

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

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4

5 **ABSTRACT**

6 Increasing Total Solids on anaerobic digestion can reduce the methane yield, by the
7 interaction of highly-complex bio-physical-chemical mechanisms. Therefore,
8 understanding those mechanisms and their main drivers becomes crucial to optimize
9 high-solids anaerobic digestion at industrial scale. In this study, seven batch
10 experiments were conducted to investigate the effects of increasing the Total Solids
11 content on high-solids anaerobic digestion of the organic fraction of municipal solid
12 waste. With an Inoculum-to-Substrate Ratio = 1.5 g VS/g VS and maximum Total
13 Solids ≤ 19.6 %, mono-digestion of the organic fraction of municipal solid waste
14 showed a methane yield of 174-236 NmL CH₄/g VS. With an Inoculum-to-Substrate
15 Ratio ≤ 1.0 g VS/g VS and maximum total solids ≥ 24.0 %, similar mono-digestion
16 experiments resulted in acidification. Co-digestion of the organic fraction of municipal
17 solid waste and beech sawdust permitted to reduce the Inoculum-to-Substrate Ratio to
18 0.16 g VS/g VS while increasing Total Solids up to 30.2 %, though achieving a lower
19 methane yield (i.e. 117-156 NmL CH₄/g VS). At each Inoculum-to-Substrate Ratio, a

21 accumulation. Thus, a 40 % lower methane yield of the organic fraction of municipal
22 solid waste was observed at a NH₃ concentration ≥ 2.3 g N-NH₃/kg Reactor Content and
23 Total Solids = 15.0 %. Meanwhile, the addition of sawdust to the organic fraction of
24 municipal solid waste lowered the nitrogen content, being the risk of acidification

25 exacerbated only at Total Solids ≥ 20.0 %. Therefore, the biodegradability of the
26 substrate, as well as the operational Total Solids and the Inoculum-to-Substrate Ratio,
27 are closely-interrelated parameters determining the success of methanogenesis, but also
28 the risk of ammonia inhibition on high-solids anaerobic digestion.

29

30 **Keywords:** High-solids Anaerobic Digestion; Organic Fraction of Municipal Solid
31 Waste; Batch Experiments; Co-digestion; Thermophilic; Methane Yield; Volatile Fatty
32 Acids.

33

34 1 INTRODUCTION

35 Anaerobic digestion (AD) is a biochemical treatment technology in which an organic
36 waste (OW) is decomposed to a mixture of gases – mainly CH₄ and CO₂ – known as
37 biogas, and a partially stabilized organic material known as digestate. Biogas has a high
38 calorific content, while the nutrient-concentrated digestate has the potential to be used
39 as soil amendment (De Baere and Mattheeuws 2013). AD takes place through a
40 sequential set of fermentative steps carried out symbiotically by different microbial
41 consortia (Gerardi 2003). The main AD steps are hydrolysis, acidogenesis, acetogenesis
42 and methanogenesis, while the AD biochemistry strongly depends on a balance between
43 volatile fatty acid (VFA) production by acidogens/acetogens and VFA consumption by
44 methanogens. When an imbalance occurs, VFA and/or H₂ accumulate, potentially
45 leading to AD failure by acidification (i.e. pH ≤ 6.0) (Motte et al. 2014; Staley et al.
46 2011). Other inhibitory substances may also accumulate during AD, such as free
47 ammonia (NH₃) and cations (e.g. Na⁺, K⁺) (Chen et al. 2008; Riggio et al. 2017).

48

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

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

60

61 HS-AD of the organic fraction of municipal solid waste (OFMSW), including food
62 waste (FW) and green/lignocellulosic waste (GW), is widely used. Indeed, the high TS
63 content (i.e. 20-50 %) and the high biodegradation potential of OFMSW are particularly
64 favorable to lower the operational costs of HS-AD (De Baere and Mattheeuws 2013). In
65 this line, batch systems for OFMSW treatment at industrial scale can be operated up to
66 40 % TS, provided that leachate is continuously recirculated as a source of
67 microorganisms and partial mixing (André et al. 2018; Riggio et al. 2017).

68

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

80

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

94

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

102

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

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

117

118

119 **2 MATERIALS AND METHODS**

120 **2.1 Organic Substrates and Inoculum**

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

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

131

132 To increase the TS content in the batch experiments, 1-2 kg of OFMSW were dried for
133 7-10 days at 55°C until constant weight right before each experiment. The resulting
134 agglomerate was further minced with mortar and pestle, homogenized to a flour-like
135 material with a particle size ≤ 2 mm, and stored in air-tight containers until use.

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

137

138 Three ‘wet’ and six high-solids inocula were used in this study, since different
139 experiments were started at different periods. All inocula were sampled from a 30 L
140 methanogenic reactor fed with OFMSW under thermophilic (55°C) conditions. Prior to
141 being used in the experiments, all inocula were degassed for 7-10 days at 55°C and
142 subsequently filtered through a 1 mm mesh to remove coarse materials. These
143 inoculums were considered ‘wet’ since TS was ≤ 5 %. To increase simultaneously the
144 TS and ISR of batch experiments, the ‘wet’ inoculums were centrifuged at 6000 rpm for
145 10 min with a bench-scale centrifuge (REMI XS R-10M, India), right before each
146 experiment – high solids inoculum. The supernatant was separated and the remaining
147 viscous material was manually homogenized. Finally, micronutrients were added to
148 each inoculum as recommended by Angelidaki and Sanders (2004).

149

150 **2.2 Batch Experiments**

151 **2.2.1 Experimental Setup**

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

162

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

172

173 **2.2.2 HS-AD Biodegradability Indicators**

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

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

185

186 **2.2.3 Sacrifice Tests**

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

197

198 **2.3 Biomethane potential of OFMSW and beech sawdust**

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

208

209 **2.4 Physical-Chemical Analyses**

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

217

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

223 μm polypropylene membrane. The VFA (acetic, propionic, butyric and valeric acids)
224 analysis of 0.45 μm pre-filtered samples was conducted with a LC-20AD HPLC
225 (Shimadzu, Japan) equipped with a Rezex ROA-Organic Acids 8+ column
226 (Phenomenex, USA) coupled to a 210 nm UV detector. The column was maintained at
227 70°C with a 0.0065 M H_2SO_4 mobile phase flowing at 0.6 mL/min. Lactate and ethanol
228 were measured by the same method but using a RID detector. However, these last
229 compounds were not detected in any of the batch conditions assessed in this study.

230

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

237

238 **2.5 Calculations**

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

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

247 or BMP tests. The overall methane or hydrogen production at the end of each
248 experiment was expressed as a normalized volume of gas ($P = 1$ bar, $T = 0$ °C)
249 measured by water displacement, divided by the VS added (VS_{added}) – including the
250 substrate and inoculum. The hydrogen production by the VS removed (VS_{removed}) was
251 also calculated in some acidified reactors.

252

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

255 The global COD conversion included the overall methane and/or hydrogen production
256 and the VFA content at the end of each experiment, divided by VS_{added} . In sacrifice tests

257 [Tests 6 and 7, Table 1], the progressive COD conversion was evaluated as the

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

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

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

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

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

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

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

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

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

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

268

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

270 2.2.2), the repeatability (i.e. average \pm standard deviation) was assessed using all

271 triplicates at each initial TS content. On the other hand, in the sacrifice tests (Section
272 2.2.3), the biogas production and composition included the average \pm standard deviation
273 of all the (remaining) replicates at a given experimental time, including that being
274 subsequently emptied. The rest of physical-chemical analyses (e.g. TS, TAN, VFA)
275 were performed in triplicate for the punctually-emptied replicate. In all these batch
276 experiments, the water loss (in terms of vapor) regarding the initial amount of water in
277 each substrate-inoculum mixture was considered negligible (i.e. $< 3\%$, data not shown).

278

279

280 **3 RESULTS AND DISCUSSION**

281 **3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum**

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

294

295 The BMP of the raw OFMSW and sawdust at 55°C was 497 ± 58 NmL CH₄/g VS_{subs}
296 [Figure 1a] and 161 ± 12 NmL CH₄/g VS_{subs} [Figure 1b], respectively, indicating the
297 lower biodegradability of sawdust than of OFMSW under anaerobic conditions.
298 Moreover, reaching the maximum methane yield took a considerably longer for sawdust
299 than OFMSW (i.e. 130 and 56 days, respectively), suggesting also a reduced hydrolysis
300 rate for lignocellulosic substrates (Pastor-Poquet et al. 2018, In Press; Vavilin et al.
301 2008). The higher standard deviation in the BMP for raw OFMSW was attributed to the
302 waste heterogeneity. The BMP values were equivalent to those observed for source-
303 sorted OFMSW and GW (Brown and Li 2013; Schievano et al. 2010).

304

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

314

315 **3.2 Batch Experiments**

316 **3.2.1 Acidified Experiments**

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

319 the TS conditions resulted in acidification (i.e. $\text{pH} \leq 6.0$), likely due to the low ISR used
320 (Angelidaki and Sanders 2004). Methanogenesis inhibition led to H_2 production and
321 VFA accumulation. The highest H_2 production with an $\text{ISR} = 0.5 \text{ g VS/g VS}$ (Test 1)
322 was achieved at the lowest TS (i.e. 10.2 %) and progressively decreased with increasing
323 TS [Figure 2b], likely due to the reduced mass transfer in high-solids conditions. The H_2
324 production (i.e. $2\text{-}20 \text{ NmL H}_2/\text{g VS}_{\text{added}} = 7\text{-}60 \text{ NmL H}_2/\text{g VS}_{\text{removed}}$) was comparable to
325 that reported by Valdez-Vazquez and Poggi-Varaldo (2009) for OFMSW (i.e. $10\text{-}50$
326 $\text{NmL H}_2/\text{g VS}_{\text{removed}}$). With an $\text{ISR} = 1.0 \text{ g VS/g VS}$ (Test 2), the H_2 production was ≤ 1
327 $\text{NmL H}_2/\text{g VS}_{\text{added}}$. A reduced H_2 production can be attributed to a higher ISR.

328

329 In both experiments, an inverse relationship between the TS removal and the initial TS
330 was observed [Figure 2c]. Meanwhile, the global COD conversion described an average
331 $0.35 \text{ g COD/g VS}_{\text{added}}$ at an initial TS of around 10 % and a similar downward trend
332 with increasing TS in both experiments [Figure 2d]. The COD conversion in acidified
333 reactors corresponded from 87 to 96 % of the VFA accumulation. This confirms that H_2
334 production and/or VFA accumulation potentially reduced the hydrolysis rate (Cazier et
335 al. 2015; Vavilin et al. 2008), playing a major role on the organic degradation at higher
336 TS, due to the low water available (García-Bernet et al. 2011).

337

338 **3.2.2 Methane-Producing Experiments**

339 Despite mono-digestion of OFMSW at an $\text{ISR} = 0.5 \text{ g VS/g VS}$ (Test 1) acidified at all
340 TS contents, methanogenesis occurred in 2 out of 3 replicates performed at 28.3 % TS,
341 leading to an average methane yield of $64 \pm 6 \text{ NmL CH}_4/\text{g VS}_{\text{subs}}$ [Figure 2a] – 87 %
342 lower than the BMP of raw OFMSW – and a 23 % TS removal [Figure 2c]. The

343 methanogenic onset observed in the two bottles at 28.3 % TS might relate to a favorable
344 mass transfer in the high-solids mixture, as discussed in Section 3.2.4, since all the
345 bottles contained exactly the same amount of substrate and inoculum.

346

347 Methanogenesis succeeded in all TS contents with mono-digestion of OFMSW using an
348 $ISR = 1.5 \text{ g VS/g VS}$ (Test 3), though only a maximum 19.6 % TS was reached under
349 these conditions [Figure 2a]. A methane yield of 236 ± 5 , 199 ± 32 , 174 ± 47 and $222 \pm$
350 $62 \text{ NmL CH}_4/\text{g VS}_{\text{subs}}$ was observed at initial TS of 10.8, 13.4, 16.4 and 19.6 %, respectively
351 [Figure 1c and 2a], i.e. 52-65 % lower than the BMP of OFMSW. These
352 methane yields corresponded to a volumetric productivity of 8.8 ± 0.2 , 9.3 ± 1.5 , $10.2 \pm$
353 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,
354 13.4, 16.4 and 19.6 %, respectively, being the higher volumetric productivity at
355 increasing TS one of the main advantages of HS-AD (Brown et al. 2012). Interestingly,
356 the standard deviation of the methane yield increased alongside the TS [Figure 2a],
357 likely due to mass transfer effects and/or a higher heterogeneity of the initial mixture, as
358 discussed in Section 3.2.4. In contrast, the TS removal decreased at increasing initial TS
359 contents [Figure 2c]. The global COD conversion was approximately $0.38 \pm 0.05 \text{ g}$
360 $\text{COD/g VS}_{\text{added}}$ at all TS, but showing a higher standard deviation at an initial TS =
361 19.6 % [Figure 2d & Table 3]. It should be noted that the TS removal (i.e. VS removal)
362 and the COD conversion yield similar information about the overall organic degradation
363 in methanogenic experiments. Nonetheless, the COD conversion was considered as a
364 more informative assessment of the VFA accumulation in these experiments, as
365 indicated in Section 2.5. Particularly, it can be observed how the COD standard
366 deviation is obscured when assessing the TS removal [Figure 2c & Figure 2d].

367

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

378

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

388

389 **3.2.3 Main Effects when Increasing the Initial TS in HS-AD**

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

397

398 The low water content of a high-solids mixture hinders the accessibility of
399 microorganisms to large portions of the substrate (Bollon et al. 2013), possibly
400 explaining the increasing standard deviation in the methane yield at $TS \geq 10\%$ [Figure
401 2a]. Particularly, ‘dry’ AD (i.e. $TS \geq 20\%$) is associated to the presence of spatially-
402 differentiated acidogenic/methanogenic centers (Staley et al. 2011; Xu et al. 2014). In
403 such systems, the convective transport is minimum, while the metabolite diffusion
404 increases in importance, since the free-to-bound water ratio is low (Bollon et al. 2013;
405 García-Bernet et al. 2011). Besides limiting the organic degradation, this phenomenon
406 also reduces the chances of acidification of all the methanogenic centers in case of
407 overload, likely explaining the methanogenesis onset observed in some replicates at
408 28.3 % TS (Test 1) and 30.2 % TS (Test 5). Homogenization devices, such as reactor
409 stirrer or leachate recirculation, might help to prevent the influence of mass transfer
410 limitations in HS-AD (André et al. 2018; Kothari et al. 2014).

411

412 **3.2.4 Maximizing the TS in HS-AD of OFMSW by Sawdust Addition**

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

428

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

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

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

446

447 **3.2.5 HS-AD Dynamics and NH₃ Inhibition**

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

458

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

461 indicators significantly slowed down in the mono-digestion sacrifice (Test 6) as NH₃
462 reached 2.3 g N/kg from day 45 [Figure 3]. Depending on the methanogens acclimation,
463 NH₃ concentrations of 0.2-1.4 g N/L have been reported inhibitory (Chen et al. 2008;
464 Fricke et al. 2007; Prochazka et al. 2012). In this study, the NH₃ increase correlated well
465 with the propionic/valeric accumulation in Test 6 [Figure 3], being the VFA buildup a
466 likely consequence of methanogenic inhibition (Demirel and Scherer 2008).

467

468 The above results indicate that the ammonia buildup most probably hampered the
469 methane production also in the mono-digestion experiment using an ISR = 1.5 g VS/g
470 VS (Test 3) [Figure 2]. Thus, the nitrogen content (i.e. TKN, TAN and NH₃) was
471 observed to increase in Test 3 alongside the higher initial TS, because of the lower
472 amount of water initially used for dilution, potentially exacerbating the NH₃ inhibition
473 and VFA accumulation at higher TS [Table 3]. With all the above, the NH₃
474 accumulation can determine the overall anaerobic degradation (i.e. methane yield, TS
475 removal and COD conversion) during HS-AD, particularly at higher initial TS contents.
476 These results complement the main bio-physical-chemical effects arising in HS-AD due
477 to the TS increase (i.e. reduced organic degradation by mass transfer effects), as
478 mentioned in Section 3.2.3. In other words, the TS increase can limit the organic
479 degradation in HS-AD of OFMSW due both to mass transfer effects and NH₃ inhibition.
480 With the aim to reduce the risk of NH₃ inhibition while increasing the TS content, a co-
481 digestion sacrifice was performed.

482

483 **3.2.6 Other Factors Influencing Acidification in HS-AD**

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

489

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

500

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

507

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

516

517 In either case, acidification in this study did not associate to a low ALK_P , nor to a high
518 $\text{ALK}_I/\text{ALK}_P$ ratio – data not shown. For example, mono-digestion Test 1 acidified at an
519 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
520 avoided in mono-digestion Test 6 with ALK_P of 2.6 and $\text{ALK}_I/\text{ALK}_P = 2.12$. Similarly,
521 methanogenesis failed to start in Test 2, operated at the same ISR than Test 6 (i.e. 1.0 g
522 $\text{VS}/\text{g VS}$), 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
523 1.51, respectively, in the acidified experiment (Test 2).

524

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

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

536

537

538 **4 CONCLUSIONS**

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

550

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556

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676
- 677

678 **TABLE CAPTIONS**

679

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

682

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

684

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

688

689

690 **FIGURE CAPTIONS**

691

692 **Fig. 1** Cumulative methane production: a) Biomethane potential (BMP) test for the
693 organic fraction of municipal solid waste (OFMSW); b) BMP test for sawdust; c) mono-
694 digestion of 55°C-dried OFMSW at an ISR of 1.50 g VS/g VS (Test 3); d) mono-
695 digestion of beech sawdust at an ISR of 0.04 g VS/g VS (Test 4); and e) co-digestion of
696 55°C-dried OFMSW and beech sawdust at an ISR of 0.16 g VS/g VS (Test 5)

697

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

700

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

705

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