Comparison of various post-treatments for recovering methane from agricultural digestate

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1. Introduction

Nowadays, biogas production through anaerobic digestion (AD) is regarded as a possible interesting energy carrier for replacing fossil fuels and reducing greenhouse gas (GHG) emissions.

Anaerobic digestion is an old and well-established biological process that involves the anaerobic degradation of organic materials into biogas, a mixture of CH_4 (50–75%) and CO_2 (25–50%), and digestate. The latter mainly constituted of water (over 90%), residual undegraded substrate, and inorganic compounds (i.e., ash). At farm scale, digestate is generally mechanically separated into liquid and solid fractions that are stored and handled separately. The liquid fraction is rich in nitrogen (N) and potassium (K), whereas the solid fraction retains great amount of phos-phorus (P) and organic matter (mainly fibres) [1].

To date, the main use of anaerobic digestate has focused on land dis-posal [2,3]. Nevertheless, digestate, produced throughout the year, has to be stored, as it cannot be used directly on agricultural lands, due to limitations imposed by its stabilization level, crop growth stage and soil type [4]. Furthermore, the increasing number of biogas plants and their concentration in certain regions might lead to an oversupply of digestate, needing the surplus of digestate to be transported to regions with nutrients deficits [5]. Indeed, farms receive back only the amount of digestate which they are allowed to use in their fields, according to the nitrate directive [6,7].

Digestate storage, mainly performed in uncovered tanks, could cause potential emission of biogas into the atmosphere, resulting in a loss of energetic efficiency and in an increased environmental impact of AD plants [8,3].

Solutions to take advantage of the residual methane potential of digestate have been firstly investigated by Balsari et al. [9] who proposed a recirculation of digestate in the digester. Such option could reduce GHG emissions and it could permit to reduce the number of out-door areas for its storage, while improving the energetic and environ-mental exploitation of the anaerobic digester [9].

The residual biodegradability of digestate depends on its compositional and structural characteristics, which vary according to the type of substrates fed to the digester and the AD plant configuration

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(i.e., with the presence or not of post-fermenters). The residual methane yields were also found to be closely correlated to other reactor parame-ters, such as the Hydraulitic Retention Time (HRT) and Organic Loading Rate (OLR) [10,11].

Some studies demonstrated that during anaerobic digestion hemicelluloses are degraded at a faster rate than cellulose, resulting in an accumulation of cellulose and lignin in the solid digestate [12–14]. Thus, treatment methods (i.e., physical, thermo-chemical, chemical, biological or various combinations of them) became fundamentals in order to break the resistant layer of residual lignin and to reduce the crystallinity of cellulose, thus increasing the availability of cellulose to anaerobic mi-croorganisms [15–20]. Generally called as "pre-treatments" when ap-plied on lignocellulosic fibres, the term "post-treatments" is used when they are applied on digested fibres. More recently, some authors have tested mechanical, thermal and chemical post-treatments on digestate and solid separated digestate [21–25]. However, the high-energy con-sumption for mechanical post-treatments, the high cost of chemicals and the possible formation of inhibiting by-products (i.e., furfural, HMF and phenol compounds) during thermo-chemical post-treatments are limiting barriers for their future industrial development [13,26].

Thus, due to the high cellulose content in agricultural digestate, a promising option is to carry out biological post-treatments, with the use of enzymes (i.e., endo-glucanase, exo-glucanase and β-glucosidase). For this purpose, different enzymatic commercial cocktails were developed at industrial scale in order to promote AD of complex solid substrates. However, according to our knowledge, the use of commercial enzymatic cocktails to enhance the methane production from digestate has not been investigated yet.

In this context, the aim of this study was to evaluate the methane production from digestate (DIG) and solid separated digestate (SS-DIG) and the feasibility of applying different kind of post-treatments (i.e., thermal, thermo-chemical and enzymatic) in order to enhance their methane recovery. Finally, preliminary energetic balances were also performed, by considering different scenarios of digestate recirculation.

2. Materials and methods

2.1. Origin of digestates

DIG and SS-DIG samples were collected from a mesophilic full-scale AD plant in the Lombardy region of Northern Italy. The plant was fed on a mixture (on the overall VS fed) of maize silage (25%), sorghum silage (11%), olive waste (11%), cow manure (8%), pig manure (18%), and tur-key poultry manure on coconut chips (26%). The operational character-istics of the anaerobic plant are presented in Table 1. DIG sample was collected at the exit of the post-fermenter and before its inlet into the solid-liquid separator, while SS-DIG was recovered from the separator (helical screw press). Both DIG and SS-DIG were stored in gas-tight con-tainers at 4 °C before their use.

Table 1
Main characteristics of the anaerobic digester plant.

Anaerobic digester parameters	
Number of reactors	2 digesters, 1 post-fermenter, 1 storage tank
Reactors volume (m^3)	Digesters: 2 × 2100 Post-fermenter: 2700 Storage tank: 2700
OLR (kg VS/m^3/day)[a]	3.4
HRT (day)[a]	36
pH[a]	7.5–7.8
Temperature (°C)[a]	43
Biogas	
Biogas (Nm^3/day)	12,000
Methane (%)	52
Total energy (MW)	0.98

[a] Referred to digesters and post-fermenter only.

2.2. Post-treatments

Thermal, alkaline and enzymatic post-treatments were performed on both DIG and SS-DIG samples. They were performed in 500 mL glass bottles closed with rubber septa. Thermal post-treatment was performed at 80 °C for 1 h under stationary conditions. Alkaline post-treatment was conducted by soaking samples in a NaOH solution at a dosage of 1 g NaOH/100 g TS, at 40 °C, for 24 h, without stirring. Alkaline dosage, post-treatment temperatures, and contact times were chosen according to our previous results [18]. Enzymatic post-treatment was conducted by using a commercial enzymatic cocktail, especially developed to enhance biogas production of agricultural substrates (MethaPlus® L 100, DSM Biogas, The Netherlands). The commercial preparation, analysed for its enzymatic activities content, was found to contain 221 IU/mL xylanase, 1740 IU/mL endo-glucanase, 7.62 IU/mL exo-glucanase and 31,900 IU/mL β-glucosidase. To perform the post-treatment, the enzymatic preparation was added to each substrate at a dosage of 0.15 mL/g TS and pH was corrected at appropriate enzyme-specific value (pH = 5) with HCl. Samples were then incubated at 40 °C for 24 h in a thermostatic incubator under stationary condition.

2.3. Analytical determinations

Total solids (TS), volatile solids (VS), ash content and chemical oxygen demand (COD) were analysed according to APHA methods [27]. TKN was determined according to Kjeldahl method [28], by using a mineraliser (BUCHI digestion unit K 438) and a BUCHI 370-K distillator/titrator. $N–NH_4^+$ concentrations were determined by using a commercial photochemical Spectroquant® test kit (Merck, Darmstadt, Germany; Hach Lange GmbH, Dusseldorf, Germany; LCK314 for COD and LCK303 for $N–NH_4$) and a spectrophotometer (HACH Lange DR6000 Hach Company, Loveland, CO., USA). Total phenols were measured according to Velioglu et al. [42] using Folin-Ciocalteu reagent. 200 μL of diluted sample was firstly filtered with a syringe filter 0.22 μm and then mixed with 1.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand for 5 min before the addition of 1.5 ml of 20% sodium carbonate. After 90 min, absorbance was measured at 750 nm using a UV-Vis Spectrophotometer. The blank contains only water and the reagents. Total phenols were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid.

Structural-carbohydrates (i.e., glucose, xylose and arabinose) from cellulose and hemicelluloses were measured using a strong acid hydrolysis method adapted from Effland [29]. Samples (100 mg) were first hydrolyzed with 12 M H_2SO_4 acid for 2 h at room temperature and then diluted to reach a final acid concentration of 1.5 M and kept at 100 °C for 3 h. The insoluble residue was separated from the supernatant by filtration on fibreglass paper (GFF, WHATMAN®), washed with 50 mL of deionized water and then placed in a crucible. The crucible and the fibreglass paper were dried at 105 °C during 24 h to determine by weighing the amount of Klason lignin. The supernatant was further filtered with nylon filters (20 μm) and analysed for the quantification of monomeric carbohydrates. All monosaccharides (i.e., glucose, xylose, arabinose) were analysed by high pressure liquid chromatography (HPLC) coupled to a refractometric detector. The analysis was carried out with a combined Water/Dionex system (Ultimate 3000), using a Biorad HPX-87H column at 50 °C. The eluent corresponded to 5 mM H_2SO_4 under a flow rate of 0.3 mL/min. A refractive index detector

(Waters 2414) was used to quantify the carbohydrates content. The system was calibrated with glucose, xylose and arabinose standards (Sigma-Aldrich®). Thereafter, cellulose and hemicelluloses were estimated as follows (Eqs. (1) and (2)):

$$\text{Cellulose}\ (\%\text{VS}) = \text{Glucose}\ (\%\text{VS})/1.11 \quad (1)$$

$$\text{Hemicelluloses}\ (\%\text{TS}) = [\text{Xylose}\ (\%\text{VS}) + \text{Arabinose}\ (\%VS)]/1.13 \quad (2)$$

where 1.11 is the conversion factor for glucose-based polymers (glucose) to monomers and 1.13 is the conversion factor for xylose-based polymers (arabinose and xylose) to monomers.

Characterization of the enzymatic activities (xylanase, endo- and exo-glucanase) present in the commercial preparation was performed as reported elsewhere [14]. β-Glucosidase enzymatic activity was measured by mixing 0.1 mL of sample with 0.9 mL of p-nitrophenyl-α-D-glucopyranoside (0.1% w/v) in citrate buffer (0.025 M, pH 4.4). A blank sample with deionized water (0.1 mL) was also prepared in the same buffer. Samples were incubated at 50 °C for 10 min and then mixed with 2 mL of 2% (w/v) Na_2CO_3. The release of p-nitrophenol was determined by using a spectrophotometer (OD 405 nm) (6705 UV/vis Spectrophotometer, Jenway, UK). One unit of enzyme (IU) was defined as the amount of enzyme which hydrolyzes 1 μmol of p-nitrophenol-α-D-glucopyranoside in 1 min.

Volatile fatty acids (VFA) composition of the liquid phase, i.e., acetic (C_2), propionic (C_3), butyric and iso-butyric (C_4 and iC_4), valeric and iso-valeric (C_5 and iC_5) and caproic (C_6) acids, were analysed by HPLC (Agilent 1260 Infinity) coupled to a refractometric detector. The analysis was carried out with a Hi-PLEX H column (Agilent®) at 60 °C. The eluent corresponded to 5 mM H_2SO_4 under a flow rate of 0.7 mL min^{-1}. A refractive index detector at 55 °C was used to quantify the VFAs. The system was calibrated with VFA standards (Sigma-Aldrich®).

2.4. Biochemical methane potential (BMP) tests

BMP tests were performed in batch mode under mesophilic conditions (35 ± 0.5 °C), using glass bottles closed with rubber septa. The total volume of each bottle was 560 mL, with a working volume of 500 mL. The inoculum used for BMP tests was a mesophilic anaerobic sludge from the waste sludge anaerobic digester of the wastewater treatment plant of Cremona (Lombardy region, Italy). The anaerobic sludge contained 24.5 g TS/L and 10.8 g VS/L. This inoculum was kept under endogenous anaerobic conditions at 35 °C for about 7 days to reduce non-specific biogas generation. DIG and SS-DIG were introduced into the flasks with the inoculum, obtaining a substrate to inoculum ratio of 1 g VS/g VS. Finally, 50 mL of mineral medium of macronutrients (as suggested by OECD 311 [30]) and tap water were also added to reach 500 mL of working volume. Thermal and alkaline pretreated samples had a final pH ranging between 9 and 9.5, while enzymatic pretreated samples had a final pH around 5. Therefore, all samples were neutralised to pH = 7–8 with a concentrated HCl or NaOH solution before adding the inoculum and the mineral medium, prior to start BMP tests.

Once the flasks were prepared, a degasification step with nitrogen gas was carried out to obtain anaerobic conditions. BMP tests were performed in duplicate and the test duration was 65 days. The methane yield (NmL CH_4/g VS) was calculated according to Eq. (3):

$$\text{BMP} = (V_{CH4,s} - V_{CH4,\ blank})/VS_s \quad (3)$$

where: ($V_{CH4,s}$–$V_{CH4,blank}$) (NmL CH_4) is the net volume (at normal temperature and pressure: 273 K, 1 atm) of methane measured at the end of the test; and VS_s (g VS) is the mass of volatile solids from substrate (i.e., treated or untreated DIG or SS-DIG). All gaseous volumes hereafter reported are referred to normal conditions.

2.5. Preliminary energetic calculations

The energetic balance was computed by considering the additional energy production by recirculating DIG (untreated and enzymatic post-treated) and SS-DIG. Only electric energy was taken into account, as it usually sold to the public grid providing extra incomes to farmer. On the contrary, thermal energy is, in most of the cases, used only for the self-consumption of the AD plant.

Three scenarios were considered: A) the recirculation of DIG; B) the recirculation of enzymatic post-treated digestate; and C) the recirculation of SS-DIG. The other post-treatment options were not considered, as they did not positively affect the methane production of both DIG and SS-DIG.

The assumptions made for the energetic balance of the overall process were:

- Biogas produced from AD process can be converted into heat and elec-tricity through a Combined Heat and Power (CHP) system, considering an electric and thermal efficiency of 40% and 41%, respectively [18].
- A solid separation efficiency of 73% and an electrical consumption of 0.4 kWh_{el}/m^3 were considered for the mechanical screw separator [25,31].
- The government incentive policy for biogas energy in Italy was con-sidered as 0.28 €/kWh_{el} [18].

3. Results and discussions

3.1. Biochemical composition of whole digestate and solid-separated digestate

Results about chemical composition of whole digestate (DIG) and solid-separated digestate (SS-SIG) are presented in Table 2. The pH values were 8.1 and 8.7 for DIG and SS-DIG, respectively. Mild alkaline pHs are common in well stabilized digestates, with higher values for the solid-separated fraction. The slightly alkaline pH values of digestates are mainly due to volatile fatty acids (VFA) degradation and ammonia (NH_3) production that occur during anaerobic digestion process, as well as the addition of strong bases or carbonates to control both pH and buffer capacity of the system. Similarly, Menardo et al. [25] have reported pH values ranging from 8.6 to 9 for solid-separated digestate of three biogas plants treating mainly manure and energy crops.

Low total solid content was observed on the raw DIG with 8.3 g TS/100 g wet biomass. After screw mechanical separation, the amount of TS on the SS-DIG increased to 21.6 g TS/100 g wet

Table 2
Chemical composition of whole digestate (DIG) and solid-separated digestate (SS-DIG).

	DIG	SS-DIG
pH	8.1	8.7
TS (g/100 g wet biomass)	8.3 ± 0.2	21.6 ± 0.1
VS (g/100 g TS)	72.7 ± 0.2	83.8 ± 0.3
COD/VS	1.24 ± 0.06	1.45 ± 0.10
Cellulose (g/100 g TS)	13.5 ± 1.8	17.5 ± 0.9
Hemicelluloses (g/100 g TS)	15.1 ± 1.4	20.3 ± 1.9
Klason lignin (g/100 g TS)	21.2 ± 1.4	24.1 ± 0.6
Ash (g/100 g TS)	14.8 ± 0.5	12.9 ± 1.1
TKN (g/100 g TS)	6.4 ± 0.5	1.0 ± 0.0
$N–NH_4^+$ (g/100 g TS)	4.8 ± 0.2	n.d.
$N–NH_4^+$/TKN (%)	76	n.d.

biomass. Ash contents of 12.9 and 14.8 g/100 g TS were observed for SS-DIG and DIG, respectively. Such results are lower than those measured by Menardo et al. [11] who reported ash contents varying from 23 to 37.9 g/100 g TS on four anaerobic digestates coming from mesophilic AD plants.

Moreover, a high content of ammonium nitrogen ($N–NH_4^+$) was observed for DIG sample (4.8 g/100 g TS). This was probably due to the composition of the substrate mixture fed to the digester that included a high percentage of animal manure with large initial nitrogen content. For the whole digestate, an $N–NH_4^+$/TKN ratio of 76% was evaluated suggesting that during the AD process a large part of proteins are converted into inorganic forms ($N–NH_4^+$). Such values are in agreement with those of Menardo et al. [11] that reported $N–NH_4^+$/TKN ratios varying from 45.4 to 77.9% for four whole digestates.

Interestingly, a high amount of holocelluloses (i.e., cellulose and hemicelluloses) was observed for both DIG (28.6% TS) and SS-SIG (37.8% TS) (Table 2), suggesting that a significant part of organic matter is not degraded during the mesophilic anaerobic process. Similar results were observed by Yue et al. [12] who reported an increase of the cellulose content on anaerobically digested fibre from 21.7 g/100 g TS to 35.7 g/100 g TS, during anaerobic mesophilic digestion in a CSTR (continuous stirred tank reactor). Furthermore a high lignin content was noticed in both DIG and SS-DIG (21.2 and 24.1 g/100 g TS, respectively), due to the recalcitrant nature of such polymers during AD process, as reported by Barakat et al. [26]. High amount of lignin in the residual digestate has also been reported in literature data after AD of straw (21.1 g/100 g TS) and dairy manure (21.4 g/100 g TS), respectively [32,33]. By comparing the DIG and the SS-DIG characteristics, it can be observed that, besides the obvious higher organic matter content, SS-DIG has a higher overall VS (18.1 against 6.0 g VS/100 g wet biomass) and an even higher COD content (26.2 against 7.5 g COD/100 g wet biomass) than DIG sample, suggesting a higher biogas potential of SS-DIG.

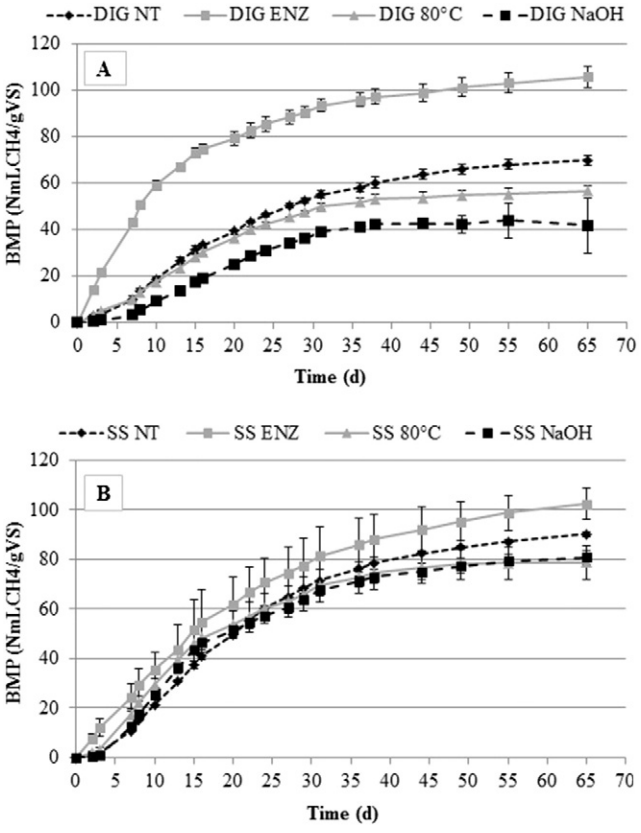

Fig. 1. Cumulative methane yields (BMP, NmL CH_4/g VS) at normal temperature and pressure conditions of untreated and post-treated DIG (A) and SS-DIG (B). Values correspond to mean ± standard deviation of measurements performed in duplicate.

3.2. Post-treatment effects on methane production from digestate

Since digestate still contains a large amount of residual undigested organic matter content and could therefore yield an attractive amount of biogas, its batch methane potential was assessed on DIG and SS-DIG samples. Methane yields (NmL CH_4/g VS) of DIG and SS-DIG samples are represented in Fig. 1A and B, respectively.

The methane yield of untreated DIG (70 ± 2.1 NmL CH_4/g VS, corresponding to 4.2 NmL CH_4/g on a wet basis) was lower than that obtained for the untreated SS-DIG (90 ± 1.2 NmL CH_4/g VS, corresponding to 16.2 NmL CH_4/g on a wet basis). Moreover, the corresponding anaerobic degradability, computed by considering the theoretical methane yield of each biodegradable compound (415 mL CH_4/g cellulose, 424 mL CH_4/g xylan and 420 mL CH_4/g proteins), resulted to be lower for DIG (33%) than for SS-DIG (41%) samples. The initial lower degradability of digestate (DIG) with respect to its solid fraction (SS-DIG) may suggest that the liq-uid phase is richer in humic substances that are less degradable compared to the lignocellulosic particulate organics that are enriched in the SS-DIG sample. Methane potentials obtained both for DIG and SS-DIG are in ac-cordance with previous literature data, as reported in Table 3. Results were also found in accordance with those of Ruile et al. [10] who investi-gated the residual methane potential of whole digestate from 21 full-scale digesters and they reported methane yields varying from 24 to 126 NmL CH_4/g VS. Conversely, Menardo et al. [11] reported lower meth-ane potential for whole digestate, ranging from 3 to 34 NmL CH_4/g VS, compared to those noticed in this study. However, as stated by the authors, the low methane yields could be partially explained by the inhibitory effect on the digestion process from the high ammonia concen-trations (higher than 2.5 g/L), recognized as inhibitory for the methano-genic archae [34,35]. In the present study, the ammonia concentrations at the end of the trials were 0.56 and 0.67 g/L for SS-DIG and DIG samples, respectively and thus lower than inhibitory concentrations for methano-genic archae (Table 4).

Due to the high content of lignocellulosic fibres remaining in both DIG and SS-DIG, various post-treatments (i.e., thermal, chemical and enzymatic) were carried out to enhance methane production from both DIG and SS-DIG samples. As shown in Fig. 1A and B, both thermal and alkaline post-treatments led to a slight reduction (around 10–20%) of methane yields compared to those of untreated samples. In this study, this reduction could not be attributed to an inhibitory effect of phenolic compounds, Na^+ ion, ammonium nitrogen concentration, nor to an accumulation of volatile fatty acids (always below the detection limit) during BMP tests. Indeed, a concentration of 0.1 g/L of total phenols was observed for all samples after BMP trials (Table 4). This concentration was not inhibitory for anaerobic digestion [13]. As for sodium, our previous study [36] demonstrates the feasibility of digesting sorghum previously pretreated with NaOH (at 10 g NaOH/100 g TS dosage), without any inhibitory effects caused by Na^+. Furthermore, the ammonia concentration at the end of the trials (Table 4) was still lower than 2.5 g/L, found inhibitory for mixed cultures during AD process [34, 35].

Some literature data confirmed the negative impact of alkaline and thermal post-treatments on methane production from digestate (Table 3). For instance, Jagadabhi et al. [37] found a low decrease of methane potentials after the application of alkaline post-treatment on whole digestate. Other authors reported a decrease of methane potentials of SS-DIG after thermal post-treatment performed at 80 °C for 3 h. According to Kaparaju et al. [23], this decrease was probably attributed to changes in the chemical composition of the solubilized compounds (SCOD, nitrogen).

Table 3
Comparison of BMP data related to untreated and post-treated digestates.

AD plant feed	Digestate sample	Post-treatment conditions	BMP test conditions	Methane yield (NmL CH_4/g VS)	Ref.
Cattle slurry (35%)	SS-DIG (screw press separator)	–	40 °C, 56 days	157 ± 7	[25]
Cattle manure (24%)		120 °C, 30 min		176 ± 5	
Triticale and sorghum silage (35%)					
Separated solid fraction (6%)					
Cattle slurry (33%)	SS-DIG (compression roller separation)	–		117 ± 11	
Cattle manure (23%)		120 °C, 30 min		98 ± 5	
Chaff rice (7%)					
Maize silage (33%)					
Separated solid fraction (4%)					
Swine slurry (76%)		–		71 ± 5	
Grass silage (8%)		120 °C, 30 min		154 ± 21	
Maize silage (16%)					
Cow manure (100%)	SS-DIG (sieve separation)	–	35 °C, 30 days	61 ± 5	[23]
		80 °C, 3 h		48 ± 2	
		NaOH (4% w/w), 20 °C, 48 h		61 ± 1	
		Freezing (−20 °C; 24 h)		47 ± 1	
		Mechanical maceration < 1 mm		51 ± 2	
Maize silage (25% VS)	SS-DIG (helical screw press)	–	35 °C, 65 days	90 ± 1	This study
Sorghum silage (11% VS)		80 °C, 1 h		79 ± 7	
Olive waste (11% VS)		Enzymes (cellulases and xylanase),		102 ± 6	
Cow manure (8% VS)		40 °C, 24 h, pH 5			
Pig manure (18% VS)		NaOH (1% w/w), 40 °C, 24 h		81 ± 3	
Turkey poultry manure on					
Coconut chips (26% VS)					
Manure (90%)	SS-DIG (decanter centrifuge)	–	38 °C, 50 days	80	[21]
Agricultural residues (5%)		Wet explosion (180 °C, 10 min)		209	
Industrial Wastes (5%)		Wet explosion (180 °C, 10 min, 6 bar O_2)		224	
Liquid manure (43.9%)	SS-DIG (decanter centrifuge)	–	37 °C, 35 days	21 ± 2	[22]
Solid manure (9%)		Ball milling, 10 min, eight ball of		58 ± 5	
Maize silage (19%)		30 mm diameter			
Grass silage (21.4%)					
Grain (6.8%)					
Grass silage (30% VS)	DIG	–	35 °C, 118 days	100 ± 6	[37]
Cow manure (70% VS)		NaOH (2% w/w) of 40% NaOH solution,		93 ± 7	
Of a CSTR laboratory scale reactor		35 °C, 65 h			
		NaOH (3% w/w) of 40% NaOH solution,		99 ± 4	
		35 °C, 65 h			
		NaOH (4% w/w) of 40% NaOH solution,		96 ± 4	
		35 °C, 65 h			
		NaOH (6% w/w) of 40% NaOH solution,		99 ± 10	
		35 °C, 65 h			
Solid fraction from swine manure	DIG	–	37 °C, 35–50 days	111 ± 11	[41]
		Aqueous ammonia soaking 22 °C,		200 ± 7	
		3 days, 32% (w/w) ammonia			
Maize silage (25% VS)	DIG	–	35 °C, 65 days	70 ± 2	This study
Sorghum silage (11% VS)		80 °C, 1 h		57 ± 2	
Olive waste (11% VS)		Enzymes (cellulases and xylanase),		106 ± 4	
Cow manure (8% VS)		40 °C, 24 h,			
Pig manure (18% VS)		pH 5			
Turkey poultry manure on		NaOH (1% w/w), 40 °C, 24 h		42 ± 12	
Coconut chips (26% VS)					

Another explanation could be the formation of toxic compounds derivated from lignocellulosic biomass during thermal and thermo-chemical pre-treatments [38].

Table 4
Chemical composition of BMP effluents from untreated and post-treated DIG and SS-DIG.

	pH	VFA (g/L)	$N–NH_4^+$ (g/L)	Total phenols (g/L)
DIG	7.0	<d.l.[a]	0.67	0.11
DIG ENZ	6.7	<d.l.[a]	0.65	0.11
DIG 80 °C	7.0	<d.l.[a]	0.47	0.11
DIG NaOH	7.0	<d.l.[a]	0.58	0.11
SS-DIG	6.8	<d.l.[a]	0.56	0.12
SS-DIG ENZ	6.2	<d.l.[a]	0.36	0.11
SS-DIG 80 °C	6.7	<d.l.[a]	0.38	0.10
SS-DIG NaOH	6.7	<d.l.[a]	0.35	0.11

[a] <d.l. = under detection limit.

Interestingly, methane yields of 102 and 106 NmL CH_4/g VS were no-ticed for enzymatically treated SS-DIG and DIG, respectively. The enzy-matic post-treatment leads to an increase in methane yield, due to the effect of the enzymes that are able to attack and solubilize cellulose and hemicelluloses, thus resulting in a better anaerobic microbial degradation.

The content of holocelluloses was previously determined to be higher in SS-DIG than DIG samples (Table 2). Consequently, a higher increase of the methane potentials could be expected from the SS-DIG fraction compared to raw DIG but interestingly the opposite was no-ticed with a higher methane increase for DIG (51%) compared to SS-DIG (13%). Such results suggested that a synergistic effect occur be-tween the anaerobic liquor and the enzymes. One plausible explanation could be the presence of soluble compounds in the liquor ($N–NH_4^+$, amino acids) that play as surfactants to bind the protein-active sites on lignin before introducing the enzyme, increasing available active en-zyme in the solution, and consequently enhance the performance of

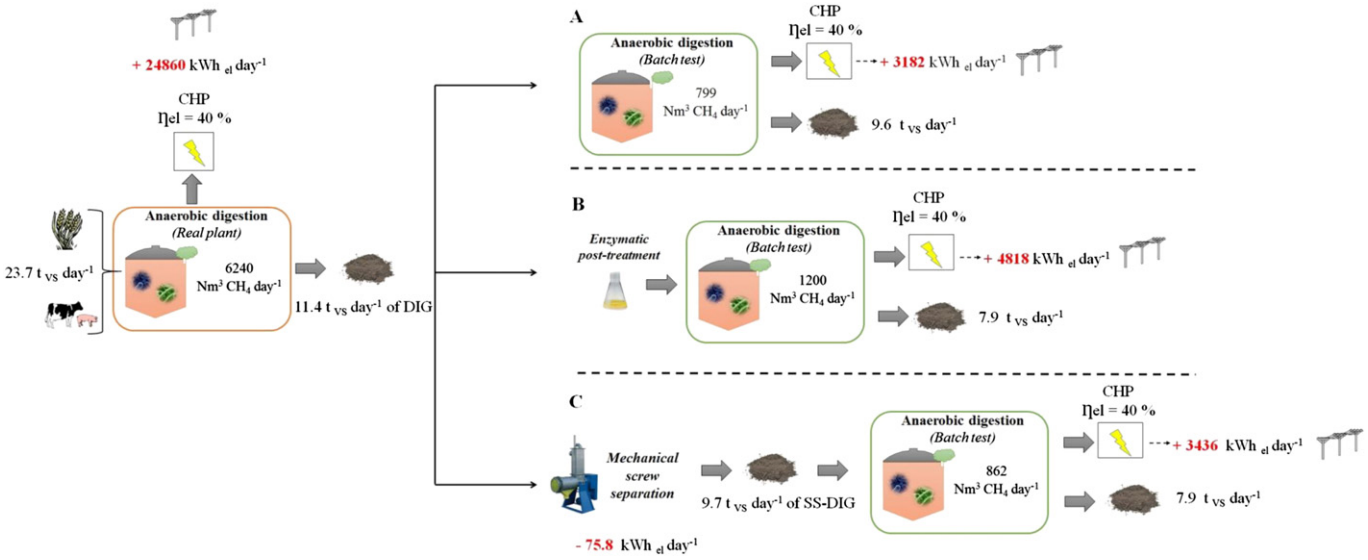

Fig. 2. Preliminary energetic balance considering (A) the recirculation of DIG; (B) the recirculation of enzymatic post-treated DIG; (C) the recirculation of SS-DIG.

enzymatic hydrolysis. For instance, some recent studies have shown that the addition of bovine serum albumin (BSA) in the medium or the use of an algal hydrolyzate (rich in soluble amino acids) could signif-icantly improve the enzymatic hydrolysis rate of rich lignin lignocellulos-ic substrates [39,40]. Further investigations are required to confirm these hypotheses.

3.2.1. Preliminary energetic evaluations

A preliminary energetic evaluation can be carried out based on the experimental data highlighted in this study. As represented in Fig. 2A and B, the recirculation of DIG and enzymatic post-treated DIG provided a supplementary electrical production of 3182 and 4818 kWh_{el}/day, re-spectively. In the case of recirculation of SS-DIG (Fig. 2C), an extra elec-trical production of 3361 kWh_{el}/day was computed, after subtracting the electrical requirement of the screw mechanical separator (75.8 kWh_{el}/day).

Thus, considering the government incentive policy for biogas energy in Italy (0.28 €/kWh_{el}), the recirculation of DIG and SS-DIG offered an extra income to farmers of 891 and 941 €/day. Moreover, the recircula-tion of digestate contributes to overcome the problems related to its dis-posal, reducing the digestate stream produced and maximising the economic value of the biomass used. In the case of scenario B (i.e., recir-culation of enzymatic post-treated DIG), an extra income to farmers of 1349 €/day was estimated. However, in this case, the economic gain has to be counterbalanced by the cost of the enzymes. In this study, an enzymatic cocktail, commonly used to enhance biogas production at full-scale agricultural AD plants was used to optimize the cost efficiency of the overall process. However, further research is needed to optimize the enzymatic dosage, for further reduce the cost of enzymatic post-treatment. Furthermore, such results have to be confirmed using con-tinuous scale anaerobic digesters, to state on the potential applicability of digestate recirculation at industrial scale and to draw more precise energetic and economic balances.

4. Conclusion

Results revealed that methane recovery from both DIG and SS-DIG is feasible. However, thermal and alkaline post-treatments did not have a beneficial effect in enhancing methane yields for both substrates. Inter-estingly, enzymatic post-treatment demonstrated positive results based on improved methane yields of both substrates, especially in the case of whole digestate. Finally, according to the energetic balances, digestate recirculation permitted to obtain an extra electrical production, which represents an extra economical income to farmers.

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