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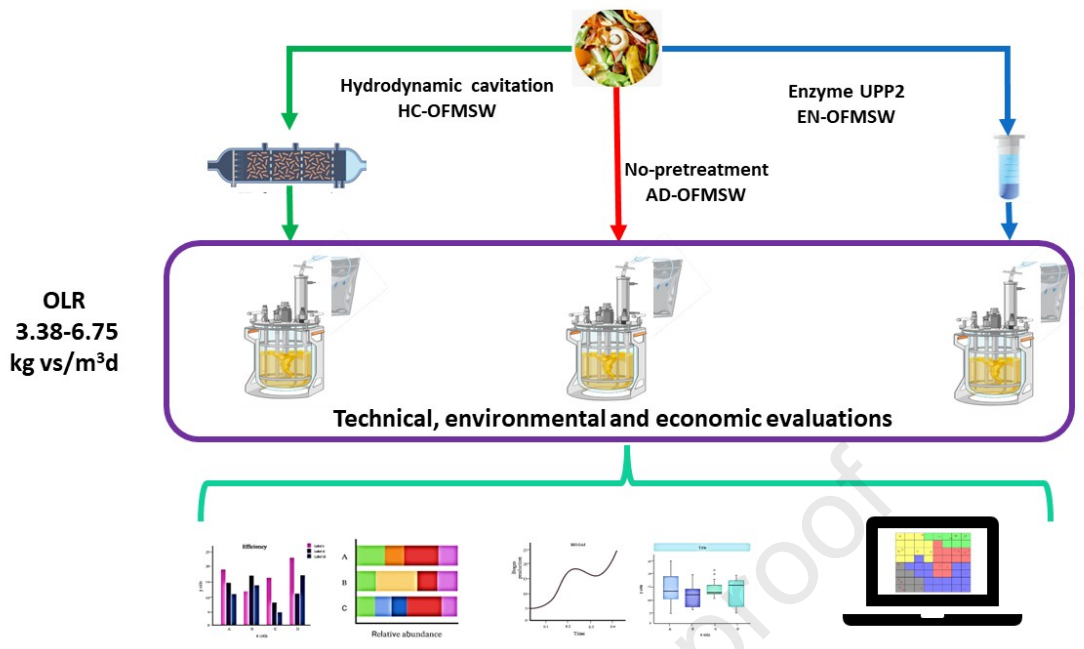
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1 **Modelling of technical, environmental, and economic evaluations of the effect of the organic**
2 **loading rate in semi-continuous anaerobic digestion of pre-treated organic fraction municipal**
3 **solid waste.**

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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/m³d. Three sAD configurations were investigated: hydrodynamic-
18 cavitated (HC-OFMSW), enzymatically pre-treated (EN-OFMSW), and non-pre-treated (AD-
19 OFMSW). Principal Component Analysis and Supervised Kohonen's Self-Organizing Maps
20 combined the experimental, economic, and environmental evaluations.

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

23 The finding highlighted that pre-treatments offset inhibition in sAD of OFMSW due to the OLR
24 increase, being economically profitable and CF negative up to 4.50 kgvs/m³d for EN-OFMSW and
25 to 5.40 kgvs/m³d for HC-OFMSW. Whereas sAD-OFMSW remained economically and
26 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
28 from 17679.30 to 43827.12 euros and from -51.08 to -407.210 kg CO₂eq/1 MWh daily produced, by
29 decreasing the OLR from 5.40 to 3.87 kgvs/m³d.

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

32

33 **1. Introduction**

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

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

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

79 $\text{COD}/\text{m}^3\text{d}$ with an optimal OLR of $8\text{ kg COD}/\text{m}^3\text{d}$. All the above-mentioned studies only investigate
80 OLR from a technical point of view.

81 The present study aims to investigate the possibility of managing the increase of the OLR of sAD
82 through the performance of pre-treatment on OFMSW before sAD in one single stage. In detail, this
83 study analyses the effect of enzymatic and hydrodynamic-cavitation pre-treatments performed in
84 (Demichelis, F. et al., n.d.) and (Demichelis et al., 2023), respectively, by increasing the OLR from
85 3.38 to $6.75\text{ kgvs}/\text{m}^3\text{d}$ corresponding to HRT from 16 to 8 d. The novelty of this study is in not limiting
86 the research to the identification of the most technically high-performing sAD configuration but
87 combining it with the sustainability analysis through statistical elaborations. The three-fold metrics
88 are adopted by including technical feasibility analysis through laboratory tests, economic viability
89 through cost-benefit analysis, and environmental sustainability by carbon footprint analysis (CF)
90 according to ISO 14067. The results of the three-fold metrics are elaborated with Principal
91 Component Analysis and Supervised Kohonen's Self-Organizing Maps. To the best of the author's
92 knowledge, no studies are currently available regarding the adoption of pre-treatment to address the
93 increased OLR in sAD of OFMSW. Additionally, there is a lack of technical, economic, and
94 environmental studies in the literature about the sAD of OFMSW considering the effect of the OLR.
95 Moreover, a standardised assessment of the three-fold sustainability metrics is not available yet but
96 is necessary as stated by (Rajendran and Murthy, 2019).

97

98 **2. Materials and methods**

99 **2.1 Substrate**

100 The organic fraction of municipal solid waste (OFMSW) was supplied by San Carlo S.p.A (Fossano,
101 Italy). To start the semicontinuous anaerobic digestion (sAD) the mesophilic digestate of cow-
102 agriculture sludge (CAS) was used as inoculum according to the previous study of (F Demichelis et
103 al., 2022). OFMSW and inoculum properties agreed with the study of (Srisowmeya et al., 2020) and
104 (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**

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

118 HC was performed with a rotor/stator HC-unit (Rotocav[®], E-PIC srl – Mongrando, Italy) at 55 °C for
119 10 min (Bruni et al., 2010). HC is a pre-treatment that promotes the formation, growth, and implosion
120 of vapor bubbles in a liquid at temperatures lower than the boiling point, which generates
121 microenvironments characterized by locally very high temperatures and intense pressure waves.

122 EN was performed with UltraPract[®] P2 (UPP2), which is a mix of cellulases, hemicellulases,
123 pectinases, and proteases, and it has been designed for biogas plants treating vegetables. UPP2 was
124 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
125 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

130 **2.3 Anaerobic digestion**

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

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

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

151 At the end of each tested HRT, the volatile solid (VS) was removed and volumetric biogas productions
 152 were measured to evaluate the performances of sAD.

153 The VS removed was evaluated with Eq. 1 (Li et al., 2018).

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

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

157

158 **2.4 Economic analysis**

159 The economic study was performed based on a cost-benefit analysis to evaluate the cost-effectiveness
 160 and to identify the barriers to implementing a cost-effective strategy for the 15 sAD investigated (3
 161 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
 165 plant with a capacity of production of 1 MWh/d of primary energy. The process was based on the data

166 obtained at the laboratory scale, but it was scaled up at the industrial level through assumption and
167 corrective factors based on (Bruno et al., 2023) (Green and Southhard, 2019) and (Turton et al., 2018).
168 The process flow diagram consisted of four phases: the first is the pre-treatment unit (enzymatic and
169 hydrodynamic cavitation pre-treatments), the second one is the sAD, the third phase is the CHP unit,
170 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).

173 (Green and Southhard, 2019) suggested assuming a scale factor equal to 90 % of the lab scale values.

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

181 In the present study, it has been tested; HRT = 16 d (OLR = 3.38 kgvs /m³d), HRT= 14 d (3.87 kgvs
182 /m³d), HRT = 12 d (OLR = 4.50 kgvs /m³d), HRT = 10 d (OLR = 5.40 kgvs /m³d), and HRT = 8 d
183 (OLR = 6.75 kgvs /m³d).

184 The corrective factors were as follows: 90 % of the OLR from 3.38 to 3.87 kgvs/m³d, 80 % for OLR
185 = 4.50 kgvs /m³d, 70 % for OLR = 5.40 kgvs/m³d, and 60 % for OLR 6.75 kgvs/m³d, based on the
186 CH₄ specific production values at the laboratory scale. The CH₄ yields, obtained by adopting the
187 scale-corrective factor, were comparable to the ones available for the industrial plants of OFMSW
188 (plant available in Piedmont, Italy) and industrial scale co-digestion of agro-waste according to (Naqi
189 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 study
191 of (Huiru et al., 2019). The composting unit was designed considering a residence time of 90 d

192 according to (Evangelisti et al., 2014). The area of the composting unit is 4 times the height of the
 193 composting 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
 195 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
 199 and considering a 5% discount for the future cash flows referring to the present value (Pleissner et
 200 al., 2016).

201 $NPV > 0$ means that sAD process is profitable.

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

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

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

$$207 \quad PBT (y) = \frac{C_0}{\text{Net cash flow per period}} \quad (4)$$

208

209 **2.5 Environmental analysis**

210 The carbon footprint (CF) analysis was performed according to ISO 14067, with the database

211 Ecoinvent 3.5 and the software SimaPro 9.5.02. The CF analysis compared the global warming

212 potential (GWP) of the 15 (3 pre-treatments x 5 OLR) tested sAD configurations focusing both on

213 the pre-treatment and variations of OLR. To compare these 15 sAD configurations, the functional unit

214 (FU) was assumed equal to 1 MWh/d of produced primary energy according to (Bruno et al., 2023).

215 The adopted approach was from grave to gate according to (Ugwu et al., 2022), which means from

216 the pre-treatment of OFMSW (grave) to bioenergy production (gate) (as reported in Figure S1). The

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

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

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

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

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

243 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
246 aligned with (Carlsson et al., 2015).. The flows and the inventory data are reported in Table S7 and
247 S8, respectively.

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

250 Life cycle impact assessment was performed with IPCC 2021 GWP 100 V0.1 which contains the
251 global warming potential climate with change factors of IPCC with a timeframe of 100 y. The
252 consistency of the results was proven through a sensitivity analysis by varying the CH₄ yields about
253 $\pm 5\%$ volume according to (F. Demichelis et al., 2022).

254

255 **2.6. Principal Components Analysis**

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

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

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

277

278 **3. Results and discussion**

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

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

286 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 kgvs/m³d), HRT = 10 d (OLR =
288 5.40 kgvs/m³d), and HRT = 8 d (OLR = 6.75 kgvs/m³d).

289 Considering the results of Figure 1, two observations can be made: the first about the variation of
290 OLR and the second one about the effect of pre-treatments.

291 Increasing OLR from 3.38 to 4.50 kgvs/m³d (HRT from 16 to 12 d) enhanced methane productions
292 and VS removals, whereas the increase of OLR from 5.40 to 6.75 kgvs/m³d inhibited the sAD
293 configurations as demonstrated by the decrease of methane productions and the drop of pH values.

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

298 Among the pre-treated sAD configurations, HC-OFMSW reached the highest methane production
299 and process stability.

300 Considering the variation of OLR from 3.38 to 4.50 kgvs/m³d, methane production of HC-OFMSW
301 rose from 0.29 to 0.38 Nm³/ kgvs d, whereas for EN-OFMSW and AD-OFMSW varied only from
302 0.24 to 0.32 Nm³/ kgvs d and from 0.19 to 0.25 Nm³/ kgvs d, respectively.

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

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

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

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

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

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

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

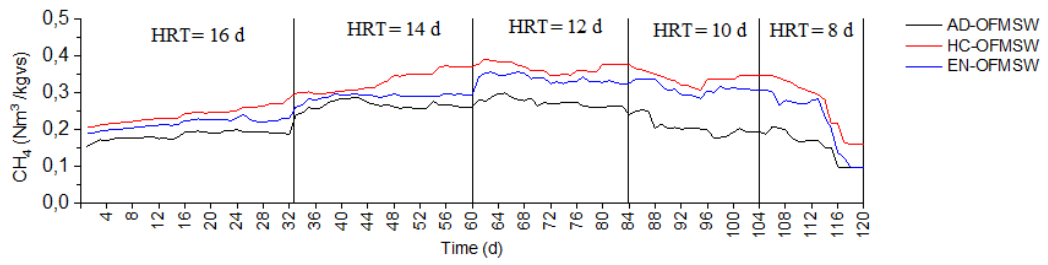
332 The VS removal trend was the same for all the three sAD configurations; it increased from OLR 3.38
333 to $3.87 \text{ kg}_{\text{vs}}/\text{m}^3\text{d}$ (from HRT=16 d to 14 d), then it decreased from OLR = 4.50 to $6.75 \text{ kg}_{\text{vs}}/\text{m}^3\text{d}$
334 (from HRT=12 d to 8 d).

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

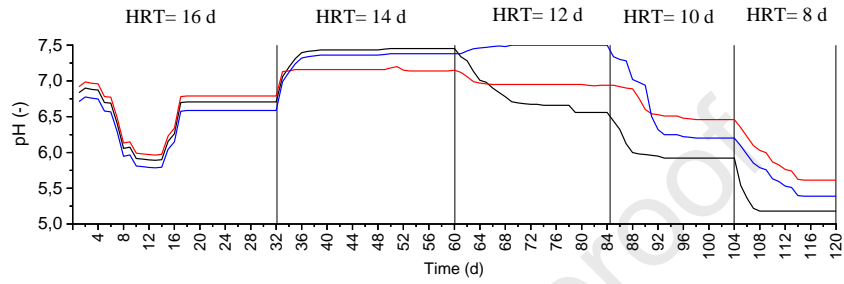
339 In HC pre-treatment, the temperature (55°C) and high pressure prevent evaporation and boost
340 hydrolysis by forming a substrate that is more biodegradable and more stable for AD also at high
341 OLR (Barber, 2016). In HC, the implosion of the bubbles at a temperature of around 50°C could
342 boost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

343 EN-OFMSW was performed with UPP2, which is a cocktail of cellulases, hemicellulases, pectinases,
344 and proteases. UPP2 can boost the AD process by degrading the lignocellulosic wastes (Nabi et al.,
345 2019). The advantage of UPP2, compared to other industrial enzymes is the shorter hydrolysis time

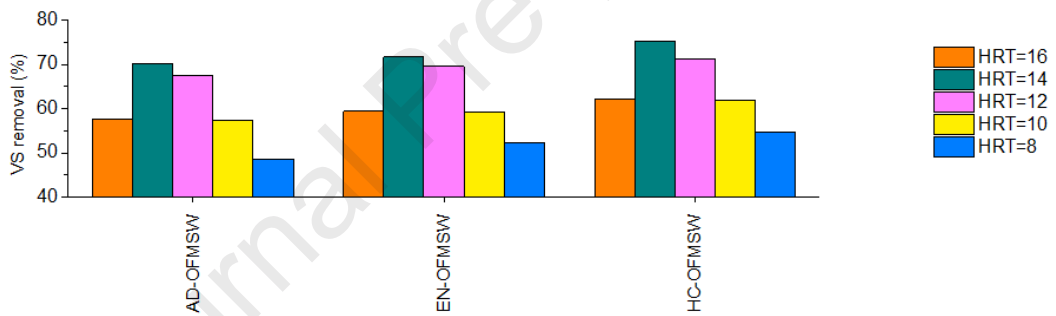
346 (2 h), moreover, the methane productions and VS removals obtained for OLR from 3.40 to 5.40
347 kgvs/m³d agreed with the ones described in the research of (Khanh Nguyen et al., 2021).
348 Considering the methane productions, pH, and VS removals, the most promising OLRs were in the
349 range of 3.38 and 3.87 kgvs/m³d, (HRT 16 and 14 d) for sAD-OFMSW, between 3.87 and 4.50
350 kgvs/m³d (HRT 16 and 12 d) for EN-OFMSW, and in the range of 3.87- 5.40 kgvs/m³d, (HRT 16 and
351 10) for HC-OFMSW. The HC-OFMSW reached the highest methane production and process stability
352 rather than the EN-OFMSW and AD-OFMSW because thermo-physic pre-treatment at T < 100 °C
353 can destroy the lignocellulosic barrier without creating recalcitrant molecule (Balasundaram et al.,
354 2022) and increasing the substrate availability to biochemical reaction (Mohammad Rahmani et al.,
355 2022).
356



357



358



359

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

362

363 3.2 Cost-benefit analysis

364 The cost-benefit analysis aimed to evaluate the cost-effectiveness and to identify the barriers to
365 implementing a cost-effective strategy for the investigated 15 sAD.

366 From an economic perspective, the choice of OLR value is difficult to choose because high OLR
367 compared to low OLR implies smaller reactor volumes, but larger amounts of digestate to daily
368 handle. Fig. 2 depicts the net present value (NPV) of the three sAD configurations in the plant life of
369 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
371 the effect of the OLR and pre-treatments.

372 Considering the OLR variation, for all three sAD configurations (AD-OFMSW, EN-OFMSW, and
373 HC-OFMSW) the NPV increased from OLR 3.38 to 3.87 kgvs/m³d.

374 For all the sAD configurations, the highest NPV and lowest PBT were observed for OLR of 3.87
375 kgvs/m³d (HRT = 14 d) according to (Choudhary et al., 2020).

376 In detail, for OLR equal to 3.87 kgvs/m³d, NPV was 43827.12 € for HC-OFMSW, 39316.77 € for
377 EN-OFMSW, and 32587.11 € for sAD-OFMSW.

378 The decrease in NPV occurred from 4.50 to 6.75 kgvs/m³d. In detail, at OLR = 6.75 kgvs/m³d all
379 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.

382 For AD-OFMSW, the NPV was positive, and the PBT ranged between 5-6 years, for OLR from 3.38
383 to 3.87 kgvs/m³d (HRT= 16-14 days).

384 For EN-OFMSW, the NPV was positive, and the PBT varied from 3-7 years, for OLR from 3.38 to
385 4.50 kgvs/m³d (HRT= 16-12 days).

386 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).

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

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

393 Indeed, the capital costs for EN-OFMSW at an OLR range of 3.87-4.50 kgvs/m³d were lower than
394 those at an OLR of 3.38 kgvs/m³d. Similarly, for HC-OFMSW, the capital costs at an OLR range of
395 3.87-5.40 kgvs/m³d were lower than those at an OLR of 3.38 kgvs/m³d.

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

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

401 Usually, the main economic issue related to pre-treatments is the possible economic unprofitability
402 since the enhancement of methane yields could not offset the efforts of the item and energy required
403 by pre-treatment (Fu et al., 2018). Whereas, in the present study, the pre-treatments were
404 economically profitable up to OLR =4.50 kgvs/m³d (HRT = 12 d) for EN-OFMSW and OLR=5.40
405 kgvs/m³d (HRT = 10 d) for HC-OFMSW. In both HC- and EN-OFMSW configurations, the pre-
406 treatment unit represented less than 15 % of capital costs and 10 % of the operational ones. As stated
407 by the technical performances, the pre-treatment allowed the process to be stable to produce enough
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 EN-
410 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 kgvs/m³d, whereas EN-OFMSW could
413 be until OLR= 4.50 kgvs/m³d.

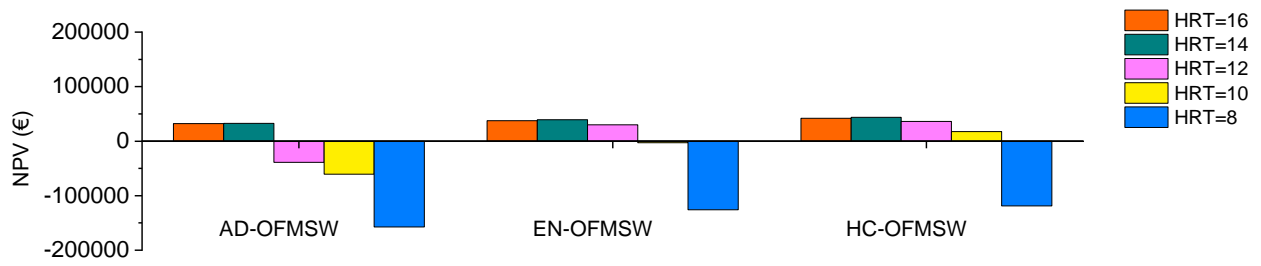
414 Usually, enzyme-assisted AD is not economically profitable due to the cost of the enzyme (Khanh
415 Nguyen et al., 2021). This cost could be overcome by the implementation of a cocktail of enzymes
416 like UPP2, which promoted a synergistic effect during the floc structure disintegration of OFMSW
417 (Salihu and Alam, 2016). However, also a cocktail of enzymes has drawbacks as the request of time
418 for the hydrolysis and correction of pH (Meegoda et al., 2018) because UPP2 required 2 h and pH =
419 7-7.5 obtained with NaOH addition.

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

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

430 EN-OFMSW was less advantageous than HC-OFMSW, because the marginal biogas increase may
431 not be able to justify the expensive cost of enzymes according to (Montgomery and Bochmann, 2014).

432 However, both pre-treatments HC- and EN-OFMSW reached economic performance higher than the
433 ones of AD-OFMSW.



434

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

437 3.3 Environmental evaluation

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

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

460 Most of the available studies about environmental evaluation focusing on GWP referred to existing
461 industrial AD plants. The present study compared the results achieved with studies concerning the

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

464 The results of CF-analysis of sAD-OFMSW agreed with most of the studies performed in batch, feed-
465 batch, and continuous AD performed with OFMSW (Kumar and Samadder, 2020), and in detail for
466 $OLR = 3.87 \text{ kgvs/m}^3\text{d}$, the GWP agreed with the study of (Mezzullo et al., 2013), which investigated
467 the same OLR, in which the avoided impacts were mainly due to the replacement of the energy and
468 fertilizer productions.

469 The results obtained in the present study by AD-OFMSW within the range of 3.38 and 4.50 $\text{kgvs/m}^3\text{d}$
470 aligned with previous studies utilising 1 MWh/d as a functional unit. Examples include the study of
471 (Fusi et al., 2016) and (Bacenetti et al., 2016) reporting -375 and 408 $\text{kg CO}_2\text{eq/FU}$ for sAD feed with
472 agro-waste and silage, respectively.

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

476 The avoided emissions achieved by HC-OFMSW in the range 3.87-5.40 $\text{kgvs/m}^3\text{d}$ were higher than
477 those obtained by (Vosooghnia et al., 2021) through ultrasound pre-treated agro-waste before AD.

478 In detail, the avoided emissions for HC-OFMSW at 4.50 $\text{kgvs/m}^3\text{d}$ were around 6 % higher than the
479 ones reached by the plant in the UK, performing thermos-mechanical pre-treatment, (Evangelisti et
480 al., 2014) and by the plant in France, performing physical pre-treatment, (Lamnatou et al., 2019)
481 under similar OLR.

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

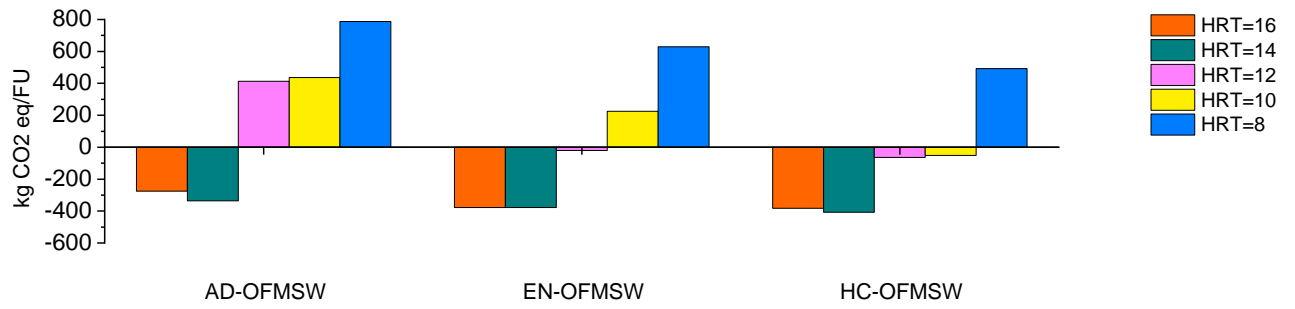
485 The energy cost to carry out HC was offset by the methane surplus produced by HC-OFMSW at
486 OLRs from 3.38 to 5.40 $\text{kgvs/m}^3\text{d}$, since for these OLRs the HC reached positive energy balance due

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

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

500 The sensitivity analysis confirmed the GWP results and the trends. HC-OFMSW and EN-OFMSW
501 played a positive effect on the increase in OLR because they buffered the inhibition and kept the
502 process stable, which means lower CO₂ eq emissions (Wei et al., 2022). The results confirmed HC-
503 OFMSW as an effective pre-treatment (Saxena et al., 2019).

504



505

506

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

507

508 3.4 Principal Component Analysis

509 Principal Component Analysis (PCA) was applied to the dataset consisting of the 30 samples (6 HRT
510 values x 3 pre-treatments x 2 replicates) described by 8 variables (biogas and methane productions,
511 VS removal, OFMSW feed, OLR, GWP, NPV, and PBT). Data were auto-scaled (mean centering
512 followed by normalization to unit variance) hence all the variables accounted for the same amount of
513 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:

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

524 The score plot proves that the samples were grouped predominantly according to the HRT (or to the
525 corresponding OLR), however, within each HRT group (or corresponding OLR) the samples were
526 clustered according to the pre-treatment. Samples with HRT=8 d (OLR = 6.75 kg_{vs}/m³ d) were at
527 positive scores on PC₁, showing higher values of OFMSW feed, GWP, PBT and OLR. This is more
528 evident for AD-OFMSW, while the trend decreases for EN-OFMSW and HC-OFMSW samples
529 respectively.

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

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

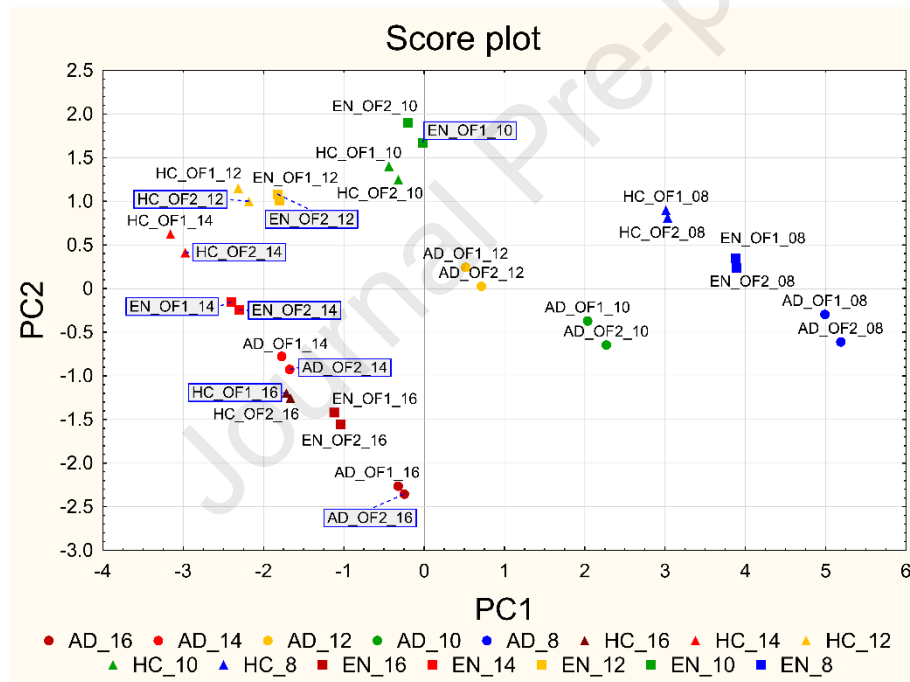
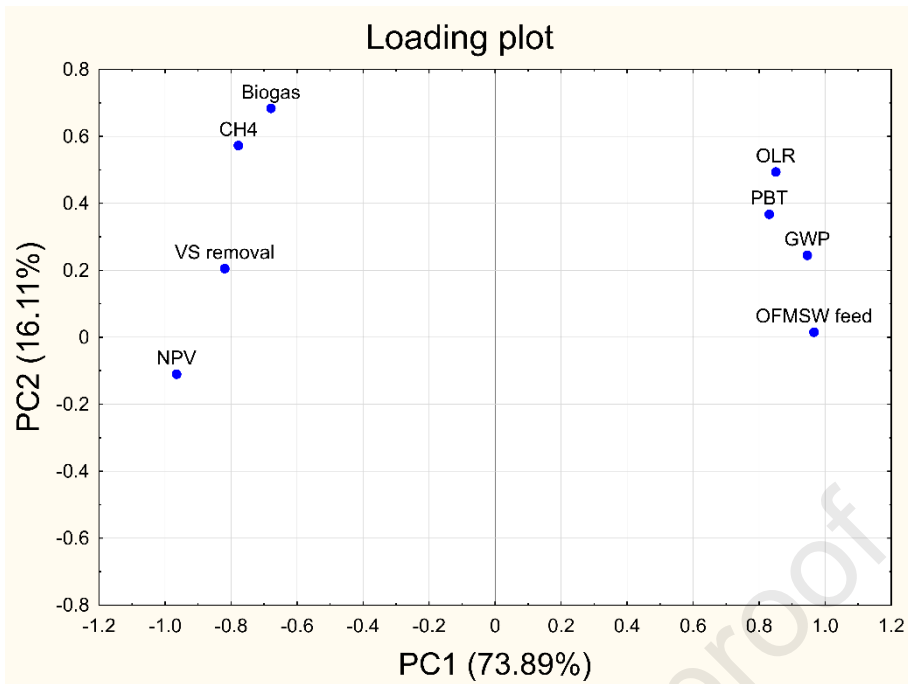
537 HRT samples were in groups with a progressive changing of their position in the score plot: from
538 right, counter clockwise, there are HRT= 8, 10, 12, 14, and 16 d. From the initial behaviour of HRT=8
539 d samples, at HRT=10 and 12 d, all the samples are at positive or slightly negative values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

543 HRT=14 d samples were characterized by the lowest values of OLR, PBT, GWP, and the best balance
544 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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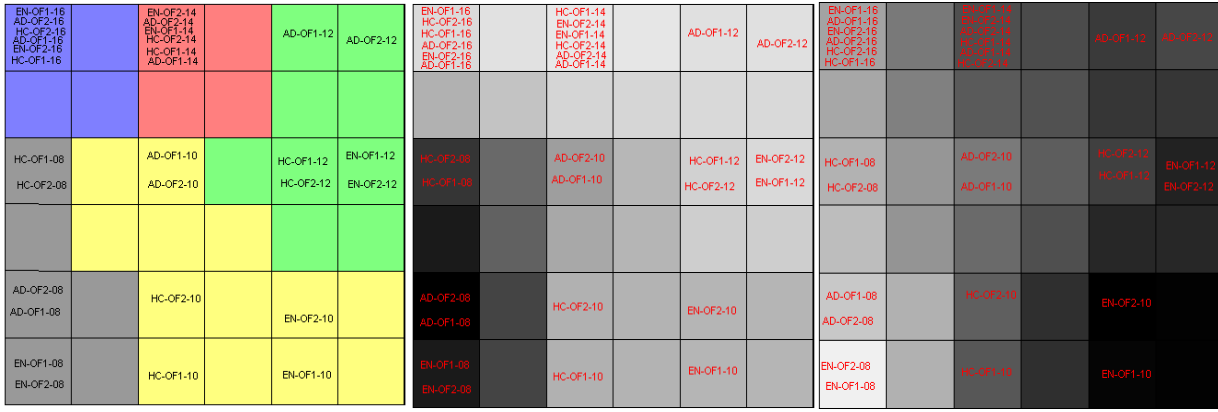


553 Fig. 4. PCA results are represented by loading plot (a) and score plot (b). where the samples are
 554 labelled according to both the treatment applied and the HRT: the treatment is indicated with a
 555 different shape of the label (AD as circles, HC as triangles, EN as squares), while the HRT is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

557 3.5 Supervised Kohonen's Self-Organizing Maps

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

- 575 • OFMSW feed, GWP and OLR exhibited almost identical behaviours, with high values for
576 HRT=8 d, smaller values for HRT=10 d, and the best values for HRT \geq 14 d.
- 577 • Biogas and CH₄ were high for HRT=10 (especially biogas) and 12 d (especially CH₄), while
578 HRT=14 d reached quite high values of biogas and very high for CH₄. VS removal was high
579 for HRT=14 and 12 d. Biogas, CH₄, and VS removal were low for HRT=8 d.
- 580 • PBT was high for all the HRT values except for HRT=14 and 16 d, for which it was very low
- 581 • NPV showed very low values for HRT=8 d (for all the sAD configuration it was negative)
582 and especially high for HRT \geq 14 d.



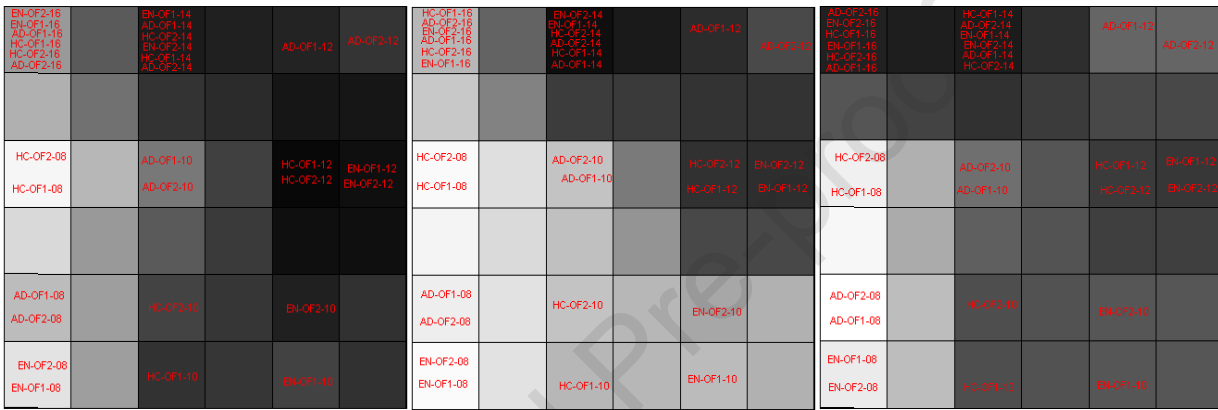
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(a) Top map

(b) OFMSW Feed

(c) Biogas



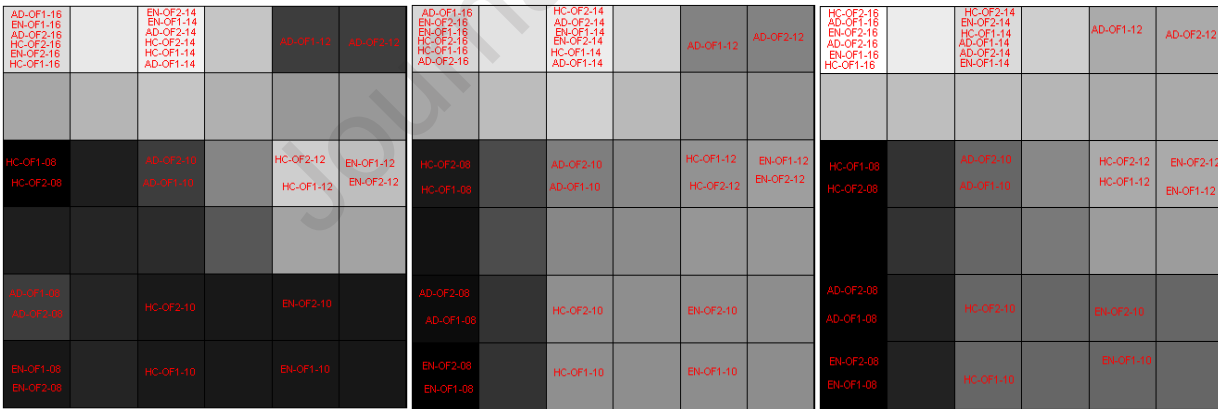
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(d) CH₄

(e) Vs Removal

(f) NPV



587

588

(g) PBT

(h) GWP

(i) OLR

589

590

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**

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

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

605 These outcomes were important because pre-treatments, conventionally considered expensive due to
606 additional energy or chemicals, emerged as viable solutions to be implemented at the industrial scale.

607 Pre-treated-AD required smaller plants compared to AD-OFMSW by treating a higher amount of
608 OFMSW per unit of volume and time and by producing higher amount of renewable energy. Hence,
609 HC-OFMSW can contribute to the decarbonization of the energy-productive system and enhance
610 waste management. These actions aligned both with the principles of Bioeconomy and contributed to
611 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
619 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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- 864

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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