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1 **High-Solids Anaerobic Digestion requires a tradeoff between Total**
2 **Solids, Inoculum-to-Substrate Ratio and Ammonia Inhibition**

3
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15

1 ABSTRACT

2 Increasing total solids on anaerobic digestion can reduce the methane yield, by the
3 interaction of highly-complex bio-physical-chemical mechanisms. Therefore,
4 understanding those mechanisms and their main drivers becomes crucial to optimize
5 high-solids anaerobic digestion at industrial scale. In this study, seven batch
6 experiments were conducted to investigate the effects of increasing the total solids
7 content on high-solids anaerobic digestion of the organic fraction of municipal solid
8 waste. With inoculum-to-substrate ratio = 1.5 g VS/g VS and maximum total solids \leq
9 19.6 %, mono-digestion of the organic fraction of municipal solid waste showed a
10 methane yield of 174-236 NmL CH₄/g VS. With inoculum-to-substrate ratio \leq 1.0 g
11 VS/g VS and maximum total solids \geq 24.0 %, similar mono-digestion experiments
12 resulted in acidification. Co-digestion of the organic fraction of municipal solid waste
13 and beech sawdust permitted to reduce the inoculum-to-substrate ratio to 0.16 g VS/g
14 VS while increasing total solids up to 30.2 %, though achieving a lower methane yield
15 (i.e. 117-156 NmL CH₄/g VS). At each inoculum-to-substrate ratio, a higher total solids
16 content corresponded a to higher ammonia and volatile fatty acid accumulation. Thus, a
17 40 % lower methane yield of the organic fraction of municipal solid waste was observed
18 at a NH₃ concentration \geq 2.3 g N-NH₃/kg reactor content and total solids = 15.0 %.
19 Meanwhile, the addition of sawdust to the organic fraction of municipal solid waste
20 lowered the nitrogen content, being the risk of acidification exacerbated only at total
21 solids \geq 20.0 %. Therefore, the biodegradability of the substrate, as well as the
22 operational total solids and the inoculum-to-substrate ratio, are closely-interrelated
23 parameters determining the success of methanogenesis, but also the risk of ammonia
24 inhibition on high-solids anaerobic digestion.

25

26 **Keywords:** High-solids Anaerobic Digestion; Organic Fraction of Municipal Solid

27 Waste; Batch Experiments; Co-digestion; Thermophilic; Methane Yield; Volatile Fatty

28 Acids.

29

30 **1 INTRODUCTION**

31 Anaerobic digestion (AD) is a biochemical treatment technology in which an organic
32 waste (OW) is decomposed to a mixture of gases – mainly CH₄ and CO₂ – known as
33 biogas, and a partially stabilized organic material known as digestate. Biogas has a high
34 calorific content, while the nutrient-concentrated digestate has the potential to be used
35 as soil amendment (De Baere and Mattheeuws 2013). AD takes place through a
36 sequential set of fermentative steps carried out symbiotically by different microbial
37 consortia (Gerardi 2003). The main AD steps are hydrolysis, acidogenesis, acetogenesis
38 and methanogenesis, while the AD biochemistry strongly depends on a balance between
39 volatile fatty acid (VFA) production by acidogens/acetogens and VFA consumption by
40 methanogens. When an imbalance occurs, VFA and/or H₂ accumulate, potentially
41 leading to AD failure by acidification (i.e. pH ≤ 6.0) (Motte et al. 2014; Staley et al.
42 2011). Other inhibitory substances may also accumulate during AD, such as free
43 ammonia (NH₃) and cations (e.g. Na⁺, K⁺) (Chen et al. 2008; Riggio et al. 2017).

44

45 Depending on the total solid (TS) content, AD can be operated under ‘wet’ (i.e. TS <
46 10 %), ‘semi-solid’ (i.e. 10 ≤ TS < 20 %) and ‘dry’ (i.e. TS ≥ 20 %) conditions
47 (Abbassi-Guendouz et al. 2012; Pastor-Poquet et al. 2018). High-solids AD (HS-AD)
48 includes the two last cases, and has some advantages such as the use of a smaller
49 digester volume, and a reduced need for water addition and dewatering operations,
50 enhancing the process economy (André et al. 2018; Kothari et al. 2014). However, HS-
51 AD also shows some drawbacks such as a high risk of reactor acidification by substrate
52 overload, and a reduced mass transfer associated to the low content of free water in the
53 system (Benbelkacem et al. 2015; Bollon et al. 2013; García-Bernet et al. 2011).

54 Moreover, as the TS content is rather high in HS-AD, a lower amount of water is
55 available to dilute potential inhibitors (i.e. NH_3) than in 'wet' AD.

56

57 HS-AD of the organic fraction of municipal solid waste (OFMSW), including food
58 waste (FW) and green/lignocellulosic waste (GW), is widely used. Indeed, the high TS
59 content (i.e. 20-50 %) and the high biodegradation potential of OFMSW are particularly
60 favorable to lower the operational costs of HS-AD (De Baere and Mattheeuws 2013). In
61 this line, batch systems for OFMSW treatment at industrial scale can be operated up to
62 40 % TS, provided that leachate is continuously recirculated as a source of
63 microorganisms and partial mixing (André et al. 2018; Riggio et al. 2017).

64

65 The operational TS of HS-AD mainly depends on the TS and volatile solid (VS) of the
66 OW, but also its biodegradability under anaerobic conditions, since AD of OFMSW
67 might yield a 30-80 % reduction of the substrate TS (Pastor-Poquet et al. 2018). Thus,
68 the presence of lignocellulosic substrates (i.e. GW or paper/cardboard) in OFMSW
69 usually permits to increase the operational TS content in HS-AD, due to the higher TS
70 content but also lower biodegradability of these substrates, in comparison to OFMSW
71 (Pastor-Poquet et al. 2018, In Press). Nonetheless, the addition of lignocellulosic
72 materials might reduce the biodegradability rate of the overall mixture due to the slower
73 hydrolysis (Brown and Li 2013; Mancini et al. 2018). On the other hand, the addition of
74 lignocellulosic substrates might reduce simultaneously the chances of NH_3 inhibition in
75 HS-AD due to the lower protein content.

76

77 Laboratory-scale batch experiments are normally used to obtain valuable information
78 about the main operating parameters and/or the AD dynamics for a given OW at
79 industrial scale. One of the main parameters is the inoculum-to-substrate ratio (ISR) to
80 be used avoiding acidification. For example, when assessing the maximum methane
81 yield of highly biodegradable substrates (i.e. FW) during a biomethane potential (BMP)
82 test, a relatively high ISR (i.e. 2-4 g VS/g VS) is recommended (Holliger et al. 2016).
83 However, as a sole parameter, the ISR is inadequate to avoid HS-AD acidification
84 (Schievano et al. 2010). Indeed, a given mixture substrate-inoculum sets simultaneously
85 the ISR (i.e. g VS/g VS) and the maximum TS, according to the VS and TS mass
86 balances, respectively. Therefore, adapted combinations of ISR (i.e. 0.25-4 g VS/g VS)
87 and FW:GW ratio (i.e. 0-100 %) are required to circumvent acidification, while
88 maximizing the TS content in HS-AD experiments (Capson-Tojo et al. 2017; Schievano
89 et al. 2010).

90

91 The effects of increasing the initial TS content on HS-AD batch tests are not yet fully
92 understood, since a higher initial TS has been reported to reduce the methane yield of
93 substrates such as cardboard (Abbassi-Guendouz et al. 2012) and OFMSW (Forster-
94 Carneiro et al. 2008b; Liotta et al. 2014), but not of lignocellulosic substrates (Brown et
95 al. 2012). Importantly, whether the TS increase inside the digester results in a lower
96 methane yield, the overall HS-AD efficiency decreases, potentially compromising the
97 OFMSW treatment economy (Fernández et al. 2010; Mata-Álvarez 2003).

98

99 This study evaluates the effects of increasing the initial TS content on the methane
100 yield, TS removal and chemical oxygen demand (COD) conversion in HS-AD

101 laboratory-scale batch bioassays at 55°C, using mono-digestion of OFMSW and co-
102 digestion of OFMSW and beech sawdust. Sawdust simulates the addition of
103 biodegradable GW (e.g. branches and leaves) to OFMSW, permitting to stabilize HS-
104 AD at high TS (i.e. ≥ 20 %). To maximize TS while avoiding acidification, different
105 ISR and/or co-digestion ratios were used. Furthermore, this study highlights the
106 important interrelationship between the initial conditions (i.e. TS and ISR) and the main
107 AD inhibitors (i.e. NH_3) in HS-AD of OFMSW, by evaluating the pH, TS, VFA and
108 ammonia dynamics during sacrifice experiments. More in particular, the interaction
109 between TS and the NH_3 content determines the overall methane yield, and set the basis
110 for an optimal HS-AD configuration when treating OFMSW at industrial scale.
111 This study was conducted at the Department of Civil and Mechanical Engineering of the
112 University of Cassino and Southern Lazio (Italy) from June 2016 to September 2017.

113

114

115 **2 MATERIALS AND METHODS**

116 **2.1 Organic Substrates and Inoculum**

117 OFMSW consisted of a mixture of household waste, restaurant waste, spent coffee
118 collected and GW (i.e. organic soil, small branches and leaves) collected in Cassino
119 (Italy). The wastes were gathered independently during one month while stored in
120 buckets at 4°C, and eventually mixed into a 100 L barrel. In total, 60 kg of waste were
121 collected with an approximated weight proportion of 45, 35, 15 and 5 % (w/w) for
122 household waste, restaurant waste, spent coffee and GW, respectively. The mixed waste
123 was minced twice to a pastry material with a particle size smaller than 5-10 mm by
124 means of an industrial mincer (REBER 9500NC), fully homogenized and stored in 5 L

125 buckets at -20°C, aiming to minimize the composition fluctuations during the
126 experimental period.

127

128 To increase the TS content in the batch experiments, 1-2 kg of OFMSW were dried for
129 7-10 days at 55°C until constant weight right before each experiment. The resulting
130 agglomerate was further minced with mortar and pestle, homogenized to a flour-like
131 material with a particle size ≤ 2 mm, and stored in air-tight containers until use.

132 Goldspan[®] beech sawdust with a 1.0-2.8 mm particle size was used as co-substrate.

133

134 Three ‘wet’ and six high-solids inocula were used in this study, since different
135 experiments were started at different periods. All inocula were sampled from a 30 L
136 methanogenic reactor fed with OFMSW under thermophilic (55°C) conditions. Prior to
137 being used in the experiments, all inocula were degassed for 7-10 days at 55°C and
138 subsequently filtered through a 1 mm mesh to remove coarse materials. These
139 inoculums were considered ‘wet’ since TS was ≤ 5 %. To increase simultaneously the
140 TS and ISR of batch experiments, the ‘wet’ inoculums were centrifuged at 6000 rpm for
141 10 min with a bench-scale centrifuge (REMI XS R-10M, India), right before each
142 experiment – high solids inoculum. The supernatant was separated and the remaining
143 viscous material was manually homogenized. Finally, micronutrients were added to
144 each inoculum as recommended by Angelidaki and Sanders (2004).

145

146 **2.2 Batch Experiments**

147 **2.2.1 Experimental Setup**

148 Seven batch experiments were performed to evaluate the effects of increasing the initial
149 TS from 10.0 to 33.6 % in HS-AD. Dried OFMSW and/or sawdust were used as organic
150 substrates under different mono- and co-digestion conditions [Table 1]. Because of
151 availability, experiments were performed in 160 or 280 mL serum bottles (Wheaton,
152 USA), all incubated at 55°C. The different TS were obtained by an adequate
153 combination of substrate, inoculum and distilled water addition. To minimize the
154 occurrence of experimental biases, each bottle contained exactly the same amount of
155 substrate and inoculum, while the amount of distilled water depended on the desired TS.
156 Thus, different medium volumes were obtained within the same set of batch
157 experiments [Table 1].

158

159 The bottles were sealed with butyl rubber stoppers and aluminum crimps, and flushed
160 with inert gas (helium or nitrogen), before adding 0.2 mL of 10 g/L Na₂S to guarantee
161 an adequate redox potential (Angelidaki and Sanders 2004). All batch assays lasted until
162 the gas production was negligible (i.e. < 1 mL/d) during three consecutive
163 measurements. The bottles were manually agitated when the gas production was
164 measured. For each experiment, blank assays were conducted in triplicate to evaluate
165 the biomethane production of the sole inoculum. Blank assays contained the same
166 amount of inoculum, while further distilled water was used to compensate for the
167 absence of substrate [Table 1].

168

169 **2.2.2 HS-AD Biodegradability Indicators**

170 Five out of seven batch experiments were aimed to evaluate the effects of increasing the
171 initial TS on the HS-AD methane yield, TS removal and COD conversion, using initial

172 TS contents from ‘wet’ (i.e. TS = 10 %) to ‘dry’ conditions (i.e. TS \geq 20 %) [Test 1-5,
173 Table 1]. Mono-digestion experiments were run with a homogeneous mixture of dried
174 OFMSW and high-solids inoculum at an ISR of 0.50, 1.00 and 1.50 g VS/g VS, for Test
175 1, 2 and 3, respectively. The ISR increase resulted in lower initial TS [Table 1]. In the
176 fourth experiment (Test 4), HS-AD of sawdust was investigated by using a mixture of
177 beech sawdust and ‘wet’ inoculum at an ISR = 0.04 g VS/g VS. In the fifth experiment
178 (Test 5), co-digestion of dried OFMSW and sawdust was performed with high-solids
179 inoculum. The OFMSW:sawdust ratio was 1:4 g TS:g TS and the overall ISR was 0.16
180 g VS/g VS. All TS conditions were evaluated in triplicate.

181

182 **2.2.3 Sacrifice Tests**

183 To evaluate the main dynamics (i.e. TS, VFA, ammonia nitrogen and COD conversion)
184 during HS-AD, two batch experiments were performed as sacrifice tests [Tests 6 and 7,
185 Table 1]. 15 replicates were used in each test. After measuring the gas volume and
186 composition, a single bottle was emptied and the content was analyzed (i.e. for VS,
187 VFA and ammonia) every 3 to 5 days during the first two weeks, and every 7 to 10 days
188 until the end of the experiment. In Test 6, dried OFMSW was used as the sole substrate
189 in presence of high-solids inoculum. The initial TS and ISR were 15.0 % and 1.00 g
190 VS/g VS, respectively. Test 7 was performed to study the co-digestion of OFMSW and
191 beech sawdust with an initial TS = 19.4 % and an ISR = 0.60 g VS/g VS. The ratio
192 OFMSW:sawdust was 1.0:1.1 g TS:g TS.

193

194 **2.3 Biomethane potential of OFMSW and beech sawdust**

195 The individual BMP of the raw OFMSW and beech sawdust at 55°C was estimated
196 according to Angelidaki and Sanders (2004) and Holliger et al. (2016). The BMP assay
197 with OFMSW was performed in 280 mL bottles using 6 replicates and an ISR = 2.00 g
198 VS/g VS, whereas the BMP of sawdust was assessed in 160 mL bottles using 3
199 replicates and an ISR = 1.00 g VS/g VS [Table 1]. In the BMP test for OFMSW, the
200 distilled water addition served to minimize the chances of ammonia inhibition. In
201 contrast, ammonia build-up was not expected in the BMP test of sawdust, due to the low
202 nitrogen content of this substrate, as shown in next section. The lower biodegradability
203 of sawdust permitted to use also a lower ISR.

204

205 **2.4 Physical-Chemical Analyses**

206 The pH and alkalinity were measured right after 1) diluting the (semi-)solid sample with
207 distilled water, 2) homogenization, 3) centrifugation at 6000 rpm for 15 min and 4)
208 supernatant titration to a pH of 5.75 and 4.3 for the carbonate (ALK_P) and total (ALK_T)
209 alkalinity, respectively (Lahav et al. 2002). The intermediate alkalinity (ALK_I) was the
210 difference between ALK_T and ALK_P . The TS and VS, total Kjeldahl (TKN) and
211 ammonia nitrogen (TAN), and specific weight (ρ_s) analyses were carried out according
212 to the standard methods (APHA 1999; EPA 2015).

213

214 The density (ρ) – containing the air-filled porosity (ε) – was approximated using a 1-2 L
215 calibrated cylinder and a ± 0.01 g precision scale. The NH_3 was approximated as in
216 Capson-Tojo et al. (2017). The COD of (semi-)solid samples was determined as
217 described by Nogueroles-Arias et al. (2012). The soluble COD (COD_s) was determined
218 with the same method by immediately analyzing the supernatant filtered through a 0.45

219 μm polypropylene membrane. The VFA (acetic, propionic, butyric and valeric acids)
220 analysis of 0.45 μm pre-filtered samples was conducted with a LC-20AD HPLC
221 (Shimadzu, Japan) equipped with a Rezex ROA-Organic Acids 8+ column
222 (Phenomenex, USA) coupled to a 210 nm UV detector. The column was maintained at
223 70°C with a 0.0065 M H_2SO_4 mobile phase flowing at 0.6 mL/min. Lactate and ethanol
224 were measured by the same method but using a RID detector.

225

226 The biogas production was evaluated with a two-vessel water displacement system. The
227 first vessel contained 4 N NaOH to capture the produced CO_2 , while the second vessel
228 was filled with distilled water to be ‘displaced’. Once measured the biogas production,
229 the reactor headspace was sampled with a 250 μL pressure-lock syringe for the analysis
230 of the biogas composition in terms of CH_4 , CO_2 , H_2 , O_2 and N_2 with a 3400 GC-TCD
231 (Varian, USA) equipped with a Restek Packed Column. The carrier gas was argon.

232

233 **2.5 Calculations**

234 Whether not stated otherwise, the above physical-chemical analyses were reported per
235 kilogram (kg) of the overall inoculum-and-substrate mixture, including water (i.e.
236 overall reactor content in wet basis).

237 The methane yields obtained in the seven batch experiments, as well as the BMP values
238 for OFMSW and for beech sawdust, were expressed as the normalized methane
239 production ($P = 1 \text{ bar}$, $T = 0^\circ\text{C}$), excluding the endogenous methane production of the
240 inoculum, divided by the added substrate VS (VS_{subs}). The Dixon’s test was applied as
241 recommended by Holliger et al. (2016) to discard any outlier in the batch experiments
242 or BMP tests. The overall methane or hydrogen production at the end of each

243 experiment was expressed as a normalized volume of gas ($P = 1$ bar, $T = 0$ °C)
244 measured by water displacement, divided by the VS added (VS_{added}) – including the
245 substrate and inoculum. The hydrogen production by the VS removed (VS_{removed}) was
246 also calculated in some acidified reactors.

247

248 The TS removal was the difference between the initial and final TS contents, divided by
249 the initial TS. Noteworthy, the TS removal is roughly equivalent to the VS removal.

250 The global COD conversion included the overall methane and/or hydrogen production
251 and the VFA content at the end of each experiment, divided by VS_{added} . In sacrifice tests
252 [Tests 6 and 7, Table 1], the progressive COD conversion was evaluated as the

253 produced methane, hydrogen and VFA at a specific time interval, divided by VS_{added} . In

254 this study, the COD conversion permitted to compare the VFA accumulation and the

255 biogas production among methanogenic and acidified experiments, but also to evaluate

256 the NH_3 inhibition between different initial TS contents in methanogenic reactors. The

257 reactor content volume (V_{Global}) for each initial mixture was obtained as $\sum(M/\rho)$, being

258 M the mass of each compound in the batch experiments (i.e. inoculum, substrate and

259 water). The liquid-solid volume (V_{Real}) for the inoculum-substrate mixture was obtained

260 as $\sum(M/\rho_s)$. ϵ was obtained as $1 - V_{\text{Real}}/V_{\text{Global}}$. In this study, all the initial batch

261 configurations were designed to be porosity free (i.e. $\epsilon = 0$; $V_{\text{Global}} = V_{\text{Real}}$), since gas

262 reduces the metabolite mass transfer in comparison to liquid media (Bollon et al. 2013).

263

264 In the HS-AD experiments used to assess the main biodegradability indicators (Section

265 2.2.2), the repeatability (i.e. average \pm standard deviation) was assessed using all

266 triplicates at each initial TS content. On the other hand, in the sacrifice tests (Section

267 2.2.3), the biogas production and composition consisted of the average \pm standard
268 deviation of all (remaining) replicates at a given experimental time, including that being
269 subsequently emptied. The rest of physical-chemical analyses (e.g. TS, TAN, VFA)
270 were performed in triplicate for the punctually-emptied replicate. In all these batch
271 experiments, the water loss (in terms of vapor) regarding the initial amount of water in
272 each substrate-inoculum mixture was considered negligible (i.e. $< 3\%$, data not shown).

273

274

275 **3 RESULTS AND DISCUSSION**

276 **3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum**

277 Table 2 shows the average composition of the raw OFMSW, dried OFMSW and
278 sawdust. The TS of the raw OFMSW was 26 %, in agreement with reported values for
279 source-sorted OFMSW (Christensen 2011; Schievano et al. 2010). The TS of the dried
280 OFMSW was 92 %. A relatively lower TAN, CODs/COD and COD/TKN ratios were
281 observed for the dried compared to the raw OFMSW, while the VS/TS was maintained
282 approximately constant and ϵ increased [Table 2]. Therefore, some volatilization of
283 organic material (e.g. VFA, TAN) occurred when drying OFMSW at 55°C. However,
284 drying was an adequate conditioning for assessing the effect of TS increase in HS-AD
285 of raw OFMSW, since the macroscopic composition was maintained relatively constant
286 [Table 2]. A similar conditioning was used by Forster-Carneiro et al. (2008a) to increase
287 the TS in HS-AD batch reactors. The TS of beech sawdust was 94 % [Table 2], similar
288 to that obtained by Brown and Li (2013) for GW.

289

290 The BMP of the raw OFMSW and sawdust at 55°C was 497 ± 58 NmL CH₄/g VS_{subs}
291 [Figure 1a] and 161 ± 12 NmL CH₄/g VS_{subs} [Figure 1b], respectively, indicating the
292 lower biodegradability of sawdust than of OFMSW under anaerobic conditions.
293 Moreover, reaching the maximum methane yield took a considerably longer for sawdust
294 than OFMSW (i.e. 130 and 56 days, respectively), suggesting also a reduced hydrolysis
295 rate for lignocellulosic substrates (Pastor-Poquet et al. 2018, In Press; Vavilin et al.
296 2008). The higher standard deviation in the BMP for raw OFMSW was attributed to the
297 waste heterogeneity. The BMP values were equivalent to those observed for source-
298 sorted OFMSW and GW (Brown and Li 2013; Schievano et al. 2010).

299

300 The average composition of the ‘wet’ and high-solids inocula is reported in Table 2.
301 Only minor deviations in macroscopic characteristics (i.e. TS and TKN) were observed
302 between ‘wet’ and high-solids inocula sampled at different times. Centrifugation
303 increased the TS content, and ALK_i/ALK_p, COD/TKN and VS/TS ratios compared to
304 the ‘wet’ inoculum [Table 2]. A similar inoculum conditioning was used by Brown and
305 Li (2013) to increase the TS in ‘dry’ co-digestion. Other inoculum pretreatments to
306 increase TS in HS-AD include inoculum filtration (Liotta et al. 2014) or drying at 105°C
307 (Capson-Tojo et al. 2017), though heating the inoculum at 105°C might result in
308 methanogenesis inhibition (Ghimire et al. 2015).

309

310 **3.2 Batch Experiments**

311 **3.2.1 Acidified Experiments**

312 Mono-digestion of OFMSW with an ISR of 0.5 and 1.0 g VS/g VS (Test 1 and Test 2)
313 allowed to increase the TS up to 33.6 and 24.0 %, respectively [Table 1]. However, all

314 the TS conditions resulted in acidification (i.e. $\text{pH} \leq 6.0$), likely due to the low ISR used
315 (Angelidaki and Sanders 2004). Methanogenesis inhibition led to H_2 production and
316 VFA accumulation. The highest H_2 production with an $\text{ISR} = 0.5 \text{ g VS/g VS}$ (Test 1)
317 was achieved at the lowest TS (i.e. 10.2 %) and progressively decreased with increasing
318 TS [Figure 2b], likely due to the reduced mass transfer in high-solids conditions. The H_2
319 production (i.e. 2-20 $\text{NmL H}_2/\text{g VS}_{\text{added}} = 7\text{-}60 \text{ NmL H}_2/\text{g VS}_{\text{removed}}$) was comparable to
320 that reported by Valdez-Vazquez and Poggi-Varaldo (2009) for OFMSW (i.e. 10-50
321 $\text{NmL H}_2/\text{g VS}_{\text{removed}}$). With an $\text{ISR} = 1.0 \text{ g VS/g VS}$ (Test 2), the H_2 production was ≤ 1
322 $\text{NmL H}_2/\text{g VS}_{\text{added}}$. A reduced H_2 production can be attributed to a higher ISR.

323

324 In both experiments, an inverse relationship between the TS removal and the initial TS
325 was observed [Figure 2c]. Meanwhile, the global COD conversion described an average
326 $0.35 \text{ g COD/g VS}_{\text{added}}$ at an initial TS of around 10 % and a similar downward trend
327 with increasing TS in both experiments [Figure 2d]. The COD conversion in acidified
328 reactors corresponded from 87 to 96 % of the VFA accumulation. This confirms that H_2
329 production and/or VFA accumulation potentially reduced the hydrolysis rate (Cazier et
330 al. 2015; Vavilin et al. 2008), playing a major role on the organic degradation at higher
331 TS, due to the low water available (García-Bernet et al. 2011). Lactate and ethanol were
332 not detected in any of the batch assessed in this study.

333

334 **3.2.2 Methane-Producing Experiments**

335 Despite mono-digestion of OFMSW at an $\text{ISR} = 0.5 \text{ g VS/g VS}$ (Test 1) acidified at all
336 TS contents, methanogenesis occurred in 2 out of 3 replicates performed at 28.3 % TS,
337 leading to an average methane yield of $64 \pm 6 \text{ NmL CH}_4/\text{g VS}_{\text{subs}}$ [Figure 2a] – 87 %

338 lower than the BMP of raw OFMSW – and a 23 % TS removal [Figure 2c]. The
339 methanogenic onset observed in the two bottles at 28.3 % TS might relate to a favorable
340 mass transfer in the high-solids mixture, as discussed in Section 3.2.4, since all the
341 bottles contained exactly the same amount of substrate and inoculum.

342

343 Methanogenesis succeeded in all TS contents with mono-digestion of OFMSW using an
344 $ISR = 1.5 \text{ g VS/g VS}$ (Test 3), though only a maximum 19.6 % TS was reached under
345 these conditions [Figure 2a]. A methane yield of 236 ± 5 , 199 ± 32 , 174 ± 47 and $222 \pm$
346 $62 \text{ NmL CH}_4/\text{g VS}_{\text{subs}}$ was observed at initial TS of 10.8, 13.4, 16.4 and 19.6 %,
347 respectively [Figure 1c and 2a], i.e. 52-65 % lower than the BMP of OFMSW. These
348 methane yields corresponded to a volumetric productivity of 8.8 ± 0.2 , 9.3 ± 1.5 , $10.2 \pm$
349 2.8 and $15.8 \pm 4.4 \text{ NmL CH}_4/\text{L Reactor Content}$ (data not shown) at initial TS of 10.8,
350 13.4, 16.4 and 19.6 %, respectively, being the higher volumetric productivity at
351 increasing TS one of the main advantages of HS-AD (Brown et al. 2012). Interestingly,
352 the standard deviation of the methane yield increased alongside the TS [Figure 2a],
353 likely due to mass transfer effects and/or a higher heterogeneity of the initial mixture, as
354 discussed in Section 3.2.4. In contrast, the TS removal decreased at increasing initial TS
355 contents [Figure 2c]. The global COD conversion was approximately $0.38 \pm 0.05 \text{ g}$
356 $\text{COD/g VS}_{\text{added}}$ at all TS, but showing a higher standard deviation at an initial TS =
357 19.6 % [Figure 2d & Table 3]. It should be noted that the TS removal (i.e. VS removal)
358 and the COD conversion yield similar information about the overall organic degradation
359 in methanogenic experiments. Nonetheless, the COD conversion was considered as a
360 more informative assessment of the VFA accumulation in these experiments, as

361 indicated in Section 2.5. Particularly, it can be observed how the COD standard
362 deviation is obscured when assessing the TS removal [Figure 2c & Figure 2d].
363

364 Mono-digestion of sawdust (Test 4) showed a methane yield of 64 ± 3 , 92 ± 3 , 94 ± 4 ,
365 81 ± 32 NmL CH₄/g VS_{subs} at initial TS of 9.8, 14.6, 19.3 and 24.1 %, respectively
366 [Figures 1d and 2a]. The methane yield at 9.8 % TS was approximately 30 % lower than
367 that obtained at higher TS. After 100 days, the methane yield was 55-70 % lower than
368 the BMP of sawdust, probably due to the lower ISR (i.e. 0.04 g VS/g VS) slowing down
369 the biochemistry (Holliger et al. 2016), and/or the higher TS used. An 8-fold-higher
370 standard deviation was observed at 24.1 % TS, likely due to inaccessible substrate
371 regions at high TS – mass transfer limitations. The TS removal at initial TS = 24.1 %
372 was around 50 % lower than that obtained at lower TS [Figure 2c]. The global COD
373 conversion showed a downward trend from 14.6 to 24.1 % TS [Figure 2d].
374

375 With co-digestion of dried OFMSW and sawdust (Test 5), methane was produced only
376 at 10.0 and 15.0 % TS, while co-digestion reactors at higher TS resulted in acidification
377 [Figure 2], potentially due to the higher organic content at higher TS. The methane yield
378 reached 138 ± 1 and 156 ± 19 NmL/g VS_{subs} at 10.0 and 15.0 % TS, respectively
379 [Figure 1e]. Interestingly, 1 out of 3 replicates performed at 30.2 % TS also showed
380 methanogenesis likely due to mass transfer effects in HS-AD, reaching a methane
381 production of 117 NmL/g VS_{subs}. The H₂ yield – during the first week – decreased with
382 increasing TS [Figure 2b]. The TS removal was also reduced at an increasing TS
383 [Figure 2c].
384

385 **3.2.3 Main Effects when Increasing the Initial TS in HS-AD**

386 The TS increase in HS-AD led to an increased biomethane volumetric productivity with
387 mono-digestion of OFMSW (Test 3), but also resulted in acidification by substrate
388 overload at higher initial TS with co-digestion of OFMSW and sawdust (Test 5).
389 Moreover, higher standard deviations in the methane yields at higher TS, as well as the
390 occurrence of methanogenesis only in some of the replicates at 28.3 and 30.2 %, were
391 observed. These last results were likely due to mass transfer effects in HS-AD
392 experiments, which influenced the occurrence of acidification and/or inhibition.

393

394 The low water content of a high-solids mixture hinders the accessibility of
395 microorganisms to large portions of the substrate (Bollon et al. 2013), possibly
396 explaining the increasing standard deviation in the methane yield at $TS \geq 10\%$ [Figure
397 2a]. Particularly, ‘dry’ AD (i.e. $TS \geq 20\%$) is associated to the presence of spatially-
398 differentiated acidogenic/methanogenic centers (Staley et al. 2011; Xu et al. 2014). In
399 such systems, the convective transport is minimum, while the metabolite diffusion
400 increases in importance, since the free-to-bound water ratio is low (Bollon et al. 2013;
401 García-Bernet et al. 2011). Besides limiting the organic degradation, this phenomenon
402 also reduces the chances of acidification of all the methanogenic centers in case of
403 overload, likely explaining the methanogenesis onset observed in some replicates at
404 28.3 % TS (Test 1) and 30.2 % TS (Test 5). Homogenization devices, such as reactor
405 stirrer or leachate recirculation, might help to prevent the influence of mass transfer
406 limitations in HS-AD (André et al. 2018; Kothari et al. 2014).

407

408 **3.2.4 Maximizing the TS in HS-AD of OFMSW by Sawdust Addition**

409 In this study, the physical-chemical characteristics of the substrate and inoculum (e.g.
410 VS/TS and biodegradability) and the operational TS and ISR were found closely
411 interrelated parameters determining the methane production or acidification in HS-AD.
412 The ISR and the maximum TS were simultaneously adjusted in mono-digestion
413 experiments according to the TS and VS balances of the substrate-inoculum mixture,
414 since only one degree of freedom is available in a binary mixture (i.e. TS or ISR).
415 Particularly, whether TS are higher in the substrate than in the inoculum, higher initial
416 TS contents of a given substrate-inoculum mixture are obtained by lowering the ISR
417 [Tests 1-3, Table 1]. Nonetheless, the ISR must be sufficiently high to avoid
418 acidification, as a function of the substrate biodegradability (Angelidaki and Sanders
419 2004; Schievano et al. 2010). For example, the high biodegradability of OFMSW
420 required a higher ISR (i.e. 1.5 g VS/g VS), yielding a lower maximum TS (i.e. 19.6 %)
421 [Figure 2]. In contrast, the lower methane potential and biodegradability rate of sawdust
422 – as an example of lignocellulosic substrate – allowed the use of an extremely low ISR
423 (i.e. 0.04 g VS/g VS) and a higher TS (i.e. 24.1 %).

424

425 In the case of co-digestion, two degrees of freedom are available in a ternary mixture
426 (i.e. TS, ISR or OFMSW:GW ratio). Thus, a great number of combinations exists
427 depending on the particular substrate and/or inoculum characteristics (e.g. VS/TS),
428 explaining the different TS, ISR and FW:GW ratios used in literature for co-digestion.

429 In this line, Brown and Li (2013) showed that, for a fixed ISR in ‘dry’ AD, the
430 acidification risk increases by increasing the FW:GW ratio, due to the higher
431 biodegradability of the inoculum-substrate mixture. Moreover, a higher FW:GW
432 exacerbates the risk of TAN buildup and NH₃ inhibition in HS-AD.

433
434 Summarizing, adding sawdust to OFMSW reduces the biodegradability and TAN
435 content of the substrate-inoculum mixture in comparison to mono-digestion of
436 OFMSW, favoring the simultaneous TS and ISR increase in HS-AD. Thus, a
437 OFMSW:sawdust ratio of 1:4 g TS:g TS was chosen in this study mainly to increase the
438 maximum TS of co-digestion up to 30 %, but reducing the chances of NH₃ inhibition
439 and acidification. Nonetheless, the addition of GW to OFMSW in industrial applications
440 depends on the availability of co-substrates, the reactor design and/or the overall
441 process economy (Christensen 2011; Kothari et al. 2014).

442

443 **3.2.5 HS-AD Dynamics and NH₃ Inhibition**

444 During the sacrifice test for mono-digestion of OFMSW (Test 6) [Figure 3], the daily
445 methane production peaked around day 28, while the cumulative methane yield
446 stabilized by day 65 reaching a value of 296 ± 13 NmL CH₄/g VS_{subs}, i.e. 40 % lower
447 than the BMP of OFMSW. Because of the organic degradation, TS showed a 34.7 % TS
448 removal. Acetic acid peaked to 8.40 g/kg (day 8) and was extensively consumed within
449 30 days from the reactor startup. Propionic, butyric and valeric acids increased
450 significantly along the experiment. TAN started at 2.4 g N/kg and reached 3.8 g N/kg.
451 At the same period, pH started at 7.3, decreased to a minimum of 6.3 and increased
452 above 8. The TAN and pH increase resulted in a NH₃ concentration up to 2.5 g N/kg.
453 The global COD conversion was 0.63 g COD/g VS_{added}.

454

455 These results suggest that the high ammonia levels were responsible for the reduced
456 methane yield, TS removal and COD conversion in HS-AD, since all biodegradability

457 indicators significantly slowed down in the mono-digestion sacrifice (Test 6) as NH_3
458 reached 2.3 g N/kg from day 45 [Figure 3]. Depending on the methanogens acclimation,
459 NH_3 concentrations of 0.2-1.4 g N/L have been reported inhibitory (Chen et al. 2008;
460 Fricke et al. 2007; Prochazka et al. 2012). In this study, the NH_3 increase correlated well
461 with the propionic/valeric accumulation in Test 6 [Figure 3], being the VFA buildup a
462 likely consequence of methanogenic inhibition (Demirel and Scherer 2008).

463

464 The above results indicate that the ammonia buildup most probably hampered the
465 methane production also in the mono-digestion experiment using an $\text{ISR} = 1.5 \text{ g VS/g}$
466 VS (Test 3) [Figure 2]. Thus, the nitrogen content (i.e. TKN, TAN and NH_3) was
467 observed to increase in Test 3 alongside the higher initial TS, because of the lower
468 amount of distilled water initially used for dilution, potentially exacerbating the NH_3
469 inhibition and VFA accumulation at higher TS [Table 3]. With all the above, the NH_3
470 accumulation can determine the overall anaerobic degradation (i.e. methane yield, TS
471 removal and COD conversion) during HS-AD, particularly at higher initial TS contents.
472 These results complement the main bio-physical-chemical effects arising in HS-AD due
473 to the TS increase (i.e. reduced organic degradation by mass transfer effects), as
474 mentioned in Section 3.2.3. In other words, the TS increase can limit the organic
475 degradation in HS-AD of OFMSW due to both mass transfer effects and NH_3 inhibition.
476 With the aim to reduce the risk of NH_3 inhibition while increasing the TS content, a co-
477 digestion sacrifice was performed.

478

479 **3.2.6 Other Factors Influencing Acidification in HS-AD**

480 In co-digestion sacrifice (Test 7) [Figure 4], methanogenesis was inhibited from day 3,
481 linked to a pH drop from 7.4 to 6.0. Thus, only a 10.3 % TS removal was observed,
482 while TAN increased from 1.5 to 3.0 g N/kg, and acetic, propionic, butyric and valeric
483 acids substantially increased. The overall H₂ production was 0.18 NmL H₂/g VS_{added} and
484 the global COD conversion was 0.18 g COD/g VS_{added}.

485

486 The pH drop observed right after starting the HS-AD batch experiments (initial 0-3
487 days) was crucial to discern about the potential acidification in Tests 6 and 7. The initial
488 pH drop is normally observed in AD when acidogenic outcompetes methanogenic
489 growth (Gerardi 2003), and becomes particularly important in HS-AD of OFMSW due
490 to the high organic content used. Both mono- (Test 6) and co-digestion (Test 7) sacrifice
491 tests showed an initial pH ≥ 7.3 (day 0) that rapidly dropped due to the VFA
492 accumulation. In mono-digestion (Test 6), the pH = 6.4 from day 3 to 11 likely
493 determined the low cumulative methane production (i.e. 6.3 NmL CH₄/g VS_{subs})
494 observed during these days, whereas the pH = 6.0 in the co-digestion sacrifice (Test 7)
495 potentially inhibited methanogenesis (Demirel and Scherer 2008; Staley et al. 2011).

496

497 The ALK_P and likely also the microbial activity of the inoculum used as a seed in a HS-
498 AD reactor played a major role to determine the acidification or methanogenesis onset,
499 since ALK_P is the main pH buffer in AD (Prochazka et al. 2012). These factors mainly
500 depend on the source reactor performance, the degassing period and the inoculum
501 pretreatment. Thus, the ALK_P of the inoculum in this study determined the initial ALK_P
502 of the inoculum-substrate mixture [Table 2], by the ALK_P mass balance.

503

504 At high TS, external buffer addition might help to circumvent HS-AD acidification. For
505 example, Liotta et al. (2014) added NaHCO_3 to stabilize the acidogenic stages in HS-
506 AD. However, whether inorganic buffering is used, particular attention is needed to
507 minimize the TS dilution, while maintain an optimal cationic (i.e. Na^+) concentration
508 for microorganisms (Chen et al. 2008). Moreover, both the NaHCO_3 concentration and
509 the NaHCO_3 -to-organics ratio (i.e. $\text{g NaHCO}_3/\text{g TS}$) need to be the same along different
510 initial TS, to allow comparison among these. Thus, NaHCO_3 addition was not used in
511 this study to reduce the ‘external’ influencers in HS-AD.

512

513 In either case, acidification in this study did not associate to a low ALK_P , nor to a high
514 $\text{ALK}_I/\text{ALK}_P$ ratio – data not shown. For example, mono-digestion Test 1 acidified at an
515 initial ALK_P of 1.7-5.6 $\text{g CaCO}_3/\text{kg}$ and $\text{ALK}_I/\text{ALK}_P = 0.88$, whereas acidification was
516 avoided in mono-digestion Test 6 with ALK_P of 2.6 and $\text{ALK}_I/\text{ALK}_P = 2.12$. Similarly,
517 methanogenesis failed to start in Test 2, operated at the same ISR than Test 6 (i.e. 1.0 g
518 $\text{VS}/\text{g VS}$), though the initial ALK_P and $\text{ALK}_I/\text{ALK}_P$ ratio were 1.5-3.8 $\text{g CaCO}_3/\text{kg}$ and
519 1.51, respectively, in the acidified experiment (Test 2).

520

521 In conclusion, other factors related to the initial inoculum-substrate mixture, and not
522 assessed here, influenced also the HS-AD acidification. Some of these might include the
523 different (micro-)nutrient or inhibitory content, but also the mass transfer, reactor
524 homogenization, reactor headspace volume, particle size and/or inoculum activity
525 (André et al. 2018; Bollon et al. 2013; Chen et al. 2008; Holliger et al. 2016; Motte et
526 al. 2014). Therefore, all these factors should be considered alongside the TS, ISR, ALK_P
527 and nitrogen content to evaluate HS-AD of OFMSW. All the above results corroborate

528 that HS-AD is an extremely complex bio-physical-chemical process, with an elevated
529 number of interrelated mechanisms and operational variables, where a thorough
530 experimental assessment is required to fully understand the overall bio-physical-
531 chemistry and eventually optimize HS-AD of OFMSW at industrial scale.

532

533

534 **4 CONCLUSIONS**

535 This study shows that both the initial TS and ISR determine the success of
536 methanogenesis in HS-AD of OFMSW. During mono-digestion of OFMSW, increasing
537 the maximum TS required a lower ISR, enhancing the risk of acidification. Meanwhile,
538 $\text{NH}_3 \geq 2.3 \text{ g N/kg}$ at 15.0 % TS resulted in VFA accumulation (i.e. 0.13-0.14 g COD/g
539 VS_{added}) and 40 % lower methane yield. Adding sawdust to OFMSW permitted to
540 increase simultaneously the TS and ISR, by reducing considerably the biodegradability
541 and nitrogen content of the mixture, in comparison to mono-digestion of OFMSW. This
542 also led to acidification occurring only at higher TS (i.e. $\geq 20 \%$). Therefore, the initial
543 inoculum-substrate mixture in HS-AD must result from a tradeoff between the
544 maximum TS and the optimum ISR, but also the buffering capacity and the nitrogen
545 content, to circumvent acidification and NH_3 inhibition.

546

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552

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675

676

677 **TABLE CAPTIONS**

678

679 **Table 1** Summary of high-solids batch experiments and biomethane potential tests
680 (BMP)

681

682 **Table 2** Bio-physical-chemical characterization of substrates and inoculum

683

684 **Table 3** Effect of total solids on the performances of high-solids anaerobic digestion of
685 the organic fraction of municipal solid waste using an inoculum-to-substrate ratio of 1.5
686 g VS/g VS (Test 3)

687

688

689 **FIGURE CAPTIONS**

690

691 **Fig. 1** Cumulative methane production: a) Biomethane potential (BMP) test for the
692 organic fraction of municipal solid waste (OFMSW); b) BMP test for sawdust; c) mono-
693 digestion of 55°C-dried OFMSW at an ISR of 1.50 g VS/g VS (Test 3); d) mono-
694 digestion of beech sawdust at an ISR of 0.04 g VS/g VS (Test 4); and e) co-digestion of
695 55°C-dried OFMSW and beech sawdust at an ISR of 0.16 g VS/g VS (Test 5)

696

697 **Fig. 2** Main anaerobic biodegradability indicators: a) methane yield; b) hydrogen yield;
698 c) total solid removal; and d) total chemical oxygen demand (COD) conversion

699

700 **Fig. 3** Sacrifice test with mono-digestion of organic fraction of municipal solid waste
701 (Test 6). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c)
702 total (TS) and volatile (VS) solids, and total (TAN) and free (FAN) ammonia nitrogen;
703 and d) chemical oxygen demand (COD) conversion

704

705 **Fig. 4** Sacrifice test with co-digestion of organic fraction of municipal solid waste and
706 beech sawdust (Test 7). a) Daily and cumulative methane production, and pH; b)
707 volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN) and free
708 (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion

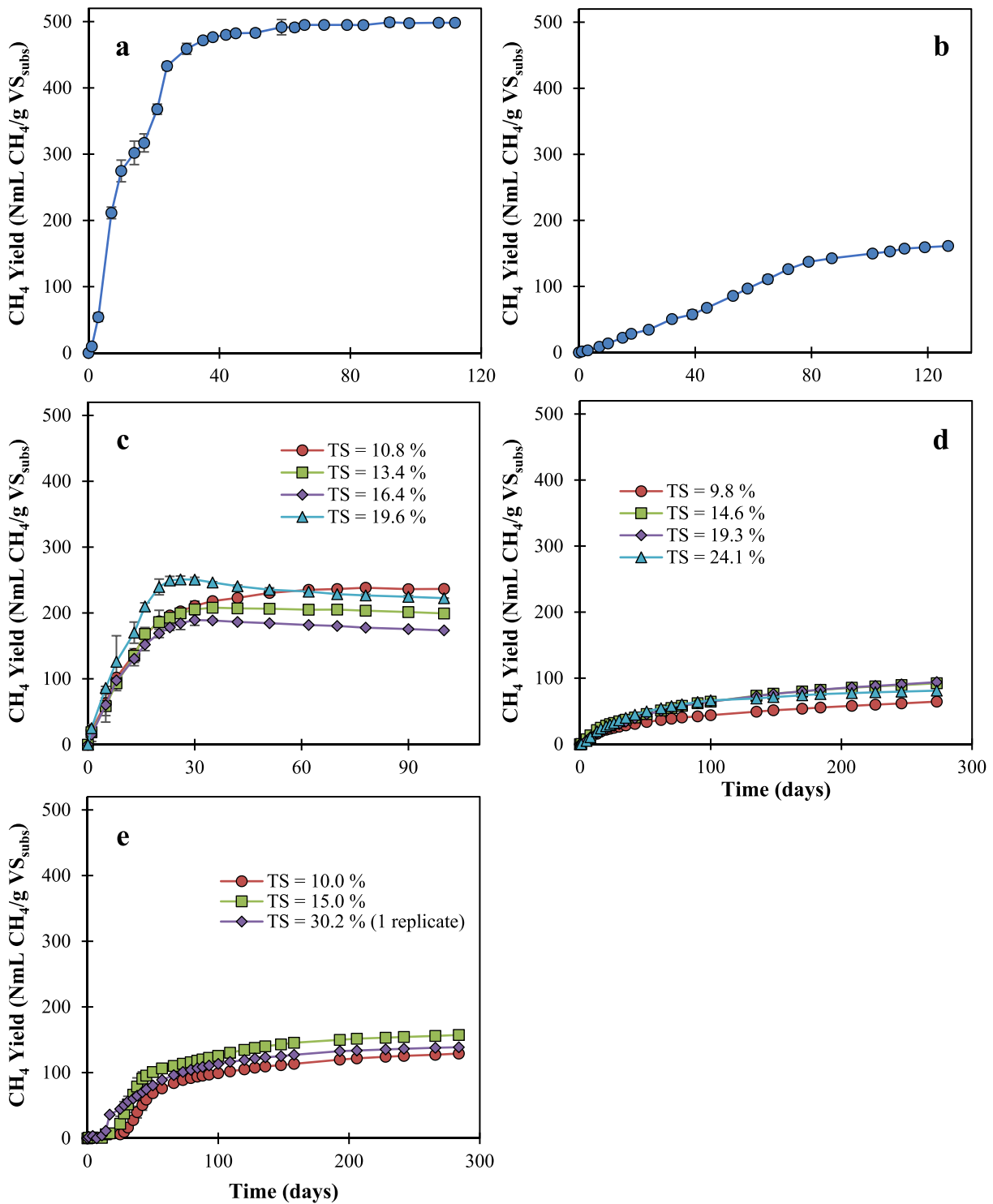


Figure 1. Cumulative methane production: a) Biomethane potential (BMP) test for the organic fraction of municipal solid waste (OFMSW); b) BMP test for sawdust; c) mono-digestion of 55°C-dried OFMSW at an ISR of 1.50 g VS/g VS (Test 3); d) mono-digestion of beech sawdust at an ISR of 0.04 g VS/g VS (Test 4); and e) co-digestion of 55°C-dried OFMSW and beech sawdust at an ISR of 0.16 g VS/g VS (Test 5).

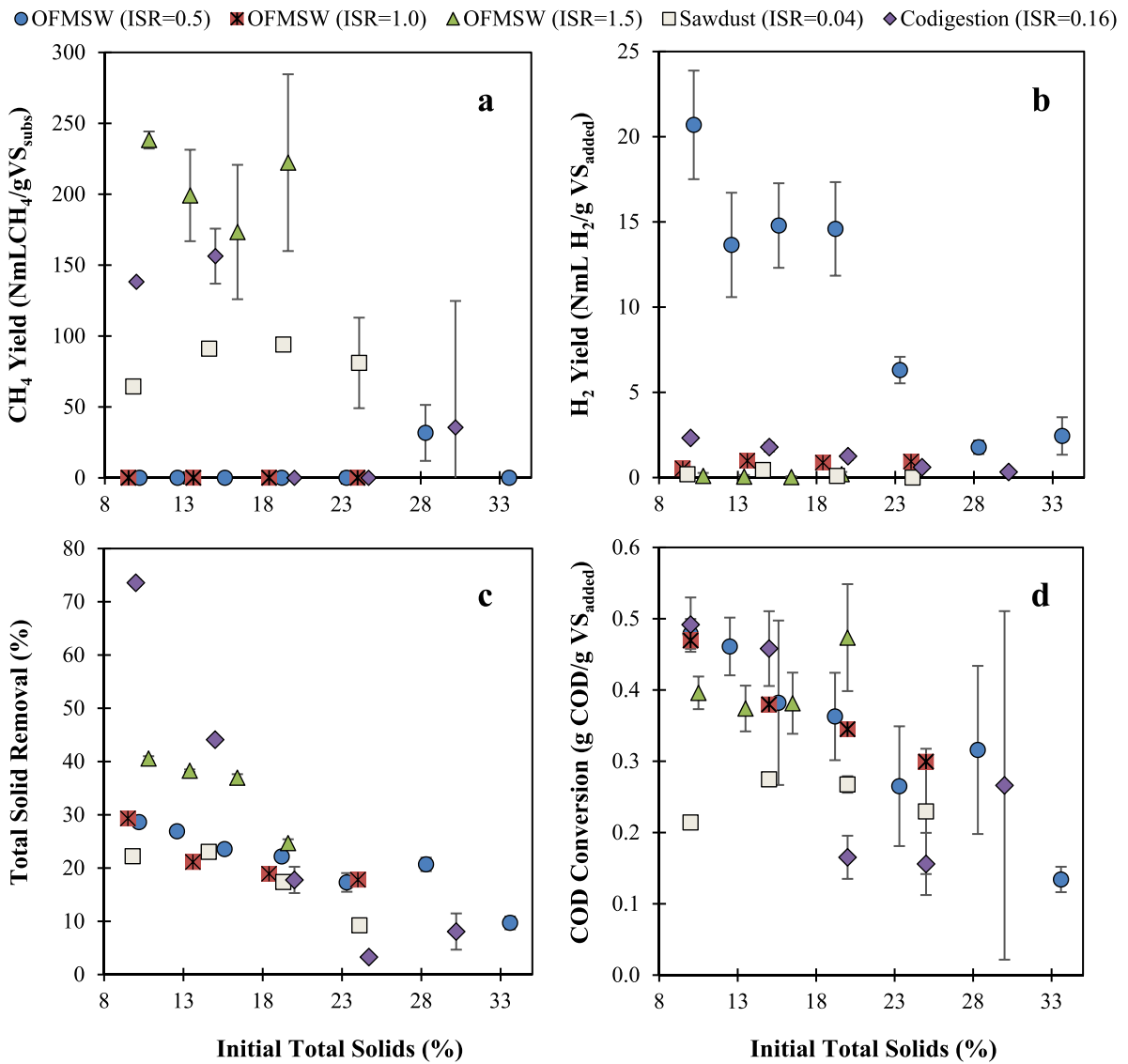


Figure 2. Main anaerobic biodegradability indicators: a) methane yield; b) hydrogen yield; c) total solid removal; and d) total chemical oxygen demand (COD) conversion.

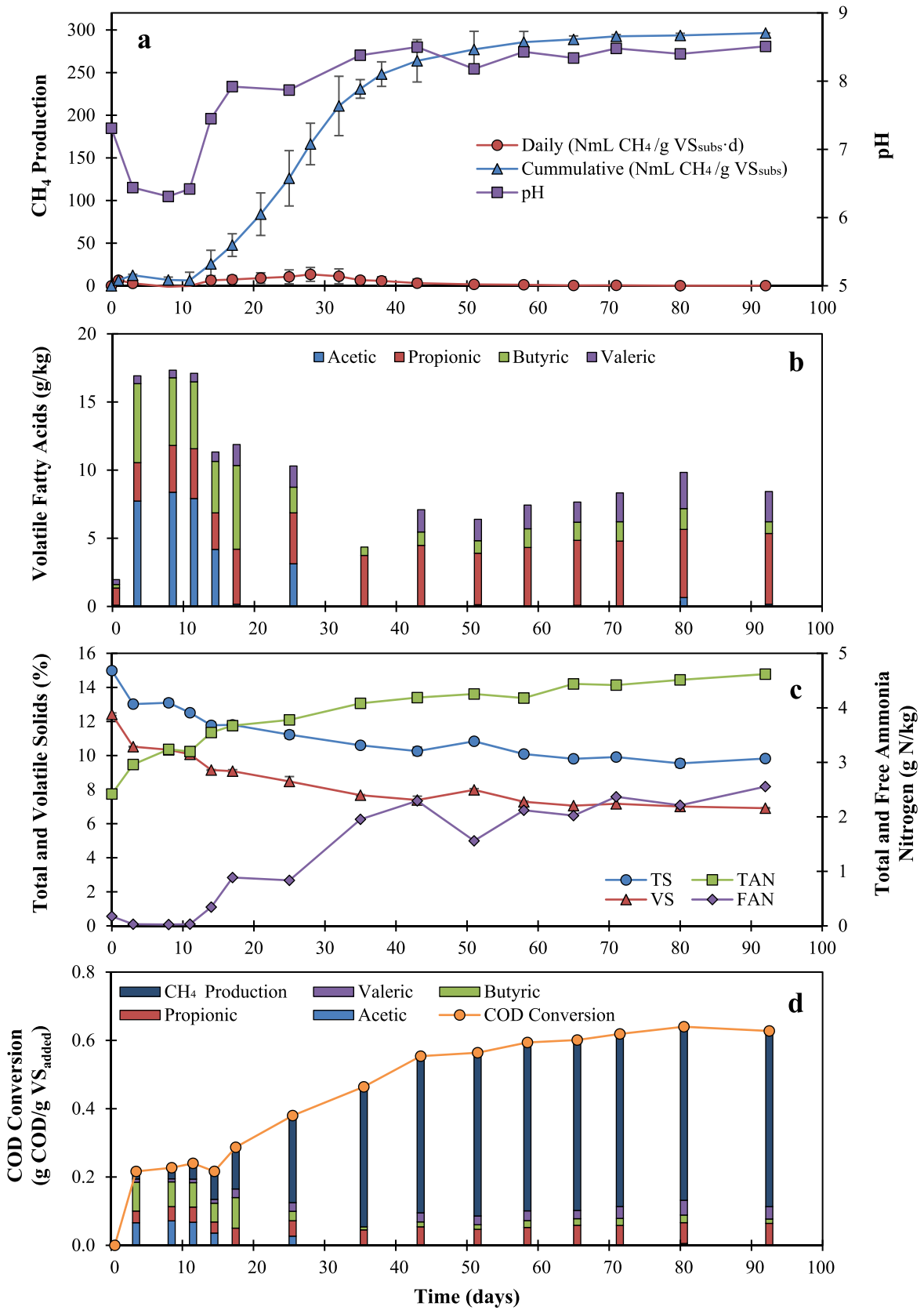


Figure 3. Sacrifice test with mono-digestion of organic fraction of municipal solid waste (Test 6). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN) and free (FAN) ammonia nitrogen; and d) chemical oxygen demand conversion.

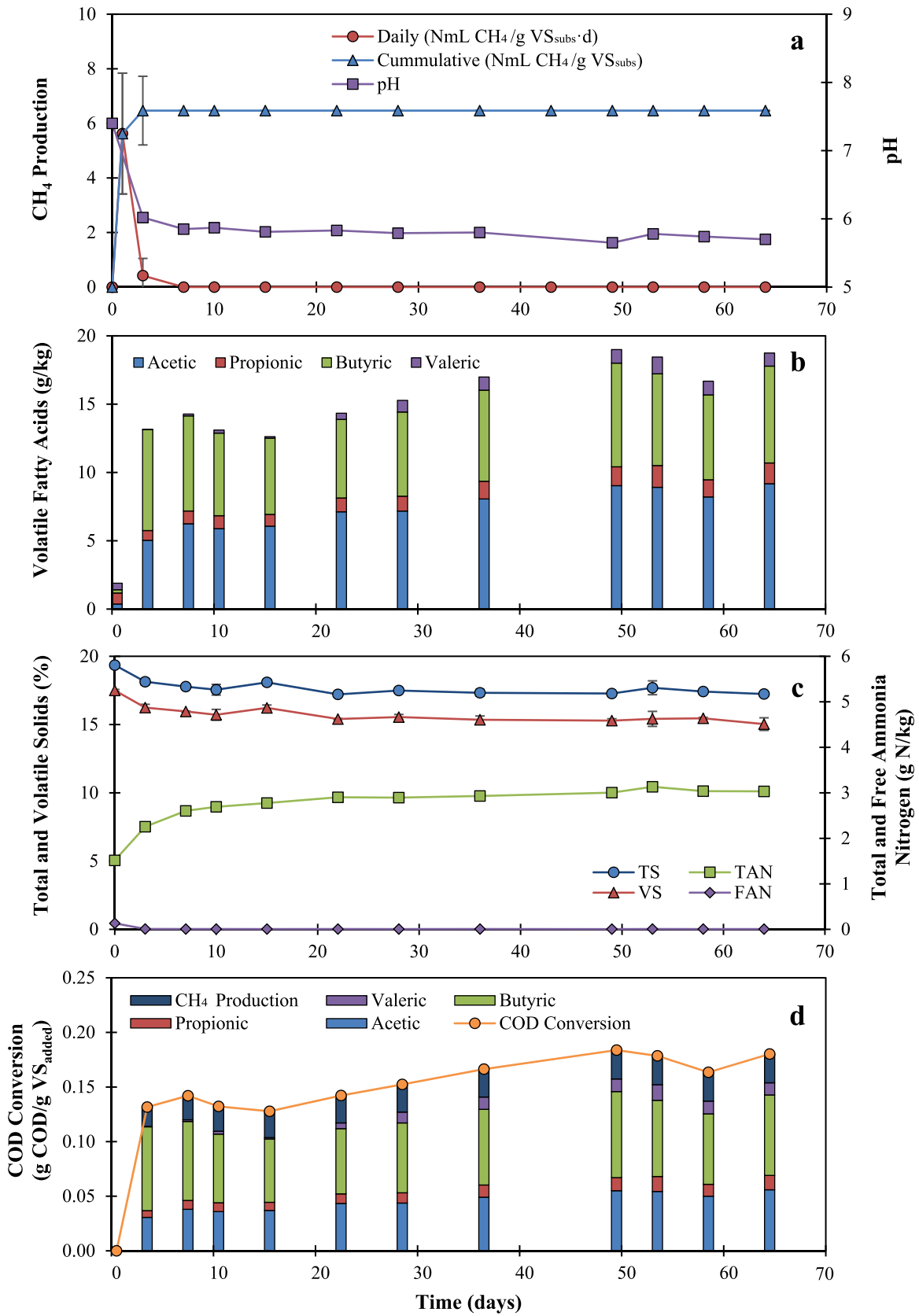


Figure 4. Sacrifice test with co-digestion of organic fraction of municipal solid waste and beech sawdust (Test 7). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN) and free (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion.

Table 1. Summary of high-solids batch experiments and biomethane potential tests (BMP).

No.	Objective	Substrate	Inoculum*	ISR (g VS/g VS)	Initial TS Content (%)	Substrate Added (g)	Inoculum Added (g)	Water Added (mL)	Water Added to the Blank (mL)	Replicates	Reactor Volume (mL)
1	Study the Main Biodegradability Indicators (i.e. CH ₄ yield, TS Removal and COD Conversion)	55°C-dried OFMSW	HS	0.50	10.2, 12.6, 15.6, 19.2, 23.3, 28.3 & 33.6	4.4	15	45, 33, 23, 15, 9, 4 & 0	50	3	280
2				1.00	9.5, 13.6, 18.4 & 24.0	2.2	15	27, 14, 6 & 0	27	3	280
3		1.50	10.8, 13.4, 16.4 & 19.6	1.2	15	13, 8, 3 & 0	14	3	160		
4		Sawdust	W	0.04	9.8, 14.6, 19.3 & 24.1	6.5	20	39, 18, 7 & 0	44	3	160
5		55°C-dried OFMSW + Sawdust	HS	0.16 (1.0:4.0)	10.0, 15.0, 20.0, 24.7 & 30.2	12.5	15	110, 65, 42, 29 & 19	120	3	280
6	Study the Main Dynamics (i.e. TS and VFA)	55°C-dried OFMSW	HS	1.00	15.0	2.6	20	14	16	15	280
7		55°C-dried OFMSW + Sawdust		0.60 (1.0:1.1)	19.4	4.2	20	10	13	15	280
-	Determine the Maximum	OFMSW	W	2.00	2.9	3.0	50	40	43	6	280
-	Methane Yield (i.e. BMP)	Sawdust	W	1.00	4.1	1.0	50	0	0	3	160

HS: High-solids inoculum; W: 'Wet' inoculum. Parenthesis refer to the ratio between organic fraction of municipal solid waste (OFMSW) and sawdust (i.e. g TS:g TS).

*Inoculums were different for each experimental setup.

Table 2. Bio-physical-chemical characterization of substrates and inocula.

	Organic Substrates			Inoculum	
	OFMSW	Dried OFMSW	Sawdust	Wet	High-Solids
TS (%)	26.2 ± 0.1	92.2 ± 1.7	93.6 ± 0.6	3.1 ± 1.0	15.6 ± 2.0
VS^a (%)	24.1 ± 0.5	85.7 ± 1.7	92.9 ± 0.3	2.2 ± 0.8	12.4 ± 1.4
COD (g O₂/g)	0.43 ± 0.02	1.38 ± 0.09	1.16 ± 0.00	0.04 ± 0.02	0.21 ± 0.05
COD_s (g O₂/g)	0.14 ± 0.01	0.38 ± 0.01	<0.01	N.A.	N.A.
TAN (g N/kg)	1.29 ± 0.06	3.45 ± 0.09	0.11 ± 0.00	3.23 ± 0.59	3.24 ± 0.65
TKN (g N/kg)	6.50 ± 1.50	25.45 ± 1.12	0.67 ± 0.45	4.40 ± 0.75	8.66 ± 1.35
pH	4.43 ± 0.11	4.37 ± 0.16	5.65 ± 0.06	8.44 ± 0.15	8.42 ± 0.52
ALK_p (g CaCO₃/kg)	N.A.	N.A.	N.A.	8.13 ± 0.99	5.90 ± 1.34
ALK_i (g Acetic/kg)	0.84 ± 0.68	0.67 ± 0.62	2.16 ± 0.68	4.13 ± 1.31	3.50 ± 1.53
ALK_i/ALK_p	N.A.	N.A.	N.A.	0.82 ± 0.22	0.99 ± 0.54
ρ_s (g/mL)	1.09 ± 0.01	1.43	1.30	1.00	1.08 ± 0.02
ρ (g/mL)	1.08 ± 0.00	0.59	0.31	1.00	1.08 ± 0.02
ε	0.01 ± 0.01	0.59	0.76	0.00	0.00 ± 0.01
VS/TS (%)	92 ± 0	93 ± 2	99 ± 1	71 ± 1	79 ± 2
COD_s/COD (%)	32 ± 2	27 ± 9	<0.01	N.A.	N.A.
COD/TKN (g O₂/g N)	67 ± 2	54 ± 1	1743 ± 4	10 ± 1	24 ± 1
TAN/TS (g N/kg TS)	4.9 ± 0.1	3.7 ± 0.1	0.1 ± 0.0	103.3 ± 0.6	20.7 ± 0.6
BMP (NmL CH₄/g VS)	497 ± 58	N.A.	161 ± 12	N.A.	N.A.

^a Per gram of raw sample on wet basis; N.A.: Not available.

Table 3. Effect of total solids on the performances of high-solids anaerobic digestion of the organic fraction of municipal solid waste using an inoculum-to-substrate ratio of 1.5 g VS/g VS (Test 3).

Initial values				Values at the end of the experiment (day 100)										
TS ₀ (%)	VS ₀ ^a (%)	TKN ₀ (g N/kg)	TAN ₀ (g N/kg)	pH	TAN (g N/kg)	NH ₃ (g N/kg)	Acetic (g COD/g VS _{added})	Propionic (g COD/g VS _{added})	Butyric (g COD/g VS _{added})	Valeric (g COD/g VS _{added})	Total VFA (g COD/g VS _{added})	CH ₄ Production (g COD/g VS _{added})	Global COD Conversion (g COD/g VS _{added})	VFA/(CH ₄ +VFA) (g COD/g COD)
10.8 ± 0.0	9.1 ± 0.0	4.94 ± 0.00	1.65 ± 0.01	8.38 ± 0.02	2.83 ± 0.09	1.35 ± 0.04	0.0058 ± 0.0016	0.0014 ± 0.0010	0.0046 ± 0.0046	0.0106 ± 0.0092	0.0224 ± 0.0147	0.3584 ± 0.0031	0.3807 ± 0.0129	0.0579 ± 0.0372
13.4 ± 0.0	11.4 ± 0.0	6.14 ± 0.00	2.05 ± 0.01	8.45 ± 0.01	3.79 ± 0.12	1.97 ± 0.06	0.0094 ± 0.0009	0.0048 ± 0.0003	0.0038 ± 0.0007	0.0178 ± 0.0020	0.0358 ± 0.0022	0.3161 ± 0.0356	0.3519 ± 0.0335	0.1027 ± 0.0159
16.4 ± 0.0	13.9 ± 0.0	7.46 ± 0.01	2.48 ± 0.01	8.43 ± 0.01	4.22 ± 0.36	2.14 ± 0.18	0.0141 ± 0.0008	0.0078 ± 0.0010	0.0059 ± 0.0005	0.0303 ± 0.0082	0.0582 ± 0.0071	0.2892 ± 0.0522	0.3473 ± 0.0463	0.1712 ± 0.0435
19.6 ± 0.0	16.6 ± 0.0	8.98 ± 0.00	2.99 ± 0.00	8.42 ± 0.04	5.39 ± 0.26	2.70 ± 0.13	0.0205 ± 0.0018	0.0276 ± 0.0030	0.0058 ± 0.0012	0.0457 ± 0.0047	0.0996 ± 0.0104	0.3412 ± 0.0678	0.4408 ± 0.0731	0.2284 ± 0.0308

^a Per gram of raw sample on wet basis.