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1	Modelling of technical, environmental, and economic evaluations of the effect of the organic
2	loading rate in semi-continuous anaerobic digestion of pre-treated organic fraction municipal
3	solid waste.
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11	Keywords: semicontinuous anaerobic digestion, pre-treatments, carbon footprint, economic
12	evaluation, Principal Component Analysis, Kohonen neural networks
13	Abstract
14	The study concerned technical feasibility, economic profitability, and carbon footprint (CF) analysis
15	of semi-continuous anaerobic digestion (sAD) of organic fraction of municipal solid waste
16	(OFMSW). The research assessed the pre-treatment effect on sAD by varying organic loading rates
17	(OLR) from 3.38 to 6.75 kgvs/m ³ d. Three sAD configurations were investigated: hydrodynamic-

cavitated (HC-OFMSW), enzymatically pre-treated (EN-OFMSW), and non-pre-treated (ADOFMSW). Principal Component Analysis and Supervised Kohonen's Self-Organizing Maps
combined the experimental, economic, and environmental evaluations.

The sAD configurations were grouped predominantly according to the OLR however, within each
OLR group the configurations were clustered according to the pre-treatments.

The finding highlighted that pre-treatments offset inhibition in sAD of OFMSW due to the OLR increase, being economically profitable and CF negative up to 4.50 kgvs/m³d for EN-OFMSW and to 5.40 kgvs/m³d for HC-OFMSW. Whereas sAD-OFMSW remained economically and environmentally viable only up to 3.87 kgvs/m³d.

27 HC-OFMSW reached the highest performance. In detail, for HC-OFMSW the NPV and CF ranged

from 17679.30 to 43827.12 euros and from -51.08 to -407.210 kg $CO_2eq/1$ MWh daily produced, by

29 decreasing the OLR from 5.40 to $3.87 \text{ kgvs/m}^3 \text{d}$.

30 These results are fundamental since pre-treatment is usually expensive due to additional energy or31 chemical requirements.

32

33 **1. Introduction**

The European biogas plant market is expected to grow from 1.87 billion euros in 2021 to 3.47 billion 34 euros in 2028 (European biogas plant market, 2023). According to the European Biogas Association, 35 in Europe 27, the use of biogas represents more than 11.5 GW of installed power generation capacity 36 (Power and renewables, 2022). Nowadays, at the industrial scale, biogas is produced from organic 37 waste like agro-waste and organic fraction of municipal solid waste (OFMSW) through anaerobic 38 digestion (AD) (Aslanzadeh et al., 2014). The performance of AD of organic waste provides two 39 benefits: 1) reduction of organic waste, and 2) production of renewable energy vector, and fertilizer. 40 At the industrial scale, anaerobic digestion is performed in continuous feeding mode (sAD), which 41 means a system fed continuously or semi-continuously with an average residence time of the substrate 42 in the reactor, expressed by the parameter hydraulic residence time (HRT). The HRT refers to the time 43 that the substrate remains in a digester, and it is calculated as the ratio between the volume of the AD 44 reactor (V) and the flow rate of a digester (Q). The parameter HRT is inversely related to the parameter 45 organic loading rate (OLR), which measures the quantity of substrate (kg of volatile solids) used to 46 feed the digester for a unit volume (V) in a day. In sAD, the OLR and HRT play a key role, and their 47 choice is critical. Currently, at the industrial scale, the working OLR represents a problem in the 48 management of the process. In general, high HRT corresponds to low OLR and vice versa, but it is 49 necessary to find a balance between the OLR and the HRT to optimise the sAD efficiency in terms of 50 biogas production, and volatile solid removal (Aslanzadeh et al., 2014), and to optimise the reactor 51 volume (Demirer and Chen, 2005). 52

The main problem related to the choice of OLR is the fact that an increase in OLR can promote higher 53 biogas production rates, but excessive OLR inhibits the process by decreasing biogas production and 54 methane content. Inhibition of the process caused by OLR consists of over-acidification and foaming. 55 Acidification in AD is a fundamental phase since AD is divided into four phases (hydrolysis, 56 acidification, acetogenesis, and methanogenesis) but over-acidification for excessive OLR leads to a 57 pH drop due to volatile fatty acid accumulation, that reduces the methane production and the quality 58 of the digestate. OLR depends on the type and concentration of the substrate, AD temperature, and 59 biogas digester. 60

To date, three macro-types of studies have been performed to investigate the effect of OLR on AD on 61 62 different substrates. The first type concerns the effect of the OLR variation on the efficiency of the biogas production from different substrates. Specifically, Babaee and Shayegan, (2016) studied the 63 OLR between 1.4 -2.75 kgvs/m³d on vegetable waste identifying an optimal OLR at 10.80 kgvs/m³d. 64 Zhou et al., (2022) investigated the variation of OLR from 1.00 to 13.80 kgvs/ m³d on food waste 65 with an optimal OLR of 10.80 kgvs/m³d. Moreover, Liu et al.,(2018) varied OLR from 1 to 2.5 66 kgvs/m³d on spirulina waste with an optimal OLR of 2.25 kgvs/m³d. The second type of study 67 concerns the effect of mixing on the OLR variation and on biogas production. In detail, Rog et al., 68 (2023) studied the OLR from 0.38 to 2.31 kgvs/m³d on activated sludge identifying the optimal OLR 69 70 of 1.98 kgvs/m³d. Nges and Liu, 2(010) at 250 rpm varied OLR from 1.6 to 20.5 kgvs/m³d on dewatered sludge with an optimal OLR corresponding to HRT of 30 d. Furthermore Leite et al., 71 (2017) studied the activated sludge at optimal OLR of 1.90 kgvs/m³d withholding mixing 2 h before 72 73 feeding. The third type of study manages the increase of the OLR by performing the two-stage AD. In detail, two-stage AD was investigated by Aslanzadeh et al., (2014) on OFMSW with OLR from 2 74 to 14 kgvs/m³d with optimal OLR of 12 kgvs/m³d. Two-stage AD was investigated by Dareioti and 75 Kornaros, (2014) on olive mill wastewater, cheese whey and liquid cow manure with HRT from 5 to 76 0.75 d, with an optimal OLR of 12.70 kgvs/m³d. Another study concerning the two-stage AD was the 77 study of Wijekoon et al., (2011) on molasses-based synthetic wastewater with OLR from 5 to 12 kg 78

COD/ m³d with an optimal OLR of 8 kg COD/ m³d. All the above-mentioned studies only investigate
OLR from a technical point of view.

The present study aims to investigate the possibility of managing the increase of the OLR of sAD 81 through the performance of pre-treatment on OFMSW before sAD in one single stage. In detail, this 82 study analyses the effect of enzymatic and hydrodynamic-cavitation pre-treatments performed in 83 (Demichelis, F. et al., n.d.) and (Demichelis et al., 2023), respectively, by increasing the OLR from 84 3.38 to 6.75 kgvs/m³d corresponding to HRT from 16 to 8 d. The novelty of this study is in not limiting 85 the research to the identification of the most technically high-performing sAD configuration but 86 combining it with the sustainability analysis through statistical elaborations. The three-fold metrics 87 88 are adopted by including technical feasibility analysis through laboratory tests, economic viability through cost-benefit analysis, and environmental sustainability by carbon footprint analysis (CF) 89 according to ISO 14067. The results of the three-fold metrics are elaborated with Principal 90 91 Component Analysis and Supervised Kohonen's Self-Organizing Maps. To the best of the author's knowledge, no studies are currently available regarding the adoption of pre-treatment to address the 92 increased OLR in sAD of OFMSW. Additionally, there is a lack of technical, economic, and 93 environmental studies in the literature about the sAD of OFMSW considering the effect of the OLR. 94 Moreover, a standardised assessment of the three-fold sustainability metrics is not available yet but 95 96 is necessary as stated by (Rajendran and Murthy, 2019).

97

98 **2.** Materials and methods

99 2.1 Substrate

The organic fraction of municipal solid waste (OFMSW) was supplied by San Carlo S.p.A (Fossano, Italy). To start the semicontinuous anaerobic digestion (sAD) the mesophilic digestate of cowagriculture sludge (CAS) was used as inoculum according to the previous study of (F Demichelis et al., 2022). OFMSW and inoculum properties agreed with the study of (Srisowmeya et al., 2020) and (Gu et al., 2020), respectively (Table 1).

- 105 All the experiments were performed with the same lot of OFMSW and inoculum, to limit the process
- 106 variability, since OFMSW strictly depends on season variability.
- 107 The OFMSW was frozen at +4 °C according to (Zeng et al., 2010) and (Gu et al., 2020) to prevent
- 108 the natural decomposition of the organic matter content and to avoid the variation of its physic-
- 109 chemical composition. Then OFMSW was defrost down for the daily feed in the anaerobic digestors.

110

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111 Table 1 Physical and chemical properties of OFMSW and inoculum TS= total solids, VS=volatile

112 solids.

	OFMSW	CAS
TS (%)	6,02 ±0.6	5.8±0.1
VS (%)	97.1±0.5	70.3±1.0
pH (-)	5.5 ±0.2	7.7±0.1
C (%)	48.1±0.5	40.6±0.6
H (%)	6.2±0.7	3.0±0.0
N (%)	3.1±0.3	7.9±0.1
S (%)	0.2±0.1	0.0±0.0
C/N (-)	15.5 ±1.4	5.1±0.1
TOC (g/kg)	24914.6±114.9	12.0±0.2

113

114 **2.2** Physical and enzymatic pre-treatments

Before semicontinuous anaerobic digestion (sAD), OFMSW was pre-treated with two different pretreatments, which were investigated in two previous studies; hydrodynamic cavitation (HC) (Demichelis et al., 2023) and enzymatic pre-treatment (EN) (Demichelis, et al., n.d.).

HC was performed with a rotor/stator HC-unit (Rotocav[®], E-PIC srl – Mongrando, Italy) at 55 °C for

117 (Demichelis et al., 2023) and enzymatic pre-treatment (EN) (Demichelis, et al., n.d.).

10 min (Bruni et al., 2010). HC is a pre-treatment that promotes the formation, growth, and implosion of vapor bubbles in a liquid at temperatures lower than the boiling point, which generates microenvironments characterized by locally very high temperatures and intense pressure waves.

EN was performed with UltraPract® P2 (UPP2), which is a mix of cellulases, hemicellulases, pectinases, and proteases, and it has been designed for biogas plants treating vegetables. UPP2 was used with a dose of 1 mL/100 g TS in the pH range 7.0 - 7.5 (GmbH, 2022) at 45 °C, (Demichelis, et al., n.d.).

126 The significant differences were calculated with Pearson test $\alpha < 0.05$, which is a correlation test to 127 investigate the presence of the linear correlation between pairs of variables, considering the 128 significance of those having p < 0.05.

129

118

130 **2.3 Anaerobic digestion**

Three sAD configurations were tested: hydrodynamic cavitated OFMSW (HC-OFMSW),
enzymatically pre-treated OFMSW (EN-OFMSW), and non-pre-treated OFMSW (AD-OFMSW) as
blank. Each sAD configuration was tested in duplicate for a total of 6 reactors.

sAD was performed in a 1 L reactor (Duran, Germany) with a working volume of 0.8 L at 6 %w/w total solid contents. The sAD was performed in mesophilic conditions and the temperature was kept at 37 °C with a 55 L water bath (Julabo Corio, C), and the anaerobic condition was guaranteed by purging nitrogen. The top of the reactor had three ports: one as the inlet to feed the pre-treated and non-pre-treated OFMSW in the reactor, the second to remove the digestate, and the third to collect the biogas into a Tedlar (Germany) 2 L gas bag. The tested organic loading rates (OLRs) were selected

by changing the hydraulic retention time (HRT) from 16 to 8 d, which means OLR variations between 140 3.38 and 6.75 kg vs/m³ d. Each HRT was maintained at least for a time equal to two HRTs to allow 141 the achievement of the pseudo-steady state. The starting HRT and the consequential OLR were 142 selected according to the results obtained in the AD of OFMSW performed in batch feeding mode (F 143 Demichelis et al., 2022). Biogas was measured with the water displacement method and its 144 composition was evaluated through SRA Micro-GC, which includes a Molsieve 5A column (for the 145 analysis of permanent gases like hydrogen, nitrogen, methane, and carbon monoxide) employing 146 argon as a carrier (column temperature: 100 °C) and a TCD detector. The injection temperature was 147 90 °C and the pressure was 30 psi. To evaluate the quality of the sAD process, the pH was daily 148 measured with a pH340 WTW pH-meter (Mettler Toledo, Germany) according to DIN 38404 C5 149 methodology. 150

- At the end of each tested HRT, the volatile solid (VS) was removed and volumetric biogas productions
 were measured to evaluate the performances of sAD.
- 153 The VS removed was evaluated with Eq. 1 (Li et al., 2018).

154
$$VS \ removed \ (\%) = 1 - \frac{VS \ output \ (1-VS \ input)}{VS \ input \ (1-VS \ output)}$$
(1)

where VS removed was the removed volatile solids (%), VS input and VS output were the volatilesolids concentrations in the OFMSW at the beginning and end of sAD.

157

158 **2.4 Economic analysis**

The economic study was performed based on a cost-benefit analysis to evaluate the cost-effectiveness and to identify the barriers to implementing a cost-effective strategy for the 15 sAD investigated (3 AD configurations x 5 OLR). A study estimate was adopted as a preliminary estimate of capital and

- 162 operational costs, and possible revenues considering the main equipment included in the process.
- 163 A detailed description of the cost-benefit analysis methodology is reported in Section 2 of SI.
- 164 The cost-benefit analysis as well as the carbon footprint analysis (paragraph 2.5) referred to the sAD
- plant with a capacity of production of 1 MWh/d of primary energy. The process was based on the data

obtained at the laboratory scale, but it was scaled up at the industrial level through assumption and
corrective factors based on (Bruno et al., 2023) (Green and Southhard, 2019) and (Turton et al., 2018).
The process flow diagram consisted of four phases: the first is the pre-treatment unit (enzymatic and
hydrodynamic cavitation pre-treatments), the second one is the sAD, the third phase is the CHP unit,
and the fourth one is the composting unit.

171 CH₄ yields of the 15 sAD configurations tested at the laboratory scale were scaled according to (Green
172 and Southhard, 2019) and (Kowalczyk et al., 2011).

(Green and Southhard, 2019) suggested assuming a scale factor equal to 90 % of the lab scale values. 173 Moreover, (Kowalczyk et al., 2011) highlighted that the scaling from a laboratory to a larger scale is 174 acceptable but variations in deviation depend on the organic loading rate. The corrective factors for 175 CH4 were derived by assuming a 10% reduction with each one-unit increase in the OLR. These factors 176 were established in alignment with the correction factor determined from the study of an AD plant 177 simulated using Aspen (Aui et al., 2019) and (Alfonso-Cardero et al., 2021). By increasing the OLR 178 the corrective factors were more and more restrictive due to the increase of viscosity and high 179 difficulties in mixing. 180

In the present study, it has been tested; HRT = 16 d (OLR = $3.38 \text{ kgvs /m}^3 \text{d}$), HRT= 14 d (3.87 kgvs/m³d), HRT = 12 d (OLR = $4.50 \text{ kgvs /m}^3 \text{d}$), HRT = 10 d (OLR = $5.40 \text{ kgvs /m}^3 \text{d}$), and HRT = 8 d(OLR = $6.75 \text{ kgvs /m}^3 \text{d}$).

The corrective factors were as follows: 90 % of the OLR from 3.38 to 3.87 kgvs/m³d, 80 % for OLR = $4.50 \text{ kgvs /m}^3 d$, 70 % for OLR = $5.40 \text{ kgvs/m}^3 d$, and 60 % for OLR $6.75 \text{ kgvs/m}^3 d$, based on the CH₄ specific production values at the laboratory scale. The CH₄ yields, obtained by adopting the scale-corrective factor, were comparable to the ones available for the industrial plants of OFMSW (plant available in Piedmont, Italy) and industrial scale co-digestion of agro-waste according to (Naqi et al., 2019).

190 The turbine was designed to treat 1 MWh/d and it was assumed equal to 50 kW in line with the study191 of (Huiru et al., 2019). The composting unit was designed considering a residence time of 90 d

according to (Evangelisti et al., 2014). The area of the composting unit is 4 times the height of thecomposting unit, according to (Ennio, 2018) and a working volume of 70 % was assumed.

- 194 The detailed methodology to calculate capital and operational cost (Table S1-S4) and to design the
- dimension of the equipment (Table S5) is provided in Section 1 of SI through equations and tables.
- 196 The cost-benefit analysis was referred to in 2023. The profitability of the tested 15 sAD configurations
- 197 (Table S6) was evaluated with the net present value (NPV) (Eq.3) and payback time (PBT) (Eq.4).
- 198 NPV quantified the profitability of the sAD configurations considering a plant lifetime equal to 20 y
- and considering a 5% discount for the future cash flows referring to the present value (Pleissner et

al., 2016).

201 NPV > 0 means that sAD process is profitable.

202
$$NPV (euro) = \sum_{t=1}^{T} \frac{C_t}{(1+d)^t} - C_0$$
 (3)

where, C_0 was the initial capital investment, C_t was the net cash flow during period *t*, *d* was the discount rate, and *t* was the sAD plant lifetime.

The PBT referred to the amount of time it took to recover the cost of an investment. It was calculated with (Eq.4)

207
$$PBT(y) = \frac{c_0}{Net \ cash \ flow \ per \ period}$$
 (4)

208

209 **2.5 Environmental analysis**

The carbon footprint (CF) analysis was performed according to ISO 14067, with the database Ecoinvent 3.5 and the software SimaPro 9.5.02. The CF analysis compared the global warming potential (GWP) of the 15 (3 pre-treatments x 5 OLR) tested sAD configurations focusing both on the pre-treatment and variations of OLR. To compare these 15 sAD configurations, the functional unit (FU) was assumed equal to 1 MWh/d of produced primary energy according to (Bruno et al., 2023). The adopted approach was from grave to gate according to (Ugwu et al., 2022), which means from the pre-treatment of OFMSW (grave) to bioenergy production (gate) (as reported in Figure S1). The

collection and transport of OFMSW to the AD plant were not considered since were the same for all
the 15 sAD configurations. The CF-analysis (ISO 14067) is geographical, and time referred. The
present study was geo-referred to Italy and in detail in the northwest of Italy, where San Carlo SpA
(Fossano, Piedmont, Italy) is located. San Carlo SpA is the waste treatment plant that supplied the
OFMSW for the present study. The study was time-referred to in 2023, as well as the cost-benefit
analysis. A detailed description of CF-analysis methodology is reported in Section 2 of SI.

In the present study, only the direct consequences of sAD of OFMSW were considered, whereas the environmental impacts of the infrastructures and capital goods were excluded (Thushari et al., 2020), because they were less important to the overall results.

The sAD included the foreground and background systems in agreement with (Clift et al., 2000). The foreground system was directly involved with the reference flow management and the background system considered energy production and chemical supply (Thushari et al., 2020).

According to (Piccinno et al., 2016), the laboratory process provides only limited indication of the possible environmental impacts. Moreover, (Carlqvist et al., 2022) stated that to better understand the environmental impact of the future system it is needed to consider an upscaled. The Life Cycle Inventory (LCI) was based on primary data obtained at the laboratory scale but then these data were scaled according to the scaling up explained for the cost-benefit analysis (paragraph 2.4 and S2 in SI).

The same boundary conditions were assumed for the cost-benefit and CF analysis to make consistent 235 the considerations derived from the study of economic and environmental sustainability. The CF 236 237 analysis included Scope 1 and Scope 2. Scope 1 concerns the direct emissions from the pre-treatments and sAD units that occurred from fuel combustion for the digester start-up, biogas combustion in the 238 CHP section to provide heat and electricity, and movement of the OFMSW and digestate in the plant. 239 Biogas combustion is generally considered carbon neutral since the emitted carbon was previously 240 absorbed from the atmosphere. However, to avoid double counting biogas combustion is considered 241 242 a positive emission since the emitted carbon was previously absorbed from the atmosphere (Aui et

al., 2019). Moreover, the fugitive CH₄ emissions during AD and CHP stages are considered equal to

244 1.5% of the CH₄ produced (Aui et al., 2019)

245 The electricity and heat generated by CHP unit and the compost were considered avoided emissions

aligned with (Carlsson et al., 2015).. The flows and the inventory data are reported in Table S7 andS8, respectively.

OFMSW was considered a zero burden according to (Lamnatou et al., 2019). The low heating value of the CH₄ was assumed to be 9.94 kWh/Nm³ according to (Rillo et al., 2020).

Life cycle impact assessment was performed with IPCC 2021 GWP 100 V0.1 which contains the global warming potential climate with change factors of IPCC with a timeframe of 100 y. The consistency of the results was proven through a sensitivity analysis by varying the CH₄ yields about \pm 5% volume according to (F. Demichelis et al., 2022).

- 254
- 255

2.6. Principal Components Analysis

Multivariate data analysis was carried out by two tools: pattern recognition through Principal 256 Component Analysis (PCA) (Massart, 1988) and classification using Supervised Kohonen's Self-257 Organizing maps (SKSOMs) (Melssen et al., 2006) (Brandi et al., 2021). The detailed methodology 258 of PCA and SOMs is reported in section 3 of SI. PCA provides the scores and the loadings. From the 259 score plot, the existence of groups of samples with similar or different behaviour is derived while 260 from the loadings plot, the correlations between the variables are highlights. Here, PCA was applied 261 after autoscaling (mean centering and normalization to unit variance) for a preliminary exploration 262 of the dataset. 263

Kohonen's Self-Organizing Maps (KSOMs) are artificial neural networks (i.e., mathematical algorithms) for solving complex problems by simulating the human brain functioning. Supervised Kohonen networks (SKN) are supervised methods for classification purposes (Melssen et al., 2006) (Brandi et al., 2021). Here, SKNs were run with the following settings: non-toroidal boundary, batch algorithm, squared topology, random initialization of weights, and learning rate decreasing linearly

from 0.5 to 0.01, a top map of 6 x 6 neurons, and 300 training epochs. To highlight the differences 269 between the HRT values (Y variable), the data were first centered according to the different pre-270 treatments and then range-scaled. The calculations were performed in cross-validation with Venetian 271 blind with 6 cancellation groups. PCA was carried out by MATLAB R2014a (The Mathworks, Natick, 272 MA, USA) using in-house-developed routines; Kohonen SOMs were built with the Kohonen and 273 CPANN toolbox for MATLAB from Milano Chemometrics (Ballabio et al., 2009). Graphical 274 representations were carried out by MATLAB, Statistica v.7 (Statsoft Inc., Tulsa, OK, USA), and 275 Excel 2016 (Microsoft Corporation, Redmond, WA, USA). 276

277

278 **3. Results and discussion**

279 **3.1 Anaerobic digestion in semicontinuous feeding mode**

The semi-continuous anaerobic digestion (sAD) of organic fraction of municipal solid waste (OFMSW) was performed in 1 L reactors, to detect the optimal working condition by varying the OLR. Three sAD configurations were tested: hydrodynamic cavitated OFMSW (HC-OFMSW), enzymatically pre-treated OFMSW (EN-OFMSW), and non-pre-treated OFMSW (AD-OFMSW) as reference. Figure 1 depicts the methane yields, the pH trends, and the volatile solids (vs) removal of the three sAD configurations at different OLRs.

- Five OLRs were tested, by changing the HRT. In detail it has been tested; HRT = 16 d (OLR = 3.38)
- 287 kgvs/m³d), HRT= 14 d (3.87 kgvs/m³d), HRT = 12 d (OLR = $4.50 \text{ kgvs/m^3}d$), HRT = 10 d (OLR = $10 \text{ kgvs/m^3}d$)
- 288 5.40 kgvs/m³d), and HRT = 8 d (OLR = 6.75 kgvs/m³d).
- Considering the results of Figure 1, two observations can be made: the first about the variation ofOLR and the second one about the effect of pre-treatments.
- Increasing OLR from 3.38 to 4.50 kgvs/m³d (HRT from 16 to 12 d) enhanced methane productions
- and VS removals, whereas the increase of OLR from 5.40 to 6.75 kgvs/m³d inhibited the sAD
- configurations as demonstrated by the decrease of methane productions and the drop of pH values.

HC- and EN-OFMSW reached higher methane production and VS removal values rather than sADOFMSW. Despite the increase in the OLR, pre-treatments can keep the anaerobic digestion process
stable and efficient since they increase the digestibility rate of the substrate by preventing the
inhibition effects (Wei et al., 2022).
Among the pre-treated sAD configurations, HC-OFMSW reached the highest methane production

and process stability.

Considering the variation of OLR from 3.38 to 4.50 kgvs/m³d, methane production of HC-OFMSW
rose from 0.29 to 0.38 Nm³/ kgvs d, whereas for EN-OFMSW and AD-OFMSW varied only from
0.24 to 0.32 Nm³/ kgvs d and from 0.19 to 0.25 Nm³/ kgvs d, respectively.

The increase of OLR from 5.4 to 6.75 kgvs/m³d (HRT from 10 to 8 d) decreased methane production for all three sAD configurations, but HC and EN pre-treatments buffered the inhibitory effects.

The effects of the variations of OLR were demonstrated by the pH and VS removal trends (in Figure 1). The pH provides information about the stability of the reaction medium since its variation is related both to the buffering capacity of the reaction system and to the variation in the equilibrium between the species, which participate in the trophic chain of the microorganisms involved in the

sAD process.

For pH values between 6.5 and 7.5, the AD process is generally considered stable (Morales-Polo et al., 2018), however, the pH can indicate unbalanced conditions of the sAD only with a certain delay compared to the evolution of the buffer effect of the substrate employed in the system.

For HC, EN, and AD-OFMSW, at OLR from 3.38 to 4.50 kgvs/m³d (HRT from 16 to 12 d), the pH was between 7 -7.5, which is the perfect range for AD (Chen et al., 2008), whereas at OLR from 5.4 to 6.75 kgvs/m³d (HRT from 10 to 8 d), the pH deeply decreased from 6.46 to 5.18, which proved the possible volatile fatty acid (VFA) accumulation (Morales-Polo et al., 2018).

High OLR can cause overloading which boosts faster hydrolysis and over-acidification of the medium, by promoting the over-accumulation of VFAs, which inhibits the methanogenesis and consequently stops AD process (Meegoda et al., 2018).

(Villa and Ferguson, 2016) investigated the effect of the overloaded grease-waste in the AD system, by finding that the quick shocks in the OLR variation could be able to cause shifts in microbial populations, and methane yields returning to normal levels after developing a tolerance to higher OLR. Hence, the study of (Villa and Ferguson, 2016) suggested that by improving the AD resistance to overloading, after the initial overloading, the system can develop diversified methanogenic microorganisms able to improve AD.

In the present study, the overloading equal to 6.75 kgvs/m^3 d inhibited the sAD, but the pre-treatment, especially of HC-OFMSW buffered the system and maintained the methane production and VS removal equal to $0.24 \text{ Nm}^3/\text{kgvs}$ d and 55.05 %w/w, respectively. These results agreed with the study of (Garuti et al., 2018) which tested OLR around 6 kgvs/m^3 d and measured, through rheological analysis, the capacity of HC to improve the mixing of the substrate without forming floating matter by increasing the OLR.

The VS removal trend was the same for all the three sAD configurations; it increased from OLR 3.38 to 3.87 kgvs/m³d (from HRT=16 d to 14 d), then it decreased from OLR = 4.50 to 6.75 kgvs/m³d (from HRT=12 d to 8 d).

Volatile solid (VS) is a fundamental parameter because it represents the measurement of the organic fraction of total solids and VS removal measures the digester efficiency and the quality of the digestate for further applications (Mei et al., 2016). Considering the sAD configurations, the highest VS removal was achieved by HC-OFMSW, followed by EN-OFMSW, and last AD-OFMSW.

In HC pre-treatment, the temperature (55°C) and high pressure prevent evaporation and boost hydrolysis by forming a substrate that is more biodegradable and more stable for AD also at high OLR (Barber, 2016). In HC, the implosion of the bubbles at a temperature of around 50 °C could boost the removal of VS and increase methane production (Calcio et al., 2018).

EN-OFMSW was performed with UPP2, which is a cocktail of cellulases, hemicellulases, pectinases, and proteases. UPP2 can boost the AD process by degrading the lignocellulosic wastes(Nabi et al., 2019). The advantage of UPP2, compared to other industrial enzymes is the shorter hydrolysis time

346 (2 h), moreover, the methane productions and VS removals obtained for OLR from 3.40 to 5.40
347 kgvs/m³d agreed with the ones described in the research of (Khanh Nguyen et al., 2021).

Considering the methane productions, pH, and VS removals, the most promising OLRs were in the 348 range of 3.38 and 3.87 kgvs/m³d, (HRT 16 and 14 d) for sAD-OFMSW, between 3.87 and 4.50 349 kgvs/m³d (HRT 16 and 12 d) for EN-OFMSW, and in the range of 3.87- 5.40 kgvs/m³d, (HRT 16 and 350 10) for HC-OFMSW. The HC-OFMSW reached the highest methane production and process stability 351 rather than the EN-OFMSW and AD-OFMSW because thermo-physic pre-treatment at T < 100 °C 352 can destroy the lignocellulosic barrier without creating recalcitrant molecule (Balasundaram et al., 353 2022) and increasing the substrate availability to biochemical reaction (Mohammad Rahmani et al., 354 2022). 355

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Fig. 1. Methane and pH daily productions and VS removal trends according to the hydraulic retention time (HRT)

363 **3.2 Cost-benefit analysis**

- The cost-benefit analysis aimed to evaluate the cost-effectiveness and to identify the barriers to implementing a cost-effective strategy for the investigated 15 sAD.
- From an economic perspective, the choice of OLR value is difficult to choose because high OLR compared to low OLR implies smaller reactor volumes, but larger amounts of digestate to daily
- handle. Fig. 2 depicts the net present value (NPV) of the three sAD configurations in the plant life of
- 20 y that produced 1 MWh/d primary energy. The payback time (PBT) is reported in Table S6 of
- 370 supplementary information. The results achieved by the cost-benefit analysis can be studied based on
- the effect of the OLR and pre-treatments.
- 372 Considering the OLR variation, for all three sAD configurations (AD-OFMSW, EN-OFMSW, and
- HC-OFMSW) the NPV increased from OLR 3.38 to 3.87 kgvs/m³d.
- For all the sAD configurations, the highest NPV and lowest PBT were observed for OLR of 3.87
- kgvs/m³d (HRT = 14 d) according to (Choudhary et al., 2020).
- In detail, for OLR equal to 3.87 kgvs/m³d, NPV was 43827.12 € for HC-OFMSW, 39316.77 € for
- EN-OFMSW, and 32587.11 € for sAD-OFMSW.
- The decrease in NPV occurred from 4.50 to 6.75 kgvs/m³d. In detail, at OLR = 6.75 kgvs/m³d all
- three sAD configurations achieved a negative NPV and PBT longer than the chosen plant life (20 y).
- 380 Considering the effect of pre-treatment, the highest NPV and shortest PBT were achieved by HC-
- 381 OFMSW, followed by EN-OFMSW, and last AD-OFMSW.
- For AD-OFMSW, the NPV was positive, and the PBT ranged between 5-6 years, for OLR from 3.38
 to 3.87 kgvs/m³d (HRT= 16-14 days).
- For EN-OFMSW, the NPV was positive, and the PBT varied from 3-7 years, for OLR from 3.38 to
- 385 $4.50 \text{ kgvs/m}^3 \text{d}$ (HRT= 16-12 days).
- In the case of HC-OFMSW, the NPV was positive, and the PBT ranged between 2-18 years, for OLR
- 387 from 3.38 to 5.40 kgvs/m³d (HRT= 16-10 days).

This trend proved that the pre-treatments could be economic profitability offsetting the inhibition due to the increase of OLR.

The possibility of pre-treated AD being economically profitable even at higher OLR was due to the possibility of reducing the working volume of the sAD reactor and consequently, the capital costs decreased.

Indeed, the capital costs for EN-OFMSW at an OLR range of 3.87-4.50 kgvs/m³d were lower than those at an OLR of 3.38 kgvs/m³d. Similarly, for HC-OFMSW, the capital costs at an OLR range of 3.87-5.40 kgvs/m³d were lower than those at an OLR of 3.38 kgvs/m³d.

However, it is important to underline that even for pre-treatments, the increase in OLR reduced theNPV, but pre-treatments were able to slow down plant impairment.

These results are of fundamental importance since usually, capital and operational costs of sAD with pre-treatment are critical and expensive due to the application of additional energy or chemicals (Michalsk and Ledakowicz, 2014).

Usually, the main economic issue related to pre-treatments is the possible economic unprofitability 401 since the enhancement of methane yields could not offset the efforts of the item and energy required 402 by pre-treatment (Fu et al., 2018). Whereas, in the present study, the pre-treatments were 403 economically profitable up to OLR = $4.50 \text{ kgvs/m}^3 \text{d}$ (HRT = 12 d) for EN-OFMSW and OLR=5.40404 kgvs/ $m^{3}d$ (HRT = 10 d) for HC-OFMSW. In both HC- and EN-OFMSW configurations, the pre-405 treatment unit represented less than 15 % of capital costs and 10 % of the operational ones. As stated 406 by the technical performances, the pre-treatment allowed the process to be stable to produce enough 407 408 methane to overcome the energy required to manage the process.

409 HC-OFMSW reached higher economic profitability than EN-OFMSW because the bottleneck of EN410 OFMSW was the cost of the enzyme UPP2, the regulation of pH for UPP2 through the addition of

411 NaOH and the duration of pre-treatment (EN-OFMSW= 2 h vs HC-OFMSW= 10 min). Hence, HC-

412 OFMSW could be economically profitable until OLR = $5.40 \text{ kgvs/m}^3 \text{d}$, whereas EN-OFMSW could

413 be until OLR= $4.50 \text{ kgvs/m}^3 \text{d}$.

Usually, enzyme-assisted AD is not economically profitable due to the cost of the enzyme (Khanh
Nguyen et al., 2021). This cost could be overcome by the implementation of a cocktail of enzymes
like UPP2, which promoted a synergistic effect during the floc structure disintegration of OFMSW
(Salihu and Alam, 2016). However, also a cocktail of enzymes has drawbacks as the request of time
for the hydrolysis and correction of pH (Meegoda et al., 2018) because UPP2 required 2 h and pH =
7-7.5 obtained with NaOH addition.

The studies of (Jiang et al., 2020) reported that microaerobic pre-treatment is also an alternative pretreatment, both for solid substrate as cellulosic substrate and liquid substrate as food waste and brown water, through which aerobic microorganisms could be enriched and produce more extracellular enzymes like cellulase to boost the hydrolysis rate. Microaerobic pre-treatment could be more economical than other biological pre-treatments since it does not require an addition of chemicals.

To conclude the cost-benefit analysis, HC-OFMSW reached the highest values of NPV and shortest PBT for all the tested OLRs. The economic profitability of HC-OFMSW until OLR = 5.40 kgvs/m^3 d disagreed with the study of (Passos et al., 2017). (Passos et al., 2017) investigated the thermochemical pre-treatment (i.e. HC-OFMSW) of dairy cow manure at OLR= 5.25 kgvs/m^3 d, resulting in more expensive than AD performed without pre-treatment.

EN-OFMSW was less advantageous than HC-OFMSW, because the marginal biogas increase may
not be able to justify the expensive cost of enzymes according to (Montgomery and Bochmann, 2014).
However, both pre-treatments HC- and EN-OFMSW reached economic performance higher than the
ones of AD-OFMSW.



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Fig. 2. Net present value (NPV) of the sAD configurations gathered per type of pre-treatmentsaccording to hydraulic retention time (HRT).

437 **3.3 Environmental evaluation**

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Carbon footprint analysis (CF) was performed according to ISO 14067 on three sAD configurations 438 by varying five OLRs from 3.38 to 6.75 kgvs/m³d. The results of CF analysis (Fig. 3) were expressed 439 on the base of the functional unit (FU) equal to 1 MWh/d of produced primary energy. The method 440 IPCC 2021 GWP 100 V0.1 was adopted to calculate the climate change impact category. The results 441 achieved through the CF analysis are evaluated according to the effect of the OLR and pre-treatments. 442 Considering the variation in OLR, all three sAD configurations (AD-OFMSW, EN-OFMSW, and 443 HC-OFMSW) exhibited a decrease in GWP impacts within the OLR range of 3.38-3.87 kgvs/m³d, 444 indicating a reduction in emissions, and detail the avoidance of emissions. The most significant 445 reductions in GWP impacts were-at OLR of 3.87 kgvs/m³d (HRT = 14); in detail: -407.21-kg CO₂ 446 eq/FU for HC-OFMSW, -377.18 kg CO2 eq/FU for EN-OFMSW and -336.44 kg CO2 eq/FU for sAD-447 OFMSW. At OLR \geq 4.50 kgvs/m³d for pre-treated sAD, and at OLR > 3.87 kgvs/m³d for AD-448 OFMSW, the GWP increased because the CH₄ production slightly decreased by increasing of the 449 OLR. Additionally, the energy requirements increased with the increase of OLR from 3.87 to 6.75 450 kgvs/m³d, primarily due to a higher daily substrate feed (+ 3.55% for AD-OFMSW, +3.2% for EN-451 OFMSW, and +2.5% for HC-OFMSW in Table S5). 452

Considering the effect of the pre-treatments, the lowest GWP impacts were achieved by HC-OFMSW, 453 followed by EN-OFMSW, and last AD-OFMSW. This rank agreed with the technical and cost-benefit 454 analyses (paragraphs 3.1 and 3.2). In detail, the GWP impacts were negative for AD-OFMSW from 455 3.38 to 3.87 kg_{vs}/m³d (HRT= 16-14 d), for EN-OFMSW from 3.38 to 4.50 kgvs/m³d (HRT= 16-12 d) 456 and for HC-OFMSW from 3.38 to 5.40 kgvs/m³d (HRT= 16-10 d). This trend proved that the pre-457 treatments could buffer the inhibition occurring with the increase of OLR because they increased the 458 content of organic matter available to be converted into methane (Deepanraj et al., 2017). 459 Most of the available studies about environmental evaluation focusing on GWP referred to existing 460

industrial AD plants. The present study compared the results achieved with studies concerning the

climate change impact category of real AD-OFMSW or real AD of agro- waste in the European 462 scenarios, to be closer to the geographical context and feedstock treated in the present study. 463

The results of CF-analysis of sAD-OFMSW agreed with most of the studies performed in batch, feed-464

OLR = 3.87 kgvs/m³d, the GWP agreed with the study of (Mezzullo et al., 2013), which investigated

batch, and continuous AD performed with OFMSW (Kumar and Samadder, 2020), and in detail for

the same OLR, in which the avoided impacts were mainly due to the replacement of the energy and 467 fertilizer productions. 468

The results obtained in the present study by AD-OFMSW within the range of 3.38 and 4.50 kgvs/m³d 469 aligned with previous studies utilising 1 MWh/d as a functional unit. Examples include the study of 470 (Fusi et al., 2016) and (Bacenetti et al., 2016) reporting -375 and 408 kg CO₂eq/FU for sAD feed with 471 agro-waste and silage, respectively.

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The GWP impacts reached by EN-OFMSW for HRT = 14-12 d agreed with the one obtained by 473 (Agostinho et al., 2015) under similar working conditions with cellulase enzyme applied on 474 lignocellulosic material before anaerobic digestion at the industrial scale. 475

The avoided emissions achieved by HC-OFMSW in the range 3.87-5.40 kgvs/m³d were higher than 476 those obtained by (Vosooghnia et al., 2021) through ultrasound pre-treated agro-waste before AD. 477

In detail, the avoided emissions for HC-OFMSW at 4.50 kgvs/m³d were around 6 % higher than the 478 479 ones reached by the plant in the UK, performing thermos-mechanical pre-treatment, (Evangelisti et al., 2014) and by the plant in France, performing physical pre-treatment, (Lamnatou et al., 2019) 480 under similar OLR. 481

These results proved that EN and HC performed in the present study were lower impactful than other 482 pre-treatments (already implemented at the industrial scale) since they can solubilise the complex 483 organic substrates improving their biodegradability even at high OLR. 484

The energy cost to carry out HC was offset by the methane surplus produced by HC-OFMSW at 485 OLRs from 3.38 to 5.40 kgvs/m³d, since for these OLRs the HC reached positive energy balance due 486

to lower energy plant and operating costs, higher process yields and energy savings due to shorter
pre-treatment process times (10 min) (Calcio et al., 2018).

The EN-OFMSW avoided emissions for OLR from 3.38 to 4.50 5.40 kgvs/m³d and achieved positive 489 emissions with OLR from 5.40 to 6.75 kgvs/m³d. The GWP impacts related to EN-OFMSW were 490 higher than the those of HC-OFMSW, since EN lasted 2 h and required the addition of NaOH to keep 491 pH around 7-7.5 for the UPP2 activity. Furthermore, EN reached lower methane production and pH 492 stability compared to HC. In the scientific literature, there are no specific studies about the 493 environmental impacts of sAD performed with a cocktail of enzymes like UPP2. However, the GWP 494 contribution of enzyme in EN-OFMSW cannot be neglected since the enzyme production represents 495 496 10.5-11% of the total GWP impact in enzymatic pre-treatment (Olofsson et al., 2017). In the CF analysis of the present study, the GWP associated with UPP2 was taken from Ecoinvent 3.5 497 considering a generic enzyme (secondary data) because no data were available for UPP 2 since it is a 498 patented commercial cocktail of cellulases, hemicellulases, pectinases, and proteases. 499

The sensitivity analysis confirmed the GWP results and the trends. HC-OFMSW and EN-OFMSW played a positive effect on the increase in OLR because they buffered the inhibition and kept the process stable, which means lower CO_2 eq emissions (Wei et al., 2022). The results confirmed HC-OFMSW as an effective pre-treatment (Saxena et al., 2019).

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Fig. 3. CF results calculated with the metho IPCC 2021 GWP 100 V0.1

3.4 Principal Component Analysis Principal Component Analysis (PCA) was applied to the dataset consisting of the 30 samples (6 HRT values x 3 pre-treatments x 2 replicates) described by 8 variables (biogas and methane productions, VS removal, OFMSW feed, OLR, GWP, NPV, and PBT). Data were auto-scaled (mean centering followed by normalization to unit variance) hence all the variables accounted for the same amount of information. The first two PCs calculated explain about 90% of the overall information (73.89 % PC1

514 and 16.11 % PC₂).

515 Considering the loading plot (Fig. 4a), different behaviours can be detected:

- GWP, OFMSW feed, PBT and OLR show positive weights on PC1, while biogas, CH4,
 VS removal and NPV are at negative weights on the same PC. These two groups of
 variables are negatively correlated with each other (when one group of variables increases
 the other one decreases and vice versa)
- NPV was negatively correlated to OFMSW feed; indeed, when OFMSW feed increased
 NPV decreased, and vice versa.
- Biogas and CH₄, were strongly correlated with each other, as is the case of PBT, OLR and
 GWP.

The score plot proves that the samples were grouped predominantly according to the HRT (or to the corresponding OLR), however, within each HRT group (or corresponding OLR) the samples were clustered according to the pre-treatment. Samples with HRT=8 d (OLR = $6.75 \text{ kg}_{vs}/\text{m}^3$ d) were at positive scores on PC₁, showing higher values of OFMSW feed, GWP, PBT and OLR. This is more evident for AD-OFMSW, while the trend decreases for EN-OFMSW and HC-OFMSW samples respectively.

HRT= 8 d and HRT=14 d exhibited opposite trends, indeed HRT=8 d samples reached low values of
VS removal, NPV, biogas, and CH4, whereas the samples with HRT= 14 d reached high values of
these variables and low values of OFMSW feed, GWP, OLR and PBT.

At HRT=14 -16 d, all the tested configurations reached high NPV, but the HC configuration depicted higher NPV values, biogas productions and CH₄ productions, compared to EN-OFMSW and above all AD-OFMSW. This trend was due to the pre-treatment unit able to cover the costs for the additional energy or chemicals and increase the process stability (Michalsk and Ledakowicz, 2014).

HRT samples were in groups with a progressive changing of their position in the score plot: from right, counter clockwise, there are HRT= 8, 10, 12, 14, and 16 d. From the initial behaviour of HRT=8 d samples, at HRT=10 and 12 d, all the samples are at positive or slightly negative values on PC₂, but the behaviour seems more dependent on the pre-treatment applied, with AD samples are in fact at more positive values on PC₁ (higher OLR, PBT, GWP, OFMSW feed; lower biogas, NPV, CH₄, and VS removal).

543 HRT=14 d samples were characterized by the lowest values of OLR, PBT, GWP, and the best balance

between CH4 and biogas productions and VS removal, on one side, and NPV, on the other.

545 HRT=16 d samples behaved like HRT=14 d samples but with lower NPV and lower biogas, CH₄, and
546 VS removal.

547 The pre-treatments reached the same trend for almost all HRT values, and HC-OFMSW samples 548 achieved better results than EN-OFMSW for all the investigated HRT, with the only exception of 549 HRT= 12 for which they exhibited similar results.

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Fig. 4. PCA results are represented by loading plot (a) and score plot (b). where the samples are labelled according to both the treatment applied and the HRT: the treatment is indicated with a different shape of the label (AD as circles, HC as triangles, EN as squares), while the HRT is indicated by a different colour (HRT 16 = brown, 14 = red, 12 = yellow, 10 = green, 8 = blue).

b

557 3.5 Supervised Kohonen's Self-Organizing Maps

To highlight more clearly the effects of HRT and OLR, notwithstanding the pre-treatment, data were 558 centred according to the pre-treatments. Supervised Kohonen self-organizing maps were applied as 559 classification method for discriminating the samples according to the HRT value. Calculations were 560 performed with a top map of 6x6 squared neurons and 300 epochs; cross-validation was applied with 561 a Venetian blind procedure with 6 cancellation groups. Fig. 5a reports the top map, where neurons are 562 563 coloured according to the class assignation; samples in the same neuron or adjacent neurons are more similar. Fig. 5b-i represents the trend of the weights of each original variable concerning the 564 565 separation of the samples identified on the top map. All the samples were correctly classified the according to HRT, both in fitting and cross-validation, as can be noticed by the top map (Fig. 5a). 566 Replications were in the same neuron or adjacent ones; furthermore, all the samples with HRT=14 d 567 or HRT=16 d were in the same neuron, showing lower variabilities if compared to the other classes. 568 These results confirmed the choice of the corrective factor for CH₄ values (paragraph 2.4). The plots 569 of the weights of each original variable (Fig. 5 b-i) are represented on a colour scale from black 570 (weights=1) to white (weights=0). For a given variable, when the colour of a neuron containing one 571 or more samples is black, those samples showed a high value of the considered variable; whereas 572 when the neuron is white, the samples showed a low value of that variable. Considering the plots of 573 the weights, it was possible to verify the differences existing between the classes. 574

OFMSW feed, GWP and OLR exhibited almost identical behaviours, with high values for
 HRT=8 d, smaller values for HRT=10 d, and the best values for HRT ≥14 d.

- Biogas and CH₄. were high for HRT=10 (especially biogas) and 12 d (especially CH₄), while
 HRT=14 d reached quite high values of biogas and very high for CH₄. VS removal was high
 for HRT=14 and 12 d. Biogas, CH₄, and VS removal were low for HRT=8 d.
- PBT was high for all the HRT values except for HRT=14 and 16 d, for which it was very low
 NPV showed very low values for HRT=8 d (for all the sAD configuration it was negative)
 and especially high for HRT ≥14 d.



Fig. 5. Supervised Kohonen's self-organizing maps: top map (a); the plot of the weights of each original variable (b-i) – black corresponds to weights = 1, white corresponds to weights = 0.

591 4 Conclusions

This study investigated the technical feasibility, economic viability, and carbon footprint-analysis 592 (CF) of semi-continuous anaerobic digestion (sAD) of the organic fraction of municipal solid waste 593 (OFMSW). The study explored the effect of pre-treatment on sAD by increasing the organic loading 594 rates (OLR) from 3.38 to 6.75 kgvs/m³d. Two pre-treated sAD configurations were investigated: 595 hydrodynamic-cavitated (HC-OFMSW), enzymatically pre-treated (EN-OFMSW), and one non-pre-596 treated (AD-OFMSW) as control. The results of the three-fold sustainability evaluations were 597 elaborated through Principal Component Analysis and Supervised Kohonen's Self-Organizing Maps. 598 599 Statistical analysis highlighted that the samples were grouped predominantly according to the OLR (or to the corresponding HRT), however, within each HRT group the samples were clustered 600 according to the pre-treatment. 601

The main finding pointed out that pre-treated sADs were technically feasible, economically profitable, and carbon negative up to 4.50 kgvs/m³d for EN-OFMSW and 5.40 kgvs/m³d for HC-OFMSW. In contrast, the sAD-OFMSW exhibited viability only up to OLR of 3.87 kgvs/m³d.

These outcomes were important because pre-treatments, conventionally considered expensive due to additional energy or chemicals, emerged as viable solutions to be implemented at the industrial scale. Pre-treated-AD required smaller plants compared to AD-OFMSW by treating a higher amount of OFMSW per unit of volume and time and by producing higher amount of renewable energy. Hence, HC-OFMSW can contribute to the decarbonization of the energy-productive system and enhance waste management. These actions aligned both with the principles of Bioeconomy and contributed to the target outlined in Mission 2 of the National Recovery Plan.

612

613 Statements and Declarations

614 Authorship contribution statement

- 615 The contributions of the authors are detailed in the following. F. Demichelis contributed to the
- 616 conceptualization of the study, she carried out the experimental tests and wrote part of the paper. F.A.
- 617 Deorsola contributed to realizing the methodology and reviewed the paper, and T. Tommasi reviewed
- 618 the manuscript. E. Robotti coordinated and realized the modelling study and contributed to writing
- and reviewing the manuscript. E. Marengo contributed to the modelling analysis and D. Fino
- 620 supervised the study.

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624 Competing interests

- 625 The authors declare no competing interests.
- 626 Data availability
- 627 Data are completely reported in the manuscript and the supplementary material.

628 Appendix

629 The appendix provided information about environmental and economic analysis.

630

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Highlights

- Semi-continuous anaerobic digestion (sAD) evaluated the organic loading rates (OLR).
- Organic fraction of municipal solid waste was physical and biological pre-treated.
- Techno-economic-environmental study on sAD was elaborated with statistical analyses.
- sAD were grouped by OLR and within each group, sAD were clustered by pre-treatment.
- Physical pretreatment was economic and environmentally viable up to 5.40 kgvs/m³d.
- Semi-continuous anaerobic digestion (sAD) investigated five organic loading rates (OLR).
- Organic fraction of municipal solid waste was physical and biological pre-treated.
- Principal component analysis and Kohonen supervised map were calculated.
- The best performances were reached at OLR = 3.87 kg/m³d

Declaration of interests

☑ 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.

□ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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