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

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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 CH_4/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 CH_4/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 NH_3 concentration $\geq$ 2.3 g N-NH_3/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

1 INTRODUCTION

Anaerobic digestion (AD) is a biochemical treatment technology in which an organic

waste (OW) is decomposed to a mixture of gases – mainly CH_4 and CO_2 – known as

biogas, and a partially stabilized organic material known as digestate. Biogas has a high

calorific content, while the nutrient-concentrated digestate has the potential to be used

as soil amendment (De Baere and Mattheeuws 2013). AD takes place through a

sequential set of fermentative steps carried out symbiotically by different microbial

consortia (Gerardi 2003). The main AD steps are hydrolysis, acidogenesis, acetogenesis

and methanogenesis, while the AD biochemistry strongly depends on a balance between

volatile fatty acid (VFA) production by acidogens/acetogens and VFA consumption by

methanogens. When an imbalance occurs, VFA and/or H_2 accumulate, potentially

leading to AD failure by acidification (i.e. pH $\leq$ 6.0) (Motte et al. 2014; Staley et al.

2011). Other inhibitory substances may also accumulate during AD, such as free

ammonia (NH_3) and cations (e.g. Na^+, K^+) (Chen et al. 2008; Riggio et al. 2017).

Depending on the total solid (TS) content, AD can be operated under 'wet' (i.e. TS <

10 %), 'semi-solid' (i.e. $10 \leq$ TS < 20 %) and 'dry' (i.e. TS $\geq$ 20 %) conditions

(Abbassi-Guendouz et al. 2012; Pastor-Poquet et al. 2018). High-solids AD (HS-AD)

includes the two last cases, and has some advantages such as the use of a smaller

digester volume, and a reduced need for water addition and dewatering operations,

enhancing the process economy (André et al. 2018; Kothari et al. 2014). However, HS-

AD also shows some drawbacks such as a high risk of reactor acidification by substrate

overload, and a reduced mass transfer associated to the low content of free water in the

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 55ºC, 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 4ºC, 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 -20ºC, 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 55ºC 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 (55ºC) conditions. Prior to

137 being used in the experiments, all inocula were degassed for 7-10 days at 55ºC 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 55ºC. 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 Na_2S 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 ≥ 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 55ºC 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 $\pm$ 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 Noguerol-Arias et al. (2012). The soluble COD (CODs) 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   70ºC 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 bar, T = 0ºC), 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 ºC)

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_{Real}/V_{Global}. In this study, all the initial batch

261   configurations were designed to be porosity free (i.e. ε = 0; V_{Global} = V_{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 ± 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 ± 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 55ºC. 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 55ºC was 497 ± 58 NmL CH_4/g VS_{subs}

291 [Figure 1a] and 161 ± 12 NmL CH_4/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 105ºC

307 (Capson-Tojo et al. 2017), though heating the inoculum at 105ºC 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. 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 ISR = 0.5 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 NmL H_2/g VS_{added} = 7-60 NmL H_2/g $VS_{removed}$) was comparable to

320   that reported by Valdez-Vazquez and Poggi-Varaldo (2009) for OFMSW (i.e. 10-50

321   NmL H_2/g $VS_{removed}$). With an ISR = 1.0 g VS/g VS (Test 2), the H_2 production was $\leq$ 1

322   NmL H_2/g VS_{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 g COD/g VS_{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 ISR = 0.5 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 NmL CH_4/g VS_{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 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 NmL CH_4/g VS_{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 ± 4.4 NmL CH_4/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 ± 0.05 g

356 COD/g VS_{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 CH$_4$/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 H$_2$ 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 NH_3 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 NH_3 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 NH_3 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 CH_4/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 NH_3 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 ISR = 1.5 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 H_2 production was 0.18 NmL H_2/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 $\geq$ 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 CH_4/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. g $NaHCO_3$/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   ALK_I/ALK_P ratio – data not shown. For example, mono-digestion Test 1 acidified at an

515   initial ALK_P of 1.7-5.6 g $CaCO_3$/kg and ALK_I/ALK_P = 0.88, whereas acidification was

516   avoided in mono-digestion Test 6 with ALK_P of 2.6 and ALK_I/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   VS/g VS), though the initial ALK_P and ALK_I/ALK_P ratio were 1.5-3.8 g $CaCO_3$/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      $NH_3 \geq 2.3$ 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. ≥ 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

552

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675

676

TABLE CAPTIONS

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

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

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)
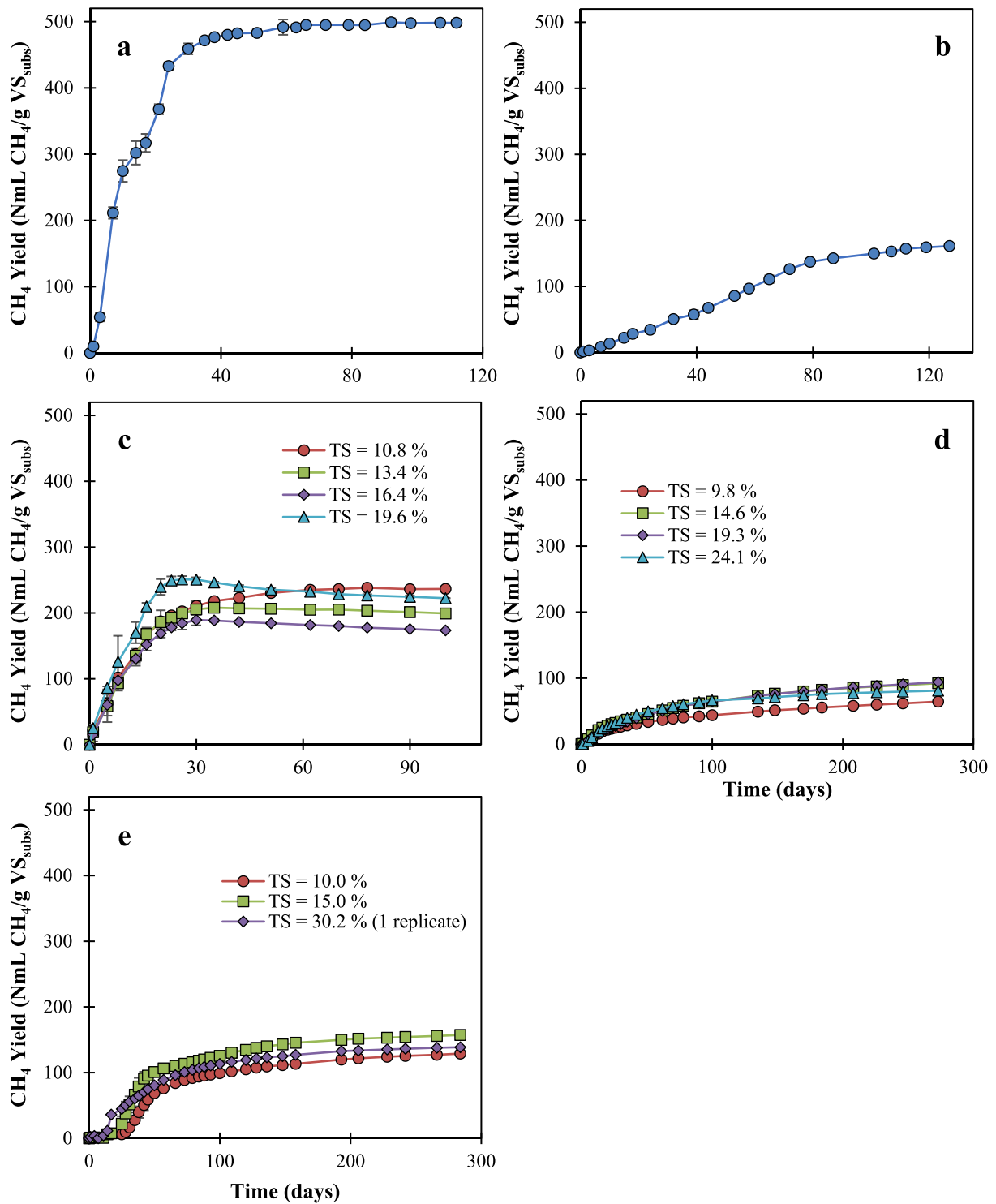
689 **FIGURE CAPTIONS**
690
691 **Fig. 1** Cumulative methane production: a) Biomethane potential (BMP) test for the
692 organic fraction of municipal solid waste (OFMSW); b) BMP test for sawdust; c) mono-
693 digestion of 55ºC-dried OFMSW at an ISR of 1.50 g VS/g VS (Test 3); d) mono-
694 digestion of beech sawdust at an ISR of 0.04 g VS/g VS (Test 4); and e) co-digestion of
695 55ºC-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;
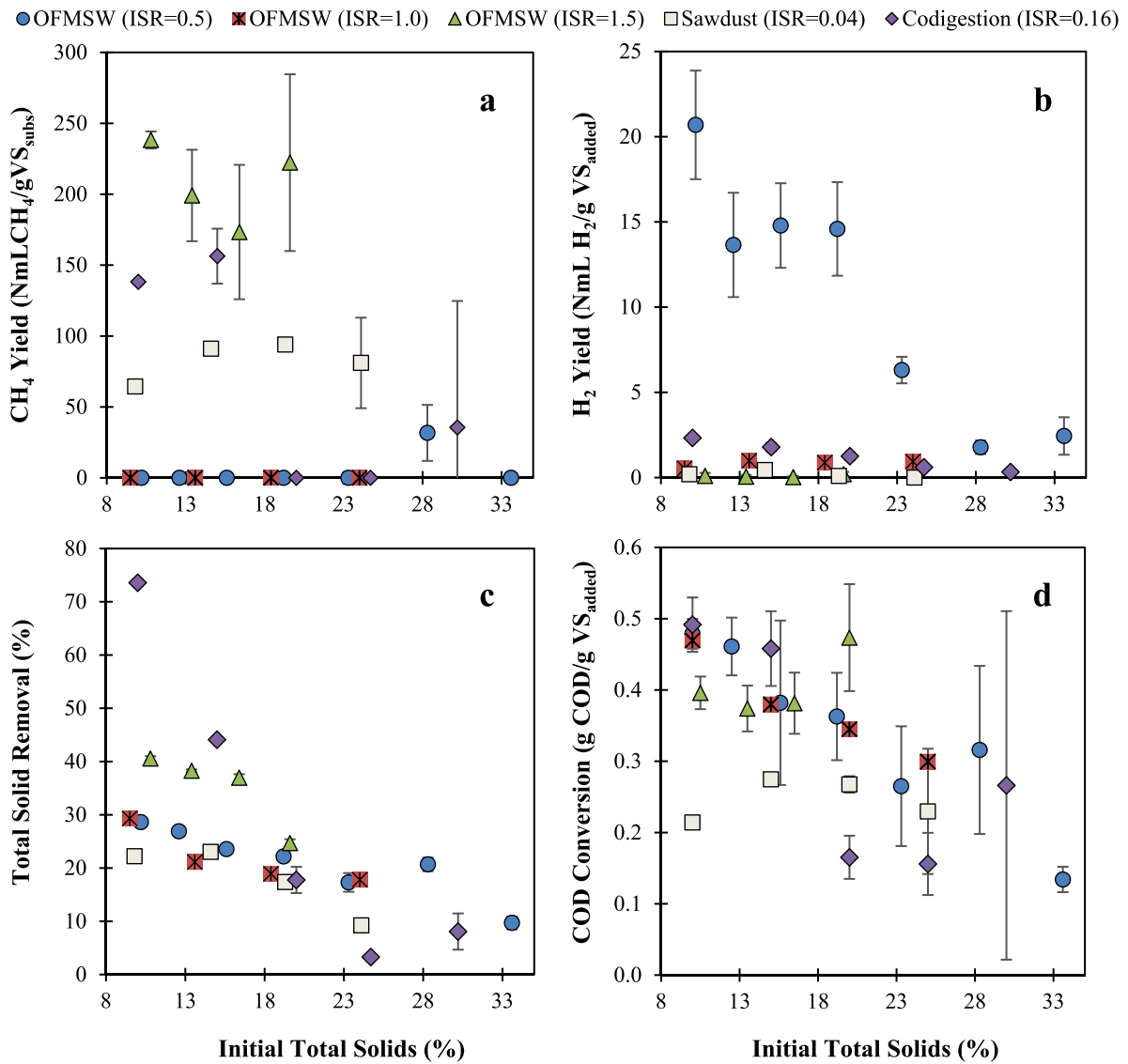698 c) total solid removal; and d) total chemical oxygen demand (COD) conversion
699
700 **Fig. 3** Sacrifice test with mono-digestion of organic fraction of municipal solid waste
701 (Test 6). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c)
702 total (TS) and volatile (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
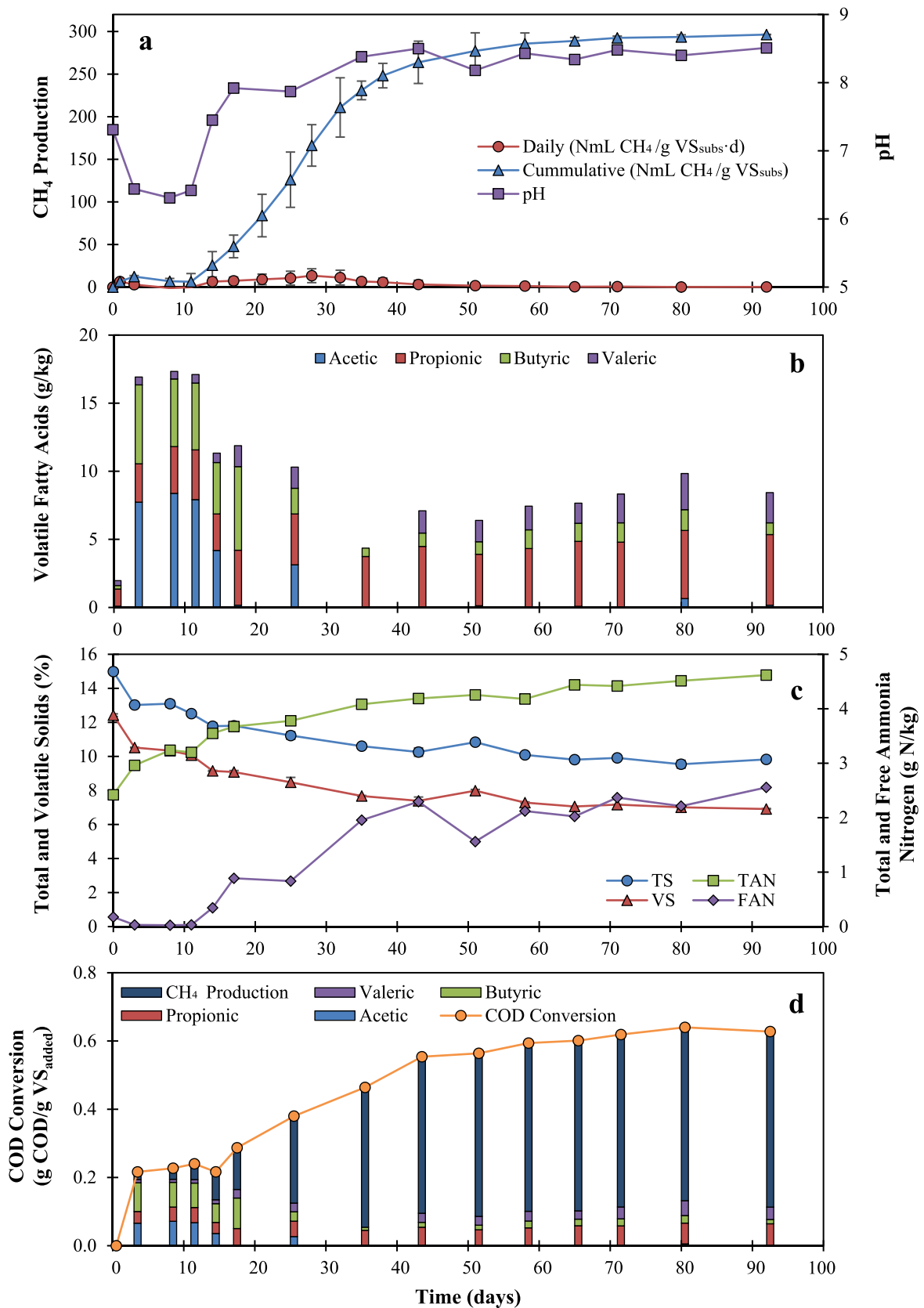708 (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion

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 55ºC-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 55ºC-dried OFMSW and beech sawdust at an ISR of 0.16 g VS/g VS (Test 5).
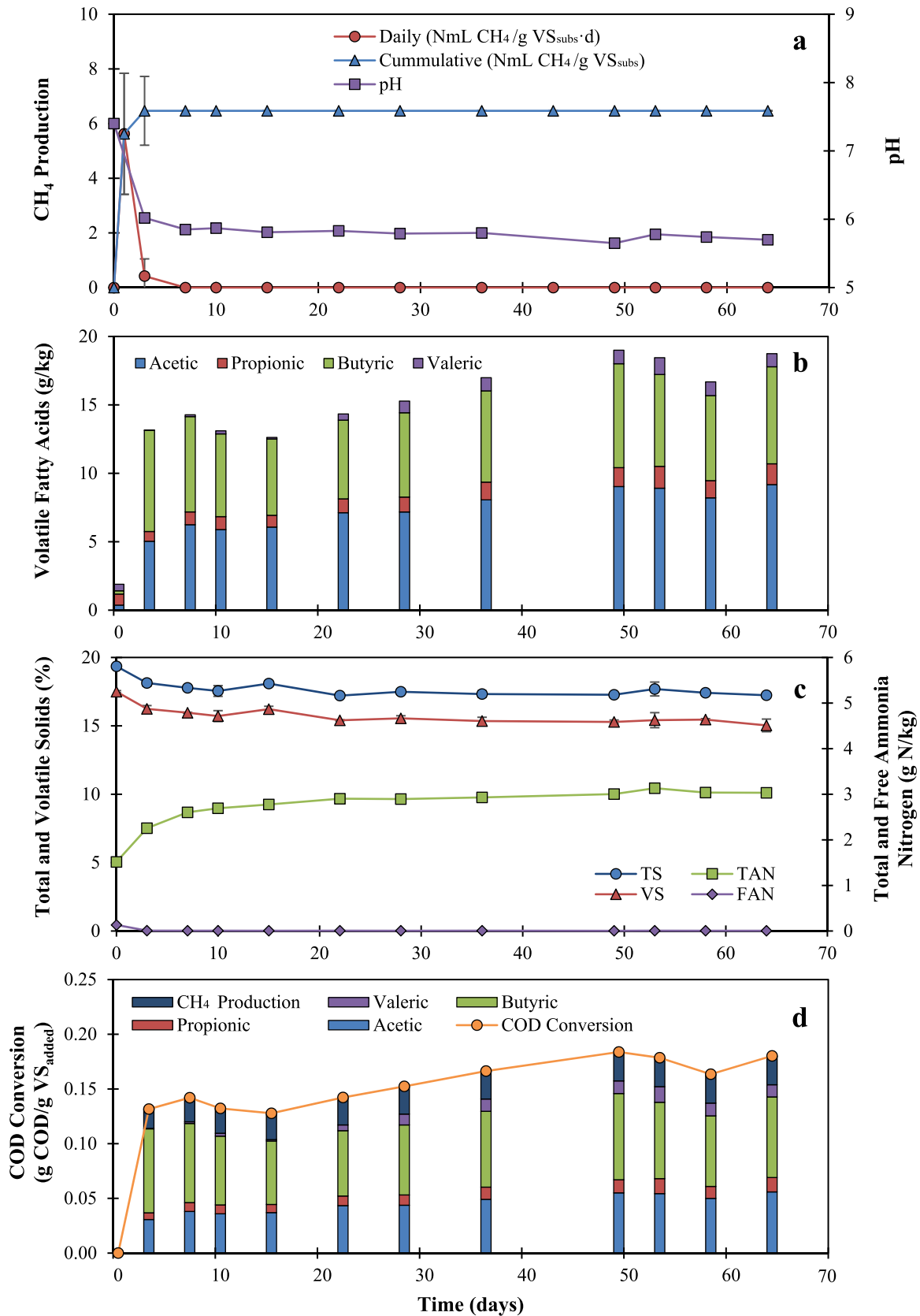
Figure 2. Main anaerobic biodegradability indicators: a) methane yield; b) hydrogen yield; c) total solid removal; and d) total chemical oxygen demand (COD) conversion.

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)	55ºC-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		55ºC-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)	55ºC-dried OFMSW	HS	1.00	15.0	2.6	20	14	16	15	280
7		55ºC-dried OFMSW + Sawdust		0.60 (1.0:1.1)	19.4	4.2	20	10	13	15	280
-	Determine the Maximum Methane Yield (i.e. BMP)	OFMSW	W	2.00	2.9	3.0	50	40	43	6	280
-		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 O_2/g)	0.43 ± 0.02	1.38 ± 0.09	1.16 ± 0.00	0.04 ± 0.02	0.21 ± 0.05
COD_S (g O_2/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 $CaCO_3$/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 O_2/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 CH_4/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.*