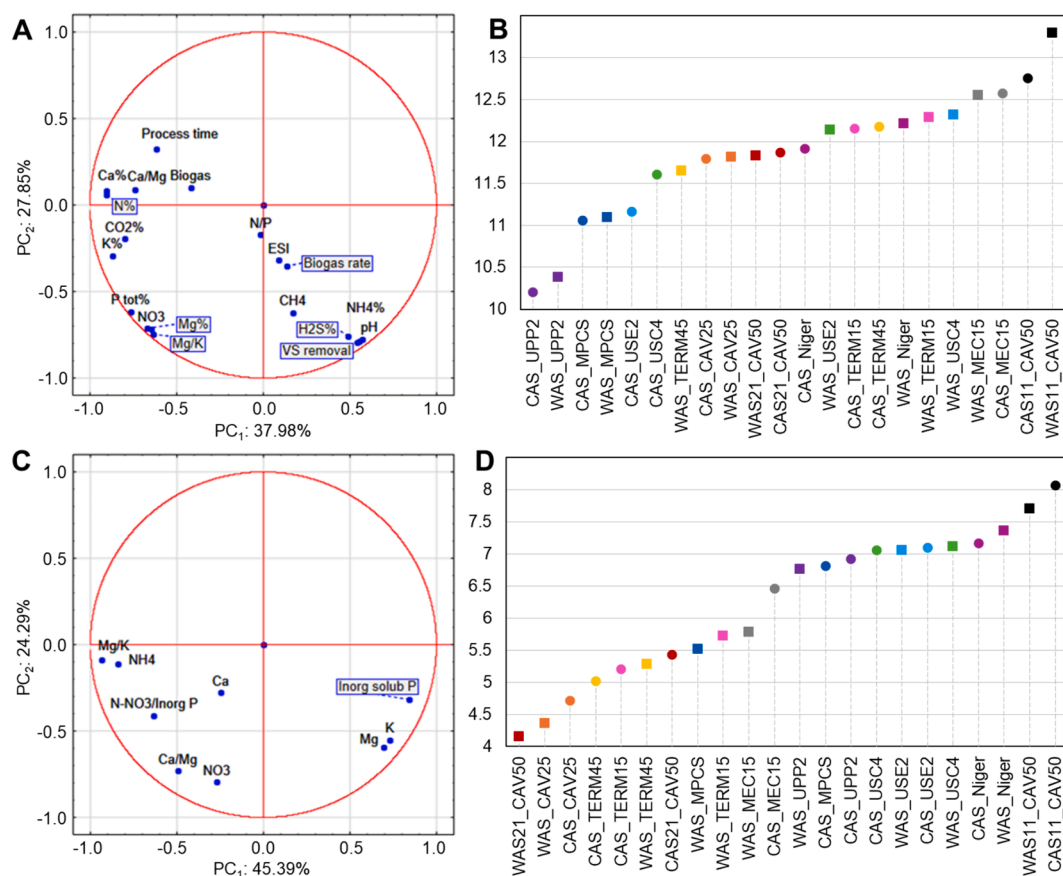


Fig. 7. Results obtained from PCA applied to the digestate dataset (A, B) and to the dataset of its liquid fraction (C, D): loading plot (A, C) and plot of the distance of each experimental configuration from the “best” result (B, D).

plot (Fig. 7C), it is possible to state that the physical pre-treatments showed a higher contribution to the distance given by Mg, K and inorganic P, while the enzymatic treatments showed the highest contribution to the distance given by Mg/K, N-NH<sub>4</sub>, N-NO<sub>3</sub>/Inorg P. From the score plot, cavitation at 25 °C corresponded to the best condition, i.e. the closest to the “optimal target result”. The distances of each sample from the “optimal target result”, as for the digestate dataset, are shown in Fig. 7D. Cavitation at 50 °C with WAS provided the best result, although the same pre-treatment on CAS inoculum showed poorer performances. Thermal treatment for 45 min also provided good results, similar for WAS and CAS, while the same treatment for 15 min showed a high difference between CAS and WAS. Among the enzymatic treatments, the best results were given by MPC5 and UPP2: while UPP2 showed similar behaviors for WAS and CAS, MPC5 is by far the best for WAS (results similar to the physical pre-treatments), while for CAS the behavior is similar to UPP2.

#### 4. Conclusions

This study examines the anaerobic digestion (AD) of organic fraction of municipal solid waste to produce energy and digestate as biofertilizer. Both enzymatic (UPP2, MPC5, *A. niger*, USC4, and USE2) and physical (mechanical, thermal, hydrodynamic cavitation) pre-treatments were applied to OFMSW. ANOVA analysis indicated no statistically significant difference between enzymatic and physical pre-treatments concerning CH<sub>4</sub> and energy production. This suggests the versatility and effectiveness of pre-treatments in the AD process of OFMSW, which is heterogeneous and partially recalcitrant to degradation. The highest CH<sub>4</sub>-producing AD configurations within physical pre-treatments did not correlate with the highest energy sustainability, highlighting the importance of considering pre-treatment energy costs. Following Regulation (EU) 2019/1009, all digestates met the macronutrient requirements for liquid organic fertilizers: N<sub>2</sub> ≥ 2.00 % dry matter, P<sub>2</sub>O<sub>5</sub> ≥ 0.44 % dry matter, and K<sub>2</sub>O ≥ 1.65 % dry matter. Enzymatic pre-treatments produced nutrient-rich digestates with higher agronomic value compared to those from physical pre-treatments.

The optimal configurations, in terms of high energy yields and suitable digestates for agricultural applications, were UPP2 among the enzymatic treatments and hydrodynamic cavitation among the physical ones. Based on the results we can conclude that enzymatic treatments led to more pronounced organic matter degradation and nutrient richer digestates compared to physical processes. On the other hand, the digestate liquid fractions from physical processes showed better nutrient balance, making them better candidates for fertigation.

This work also highlights the importance of OFMSW characterization for its elemental and biochemical composition to better understand the effects of pre-treatments and nutrient content in digestates. Future research should be devoted to scale up these results in pilot plants to assess the effect of pretreatments on CH<sub>4</sub> production and energy sustainability, and to evaluate the soil application effects of large quantities of digestate.

#### CRedit authorship contribution statement

**Alice Boarino:** Writing – original draft, Visualization, Validation. **Francesca Demichelis:** Writing – original draft, Investigation, Conceptualization. **Daniela Vindrola:** Investigation. **Elisa Robotti:** Writing – review & editing, Formal analysis. **Emilio Marengo:** Formal analysis. **Maria Martin:** Supervision. **Fabio Deorsola:** Supervision, Funding acquisition. **Elio Padoan:** Supervision, Project administration, Conceptualization. **Luisella Celi:** Supervision, Project administration, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2024.08.023>.

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