Semi-continuous mono-digestion of OFMSW and Co-digestion of OFMSW with beech sawdust: Assessment of the maximum operational total solid content

Vicente Pastor-Poquet, Stefano Papirio, Eric Trably, Jukka Rintala, Renaud Escudié, Giovanni Esposito

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2 **OFMSW with Beech Sawdust: Assessment of the Maximum**

3 **Operational Total Solid Content**

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5 Vicente Pastor-Poquet [a,b,c,*], Stefano Papirio [d], Eric Trably [b], Jukka Rintala [c], Renaud

6 Escudié [b], and Giovanni Esposito [a]

7

8 [a] Department of Civil and Mechanical Engineering, University of Cassino and Southern

9 Lazio, via Di Biasio 43, 03043 Cassino (FR), Italy

10 * Corresponding author. E-mail: vicente.pastor.poquet@gmail.com

11 [b] LBE, Univ Montpellier, INRA, 102 avenue des Etangs, 11100, Narbonne, France

12 [c] Department of Chemistry and Bioengineering, Tampere University of Technology,

13 Korkeakoulunkatu 10, FI-33720 Tampere, Finland

14 [d] Department of Civil, Architectural and Environmental Engineering, University of

15 Napoli Federico II, via Claudio 21, 80125 Napoli, Italy

16

ABSTRACT

In this study, mono-digestion of the organic fraction of municipal solid waste (OFMSW) and co-digestion of OFMSW with beech sawdust, simulating green waste, were used to investigate the maximum operational total solid (TS) content in semi-continuous high-solids anaerobic digestion (HS-AD). To alleviate substrate overloading in HS-AD, the effluent mass was relatively reduced compared to the influent mass, extending the mass retention time. To this aim, the reactor mass was daily evaluated, permitting to assess the reactor content removal by biogas production. During mono-digestion of OFMSW, the NH_3 inhibition and the rapid TS removal prevented to maintain HS-AD conditions (i.e. TS $\geq$ 10 %), without exacerbating the risk of reactor acidification. In contrast, the inclusion of sawdust in OFMSW permitted to operate HS-AD up to 30 % TS, before acidification occurred. Therefore, including a lignocellulosic substrate in OFMSW can prevent acidification and stabilize HS-AD at very high TS contents (i.e. 20-30 %).

1 INTRODUCTION

Anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW), including food waste (FW) and green waste (GW), is a particularly suited treatment biotechnology for energy and by-product recovery (Clarke, 2018; Mata-Álvarez, 2003). In AD, an organic waste is degraded to biogas, mainly composed by CH_4 and CO_2, and a partially stabilized organic digestate, by consortia of different microorganisms working in absence of oxidative species (i.e. O_2 and NO_3^-) (Astals et al., 2015; Gerardi, 2003).

The sequential steps in AD include hydrolysis, acidogenesis, acetogenesis and methanogenesis, during which different inhibitory substances can be formed leading to inhibitory effects for the anaerobic microorganisms and/or even a complete AD failure. Depending on the concentration, free ammonia (NH_3), hydrogen sulfide (H_2S) and free ions (i.e. Na^+) are some of the inhibitory substances in AD, affecting predominantly the methanogenic stage, either acetoclastic and/or hydrogenotrophic, and potentially resulting in the buildup of volatile fatty acids (VFA) and H_2 in the system (Astals et al., 2015; Chen et al., 2008). Meanwhile, the acetoclastic activity results into inorganic carbon (i.e. HCO_3^-) release in AD, as an important source of pH buffering, minimizing the risk of reactor acidification (i.e. $pH \leq 6.0$) by VFA accumulation (Gerardi, 2003).

The interrelationship between the organic waste characteristics, operational conditions and reactor design determines the AD potential (Karthikeyan & Visvanathan, 2013; Mata-Álvarez, 2003). AD can be differentiated depending on the operational total solid (TS) content into 'wet' (i.e. TS < 10 %) and high-solids AD (HS-AD, i.e. TS ≥ 10 %)

59 (Benbelkacem et al., 2015). HS-AD allows the use of a smaller reactor, reducing the

60 need for water addition and minimizing the digestate production (Karthikeyan &

61 Visvanathan, 2013; Pastor-Poquet et al., 2018). However, HS-AD drawbacks include

62 the pervasive chances of reactor acidification due to substrate overload (Benbelkacem et

63 al., 2015). Overloading is the consequence of the slow-growing methanogens being

64 unable to cope with the rapid VFA and/or H_2 buildup resulting from

65 acidogenesis/acetogenesis in HS-AD (Pavan et al., 2000). Furthermore, overloading is

66 in many cases related to the presence of methanogenic inhibitors (Drosg, 2013), such as

67 NH_3, due to the high protein content of OFMSW (Kayhanian, 1999).

68

69 HS-AD of OFMSW is a mature technology, with most of the recently-constructed

70 industrial plants targeting the semi-continuous HS-AD process (Mattheeuws, 2016).

71 The focus of semi-continuous HS-AD lies on the maximization of the organic loading

72 rate (OLR) that optimizes the methane yield and ensures an adequate organic removal at

73 high TS contents (Benbelkacem et al., 2015; Hartmann & Ahring, 2006). In this line,

74 depending on the organic waste used in HS-AD, the operational TS content is

75 substantially lower than the feed TS, as the organic substrate is converted to biogas by

76 methanogenesis (Pastor-Poquet et al., 2018).

77

78 Therefore, HS-AD lies on a balance between maximizing the OLR and TS content,

79 while minimizing the chances of reactor failure. Particularly, in order to startup HS-AD,

80 the OLR needs to be increased relatively slowly, permitting the methanogens to grow

81 and adapt to the new conditions. The transient (non-steady) OLR modification in HS-

82 AD aims to find an optimum stationary (steady-state) operation to be used, avoiding

83 acidification and maximizing the economy of the process (Angelidaki et al., 2006;

84 Bolzonella et al., 2003). However, the risk of inhibition and failure is undesirably high

85 under HS-AD startup, potentially requiring the implementation of recovering strategies

86 (i.e. reactor content dilution) to minimize the influence of inhibitory substances, or even

87 restarting the process when a significant methanogenic imbalance occurs (Fricke et al.,

88 2007; Kayhanian, 1999).

89

90 This study evaluated the highest tolerable TS content in semi-continuous HS-AD of

91 OFMSW, by gradually increasing the OLR in semi-continuous reactors operated at

92 55ºC, until process failure occurred by acidification. Two feeding strategies were used:

93 mono-digestion of OFMSW and co-digestion of OFMSW and beech sawdust – as a

94 model lignocellulosic substrate, simulating the inclusion of GW in OFMSW. Aiming to

95 minimize the risk of substrate overload, the mass retention time (MRT) was relatively

96 extended by reducing the effluent compared to the influent mass, according to the daily

97 mass content removal by biogas production observed in the semi-continuous reactors.

98

99

100 **2 MATERIALS AND METHODS**

101 **2.1 Substrates and Inoculum**

102 The substrates used in this study were OFMSW and beech sawdust. OFMSW consisted

103 of a mixture of household waste collected in Cassino (Italy), restaurant waste, spent

104 coffee, and garden waste collected at the university facilities, with an approximated wet

105 weight proportion of 45, 35, 15 and 5 % (w/w), respectively. OFMSW was minced

106 twice to a particle size ≤ 5-10 mm by an industrial mincer [REBER 9500NC, Italy],

107 fully homogenized manually and stored in 5 L buckets at -20ºC. During mincing and

108 homogenization, no extra water was added to the raw substrate. A single 5 L bucket of

109 OFMSW was thawed at room temperature overnight, as required to feed the semi-

110 continuous reactors. Goldspan® beech sawdust with 1.0-2.8 mm particle size was used

111 as co-substrate, to simulate biodegradable green/lignocellulosic waste.

112

113 The inoculum for semi-continuous experiments was obtained from a pre-adapted 'wet'

114 AD (i.e. TS ≤ 5 %) source reactor operated at 55ºC. The pre-adaptation of 20 L sludge,

115 collected from a mesophilic (35ºC) digester treating buffalo manure and mozzarella

116 whey (Capaccio, Italy), consisted of a 4-month progressive feeding of tap-water-diluted

117 OFMSW at 55ºC, in order to adapt the inoculum to the new substrate and temperature.

118

119 Prior to start the mono-digestion experiments, the source reactor was kept unfed for 1

120 month to consume/reduce the organic content, while continuing with the inoculum

121 adaptation to the new substrate. Subsequently, the feeding with diluted OFMSW was

122 resumed to recover methanogenesis. After 7 and 15 days from the feeding restart, 4 kg

123 of sludge were taken from the source reactor, filtered through a 1 mm mesh and used to

124 inoculate the mono-digestion reactors "A" and "B", respectively. Therefore, the

125 inoculum was slightly different in reactors A and B, as shown in section 3.1.

126

127 During the mono-digestion experiments, the source reactor was periodically fed with

128 diluted OFMSW and the mono-digestion reactors effluents, to maintain the reactor

129 volume and methanogenic activity. Once the mono-digestion experiments ended, the

130 source reactor was kept unfed for 1 month to serve as inoculum for the co-digestion

131 experiments. Thus, 3.4 kg of reactor content were filtered through a 1 mm mesh and

132 used to inoculate each co-digestion reactor "A", "B" and "C".

133

134 **2.2 Experimental Setup**

135 The laboratory-scale semi-continuous reactors consisted of 5 L polyethylene

136 terephthalate (PET) bottles with a modified head allowing the (semi-)solid waste input,

137 reactor content withdrawal and biogas measurement [Figure 1]. The reactor port was a

138 polyvinyl chloride (PVC) flexible hosepipe with two valves, easing the reactor

139 loading/unloading while avoiding air intrusion. The biogas output, containing a

140 sampling septum, was connected to 5 L Tedlar® bags [Sigma-Aldrich, USA]. All

141 reactors were maintained at 55ºC within a temperature-controlled TCF 400 oven

142 [ARGOLAB, Italy].

143

144 **2.3 Operation Strategy**

145 Two semi-continuous reactors for mono-digestion of OFMSW or three reactors for co-

146 digestion of OFMSW and sawdust were operated simultaneously in a drag-and-fill

147 mode. The semi-continuous reactors (i.e. kg) and the reactor influents/effluents (i.e. g)

148 were weighed on a $\pm$ 0.01 precision scale. The OLR was evaluated as the daily substrate

149 addition in terms of volatile solids (VS) divided by the reactor mass content (i.e. g

150 VS/kg·d), while the MRT was evaluated as the quotient between the reactor mass and

151 the daily effluent mass (i.e. days). Since the reactors were fed a maximum of 5 days per

152 week, 7-days moving average OLR and MRT were estimated. Moving-average

153 operational variables are well suited indicators of the immediately preceding operations

154 (i.e. feeding, dilution, reactor content removal) to discern about the risk of VFA buildup

155 in semi-continuous digesters. Moreover, expressing the operational conditions as a

156 moving-average eases the comparison of digesters, when feeding days are not the same

157 or an important mass removal occurs.

158

159 During each drag-and-fill operation, the reactor content was 1) homogenized before

160 opening the system, 2) sampled and 3) analyzed mainly for pH and alkalinity – since pH

161 had to be maintained over 6.5, as an important methanogenic inhibition might take place

162 below this threshold (De Vrieze et al., 2012; Gerardi, 2003). Depending on the pH and

163 alkalinity, 4) the proper amount of substrate was used or diluted as needed, 5) prior to

164 be fed to the reactors. Finally, 6) the reactor content was homogenized once again,

165 while the Tedlar® bags were checked for biogas production and subsequently emptied.

166

167 To increase the reactor TS content from 'wet' AD (i.e. TS < 5 %) to HS-AD (i.e. TS $\geq$

168 10 %), the OLR was controlled by increasing/decreasing the daily amount of substrate

169 and/or tap water addition based on the methanogenic activity, and aiming to minimize

170 the substrate overload. To evaluate the differences in the reactor performance, mono-

171 digestion reactors were fed in parallel using different OLR/MRT in each reactor, as

172 shown in section 3.2. Subsequently, co-digestion reactors were also operated in parallel

173 at three different OLR/MRT. In each reactor, the methanogenic activity was roughly

174 associated with the relative increase of the pH and inorganic carbon alkalinity (ALK_P),

175 the reduction of the reactor mass content and the biogas production compared to

176 previous operational values, as also mentioned in section 3.2. For example, a relative

177 pH and ALK_P increase of approximately 0.5 pH units and 0.3 g $CaCO_3$/kg, respectively,

178 alongside a reactor mass removal of about 30-50 g/d and a specific biogas production

179 higher than 250 mL/kg reactor content·d were associated with ongoing methanogenesis,

180 indicating that the OLR could be maintained or relatively increased. Similarly, the

181 relative increase of intermediate alkalinity (ALK_I) (i.e. 0.5 g Acetic Acid/kg) was used

182 as a preliminary indicator of the potential VFA buildup and risk of substrate overload

183 (Lahav et al., 2002).

184

185 All these parameters were further complemented with the user's evaluation of the

186 previous operation, in order to decide for the daily feed/dilution to be used. Thus, all

187 reactors were started with a low OLR (i.e. 2 g VS/kg·d) that was gradually increased to

188 increase the TS content. As reactor performance deteriorated with increasing OLR, the

189 reactor feeding was reduced/stopped to prevent acidification (i.e. pH ≤ 6.0).

190

191 The reactor mass was maintained constant by reducing the effluent compared to the

192 influent mass, according to the observed reactor mass content removed by biogas

193 production from the previous operation. With this strategy, the MRT was relatively

194 extended, aiming to promote the methanogenic adaptation in case of overloading. Semi-

195 continuous reactors were fed until acidification occurred. From this point, feeding was

196 stopped and reactor dilution and/or inorganic salt addition (i.e. $NaHCO_3$ and $FeCl_2$)

197 were tested as recovering strategies. A summary of the weekly operational variables is

198 presented as Supplementary Information.

199

200 **2.4 Bio-Physical-Chemical Analyses**

201 The pH, ALK_P and ALK_I were determined from the supernatant of solid and semi-solid

202 samples (Lahav et al., 2002), after diluting the sample with distilled water,

203 homogenization and centrifugation at 6000 rpm for 15 min (EPA, 2015). The TS and

204 VS content, total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total H_2S were

205 determined by the standard methods (APHA, 1999). The NH_3 was approximated as a

206 function of TAN and pH (Astals et al., 2015). The VFA (acetic, propionic, butyric and

207 valeric acids) were measured with an LC-20AD HPLC [Shimadzu, Japan], mounting a

208 Rezex ROA-Organic Acids 8+ column coupled to a 210 nm UV detector, and using

209 0.0065 M H_2SO_4 at 0.6 mL/min as mobile phase. The biogas composition (CH_4, CO_2

210 and H_2) was analyzed with a 3400 GC-TCD [Varian, USA], using argon as carrier gas.

211

212 The biomethane potential (BMP) test for OFMSW used 3.0 g of substrate, 50.0 g of

213 source inoculum, 40.0 g of distilled water and 0.10 g of $NaHCO_3$ in 280 mL bottles (6

214 replicates), with an inoculum-to-substrate ratio (ISR) of 2.0 g VS/g VS. The BMP test

215 for sawdust used 1.0 g substrate and 50.0 g of inoculum in 160 mL bottles (3 replicates),

216 with an ISR of 1.0 g VS/g VS. BMP tests were performed according to Angelidaki and

217 Sanders (2004) and Holliger et al. (2016). In the BMP test for OFMSW, the distilled

218 water and $NaHCO_3$ addition served to minimize the chances of inhibition (i.e. by NH_3)

219 and acidification, respectively. In contrast, NH_3 build-up and acidification were not

220 expected in the BMP test of sawdust, due to the low nitrogen content and the reduced

221 biodegradability of sawdust, as thoroughly discussed in next section, permitting also to

222 use a lower ISR. Both BMP tests lasted longer than 100 days. Blank assays included the

223 inoculum and further distilled water compensating for the absence of substrate, using

224 three replicates in each BMP. Inoculum activity assays using a reference substrate were

225 not performed.

226

227 The BMP was the normalized methane production (P = 1 bar, T = 0ºC), excluding the

228 methane production of the inoculum, per unit of substrate VS added. The gas production

229 was evaluated with a two-vessel displacement system, with the first vessel containing 4

230 N NaOH to capture CO_2 and the second vessel containing water to be 'displaced'. The

231 bottles were sealed with butyl rubber stoppers and aluminum crimps and flushed with

232 helium, before adding 0.2 mL of 10 g/L Na_2S piercing the septum to ensure an adequate

233 redox potential (Angelidaki & Sanders, 2004). All bottles were incubated at 55ºC and

234 agitated only while measuring the gas production.

235

236 **2.5 Statistical Analyses**

237 The Dixon's test for BMP outliers was applied as recommended by Holliger et al.

238 (2016). The unpaired t-test of Microsoft Excel 2016 (Microsoft, USA) was applied to

239 determine the statistical significance of experimental data, using the two-tail p-value at

240 95 % confidence.

241

242

243 **3 RESULTS AND DISCUSSION**

244 **3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum**

245 OFMSW showed a TS of 26 %, a VS/TS ratio of 0.93 and a TKN of 24.8 g N/kg TS, in

246 agreement with real source-sorted OFMSW (Angelidaki et al., 2006; Bolzonella et al.,

247 2006; Jokela & Rintala, 2003). The high VS/TS ratio of OFMSW (i.e. > 0.9) indicated

248 minimal presence of inert materials (Pavan et al., 2000). Sawdust showed a TS of 94 %

249 and a VS/TS ratio of 0.99, similar to those obtained by Brown and Li (2013) for 40ºC-

250 dried yard waste, suggesting that beech sawdust could simulate GW. The BMP of

251 OFMSW and sawdust was 497 and 161 NmL CH_4/g VS, respectively. Table 1 shows

252 the bio-physical-chemical characterization of OFMSW and sawdust. Despite the

253 thorough mincing and homogenization, minor modifications were observed in the

254 OFMSW characterization (i.e. TS, TKN or BMP), mainly attributed to the substrate

255 heterogeneity.

256

257 The inoculum in mono-digestion reactors A and B showed a common TS and TKN of

258 2.8 % and 161 g N/kg TS, respectively. An initial acetic acid concentration of 2.30 and

259 3.30 g/kg was observed in reactors A and B, respectively, being this difference

260 associated with the later inoculation of reactor B than reactor A. The inoculum used in

261 co-digestion reactors showed a TS of 2.5 %, a TKN of 139 g N/kg TS and an acetic acid

262 concentration of 0.02 g/kg. The inoculum compositions are shown in Table 2.

263

264 **3.2 Semi-continuous Operation – Increasing the TS Content**

265 **3.2.1 Mono-digestion of OFMSW**

266 Mono-digestion results are summarized in Figures 2 and 3. The weekly-averaged results

267 were also included as Supplementary Information. The 7-days average OLR in reactors

268 A and B was varied from an initial 2.4 (day 6) and 6.0 (day 13) g VS/kg·d to 4.9 and 5.5

269 g VS/kg·d, respectively, on day 17 [Figure 2a]. Thus, a common OLR (i.e. around 5 g

270 VS/kg·d) was achieved, aiming to compensate for the 1-week-lagged inoculation in

271 reactor B. After two days with no feed, feeding was resumed in reactor B on day 20, but

272 not in reactor A due to the low pH (i.e. 6.4) [Figure 3a]. As pH recovered in reactor A

273 (i.e. from 6.4 on day 21, to 7.6 on day 29) due to methanogenesis activity, feeding was

274 resumed. During the same period, ALK_P in reactor A increased alongside pH from 1.1

275 to 2.7 g $CaCO_3$/kg (data not shown), as an indicator of ongoing methanogenesis. By day

276 45, a maximum OLR of 6.8 and 8.5 g VS/kg·d was reached in reactors A and B,

277 respectively. After day 48, the OLR required progressive reduction to minimize the risk

278 of acidification. The last feeding in reactors A and B was implemented on days 78 and

279 73, respectively, as both reactors showed pH ≤ 6.5 and CH_4 content ≤ 40 % [Figure 2f].

280 From this point, mono-digestion reactors were left unfed aiming to promote the

281 recovery of methanogenesis.

282

283 The OLR in reactor B was averagely about 1.5 g VS/kg·d higher than that used in

284 reactor A during the whole experiment ($p < 0.001$), explaining the relatively faster

285 acidification observed in reactor B. Thus, prior to the occurrence of acidification,

286 reactor B was fed with an average 35 g VS/d, significantly higher than the 26 g VS/d

287 used for reactor A ($p = 0.03$). The initial MRT was 55 (day 6) and 29 days (day 13) for

288 reactors A and B, respectively, and was gradually increased to maintain the

289 methanogenic performance at higher OLR [Figure 2b]. Noteworthy, the MRT and OLR

290 in these semi-continuous reactors did not show an inverse pattern, since the dilution as

291 well as the influent and effluent mass flows used were different to account for the

292 organic removal.

293

294 Uncoupling the influent and effluent mass flows in the semi-continuous reactors, based

295 on the HS-AD reactor content removal by methanogenesis, permitted to increase the

296 MRT and OLR simultaneously. In this study, the MRT was considered as a more suited

297 indicator in HS-AD than the hydraulic retention time (HRT), since both the specific

298 weight of the influent/effluent and the reactor mass content varied, in contrast to 'wet'

299 AD (Pastor-Poquet et al., 2018). As an example, the occurrence of methanogenesis led

300 to a 60 g removal of the reactor mass content in both mono-digestion reactors from day

301 37 to 41 (data not shown). Prior to the occurrence of reactor acidification, the weekly

302 effluent mass was significantly higher than the influent (i.e. 18 %; p = 0.03) to maintain

303 the mono-digestion reactors mass content constant.

304

305 The MRT-uncoupling concept was proposed by Richards et al. (1991) and was used by

306 Kayhanian and Rich (1995) to operate a pilot-scale semi-continuous HS-AD reactor fed

307 with OFMSW. In this study, uncoupling the influent and effluent in HS-AD promoted

308 the methanogenic adaptation to overloading conditions and/or the buildup of inhibitors

309 (i.e. NH_3) during the OFMSW degradation. Noteworthy, the MRT must be longer than

310 the doubling time of methanogens (i.e. 20-30 days) to avoid their 'washout' from

311 continuous HS-AD reactors, while the methanogenic doubling time might lengthen

312 considerably in presence of inhibitory substances (i.e. NH_3) (Drosg, 2013; Gerardi,

313 2003; Rittman & McCarty, 2001). Therefore, extending the MRT resulted in a more

314 stable HS-AD operation (Hartmann & Ahring, 2006; Rajagopal et al., 2013), though the

315 sole implementation of influent-effluent uncoupling was not sufficient to avoid HS-AD

316 overloading and acidification during mono-digestion of OFMSW.

317

318 **3.2.2 Co-digestion of OFMSW and Sawdust**

319 Co-digestion results are summarized in Figures 4 and 5. The 7-days average OLR was

320 increased from 4.5-4.9 g VS/kg·d (day 6) up to 10.9, 12.1 and 12.6 g VS/kg·d (day 23)

321 in reactors A, B and C, respectively. To avoid acidification, feeding was stopped in

322 reactors A and B from day 26, while the OLR was only reduced to 5.0 g VS/kg·d in

323 reactor C [Figure 4a]. As pH recovered (i.e. $\geq$ 7.0) [Figure 5], feeding was resumed in

324 reactors A and B. A maximum OLR of 14.8 g VS/kg·d was reached in reactor C (day

325 47) using a sawdust/OFMSW ratio of 2.1 g VS/g VS, prior to the occurrence of reactor

326 acidification (day 56). The maximum OLR in reactor B was 15.1 g VS/kg·d (day 55)

327 using a sawdust/OFMSW ratio of 1.6 g VS/g VS, while an OLR of 16.0 g VS/kg·d was

328 reached in reactor A during the same period, using a sawdust/OFMSW ratio of 1.3 g

329 VS/g VS. The last feeding in reactors A and B was performed on day 76, as a slight but

330 continued drop in pH [Figure 5] and CH_4 [Figure 4f] was observed in both reactors.

331

332 The average OLR used for co-digestion was two times higher than that for mono-

333 digestion (i.e. 8.3 *vs.* 4.5 g VS/kg·d, respectively; $p < 0.001$), due to the lower

334 biodegradability of sawdust, though the OLR only due to OFMSW was similar in both

335 cases (i.e. 4.1 *vs.* 4.5 g VS/kg·d, respectively; $p = 0.07$). Thus, a maximum OLR of 7.5-

336 8.0 g VS/kg·d related to the sole supplementation of OFMSW was used in the three co-

337 digestion reactors on day 21, while the OLR solely due to OFMSW was subsequently

338 maintained below 6.0 g VS/kg·d, as sawdust was increased in the feeding mixture

339 [Figure 4a]. In terms of average VS fed, reactor C was operated under relatively more

340 stressing feeding conditions than reactors A and B (i.e. 53 *vs.* 44 g VS/d, respectively; p

341 = 0.15), being again the fastest occurrence of reactor acidification related to the highest

342 VS fed.

343

344 The initial MRT was higher than 168 days (day 6) and was decreased to 30 days (day

345 17), similarly in the three reactors [Figure 4c]. From this point, the MRT reached an

346 average of 85 days (day 35) and was subsequently reduced to an average of 37 days

347 (day 53) in all reactors, before being progressively increased to minimize the substrate

348 overload. The MRT was significantly lower in co-digestion than mono-digestion (i.e. 69

349 *vs.* 92 days, respectively; $p < 0.001$), as lower MRT were predominantly linked to the

350 higher OLR used in co-digestion.

351

3.3 Influence of the Substrate Composition on the TS Increase

353 The OLR/MRT control in the mono-digestion reactors fed with OFMSW permitted to

354 increase the TS content, balancing the VFA accumulation with the rapid organic

355 degradation observed [Figures 2 and 3]. Reactors A and B were started at TS = 2.8 %

356 and reached a maximum of 10.7 (day 79) and 11.7 % (day 69), respectively [Figure 2c],

357 being these TS slightly higher than the lower HS-AD threshold (i.e. TS $\geq$ 10 %). The

358 highest TS in the semi-continuous reactors did not coincide with the maximum OLR,

359 but were predominantly associated with low pH (i.e. $\leq$ 6.5), when methanogenesis was

360 potentially inhibited. In this line, a gradual increase of the VS/TS ratio (data not shown)

361 was observed in both reactors from 0.69 (day 0) to 0.82 (day 40), reaching a maximum

362 value of 0.87, prior acidification occurred on days 79 and 76 in reactors A and B,

363 respectively.

364

365 The highest TS and VS/TS observed in semi-continuous HS-AD of OFMSW were

366 associated with acidification and indicate a reduced VS degradation alongside inhibitory

367 conditions. Particularly, the lowest HS-AD threshold (i.e. TS = 10 %) using OFMSW

368 was reached only under extreme overloading. A more stable HS-AD fed with an easily

369 biodegradable OFMSW (i.e. FW) is also associated with a TS increase alongside

370 overloading/inhibitory conditions. For example, Tampio et al. (2014) reported a TS

371 increase from 7 to 8 % during 400 days of semi-continuous AD fed with FW, though TS

372 rapidly reached 11 % during the next 50 days of operation, when reactor inhibition was

373 likely occurring. In the same line, Bolzonella et al. (2003) reported a TS increase from 5

374 to 15 % during the initial 60 days of continuous AD pilot-scale startup fed with

375 OFMSW, being the maximum TS associated with the highest total VFA observed (i.e.

376 2.8 g Acetic Acid/L). All these results were likely related to methanogenesis inhibition,

377 since the VFA accumulation affects the hydrolysis/acidogenesis rates, hampering the

378 organic removal in HS-AD (Vavilin et al., 2008).

379

380 The maximum TS obtained in this study for semi-continuous HS-AD of OFMSW

381 should be considered as indicative (only) of those obtainable in steady-state digesters,

382 since the transient/acidification conditions potentially reduced the VS removal. Thus,

383 the operational TS content of stable digesters fed with the easily biodegradable content

384 of OFMSW (i.e. FW) might be lower than those observed along non-steady-state

385 conditions. This is a further indication that a steady-state semi-continuous reactor using

386 an easily biodegradable OFMSW as a substrate might not be operated within the HS-

387 AD threshold (i.e. TS $\geq$ 10 %).

388

389 Co-digestion permitted to increase TS from 2.5 % (day 0) up to a maximum of 33.2

390 (day 79), 26.7 (day 76) and 27.0 % (day 57) in reactors A, B and C, respectively [Figure

391 4c]. Hence, the maximum TS reached in co-digestion before the reactors acidified (i.e.

392 29.0 $\pm$ 2.8 %) was considerably higher than the lower HS-AD threshold (i.e. TS $\geq$ 10 %)

393 and the maximum TS of mono-digestion (i.e. 11.5 $\pm$ 0.5 %), due to the addition of

394 sawdust to OFMSW. The highest TS was related again to acidified (i.e. pH < 6.5) or

395   acidifying (i.e. downward trend on pH/CH_4 content) conditions, as observed for mono-

396   digestion. Thus, the VS/TS ratio in co-digestion (data not shown) increased from 0.65

397   (day 0) to 0.90 (day 40), reaching a maximum of 0.95 before reactors acidified (day 76),

398   due to both the higher VS/TS ratio of sawdust and the reduced VS removal during

399   inhibitory conditions.

400

401   These results showed that the particular characteristics of OFMSW determined the

402   maximum operating TS content in semi-continuous HS-AD. Co-digestion of OFMSW

403   and sawdust resulted in approximately three times higher TS than mono-digestion (i.e.

404   29.0 and 11.5 %, respectively). The inclusion of sawdust in OFMSW favored the rapid

405   TS and OLR increase compared to mono-digestion due to the higher TS and the lower

406   biodegradability of sawdust, as demonstrated by the substantially lower BMP of

407   sawdust than that of OFMSW (i.e. 161 and 497 NmL CH_4/g VS, respectively) [Table

408   1]. Indeed, lignocellulosic materials (i.e. GW) are normally associated with a reduced

409   biodegradation rate, compared to more easily degradable substrates (i.e. FW), due to the

410   high lignin content hampering hydrolysis (Brown & Li, 2013; Mancini et al., 2018a;

411   Vavilin et al., 2008), being also beneficial to limit the VFA buildup in HS-AD. On the

412   other hand, TAN was 22 % lower during co-digestion than mono-digestion (i.e. 2.9 *vs.*

413   3.7 g N/kg, respectively; $p < 0.001$) [Figures 2 and 4], due to the lower TKN of sawdust

414   [Table 1]. Noteworthy, the TAN accumulation was likely promoting methanogenic

415   inhibition in this study, as further discussed in section 3.5. Therefore, using sawdust –

416   as GW – was also adequate to adjust the carbon-to-nitrogen (C/N) ratio in HS-AD of

417   OFMSW.

418

419

3.4 Main Indicators of Substrate Overload

3.4.1 Evolution of pH and VFA

422 pH in mono-digestion reactor A decreased from 8.1 to 6.4 due to the rapid acetic acid

423 buildup (i.e. from 3.00 to 9.00 g/kg) observed during the initial 20 days of operation

424 [Figure 3a]. As feeding was stopped from day 20 to 29, pH reached 7.6, while acetic

425 acid decreased below 0.70 g/kg right afterwards (day 34). Propionic, butyric and valeric

426 acids gradually increased from < 0.15 g/kg (day 0) to 5.00, 4.00 and 1.10 g/kg (day 79),

427 respectively. From day 79, pH dropped from 7.1 to 6.1, linked to a sudden acetic acid

428 increase from 3.00 to 5.00 g/kg, and the subsequent CH_4 content drop from 56 to 37 %

429 [Figure 2f].

430

431 Mono-digestion reactor B was relatively more stressed than reactor A, as indicated by

432 the wider acetic acid fluctuations (i.e. $\pm$ 4.00 g/kg) and the rapid accumulation of

433 propionic acid from 1.20 (day 7) to 5.80 g/kg (day 73) [Figure 3b]. The VFA fluctuation

434 is in line with the fact that methanogens grow relatively slower than the

435 hydrolytic/acidogenic microorganisms in AD (De Vrieze et al., 2012; Gerardi, 2003).

436 Thus, the higher OLR used in reactor B led to a more pronounced

437 methanogenic/acidogenic imbalance, exacerbating the VFA accumulation. The VFA

438 buildup led to a pH decrease from 8.4 to 6.2 in reactor B during the whole experimental

439 period, while a significant acetoclastic inhibition occurred from day 70 to 73, when

440 acetic acid abruptly increased from 2.70 to 5.80 g/kg.

441

442   pH in co-digestion reactor A gradually decreased from 8.7 to 6.4 along the experimental

443   period, showing a minimum of 6.1 associated with a peak of acetic acid of 8.30 g/kg

444   (day 26) [Figure 5a]. Acetic acid was considerably consumed (i.e. < 0.36 g/kg) by day

445   47 due to ongoing methanogenesis, and progressively increased thereafter by

446   overloading. Similarly, pH in reactor B showed a minimum of 6.3 when acetic acid

447   peaked at 8.20 g/kg (day 26) [Figure 5b], while the acetic acid was extensively

448   consumed (i.e. < 0.35 g/kg) by day 41 prior to increase again steadily. In reactor C,

449   acetic acid had a similar evolution with a maximum of 7.20 g/kg (day 26) [Figure 5c],

450   while pH dropped to 6.0 on day 57, associated with a sharp acetic acid build-up from

451   1.00 to 3.70 g/kg. Propionic, butyric and valeric acids increased from 0.50, 0.14 and

452   0.00 g/kg (day 0) to a maximum range of 3.00-3.50, 2.90-3.20 and 2.50-2.60 g/kg,

453   respectively, obtained right after acidification occurred on day 79 in reactors A and B,

454   and on day 56 in reactor C. The pH was relatively lower (i.e. 2 %; p = 0.13) and total

455   VFA was relatively higher (i.e. 5 %; p = 0.25) during mono-digestion than co-digestion,

456   likely due to the faster degradation rates but also the higher release of inhibitory

457   compounds related to OFMSW than sawdust, as discussed in section 3.3.

458

459   Feeding the reactors a maximum of 5 days per week influenced the reactor dynamics,

460   since pH increased and VFA – mainly acetic acid – decreased during the periods with

461   no feed. The pH and VFA modifications [Figures 3 and 5] were associated with the TS

462   removal, as mentioned before, and also affected the biogas production/composition, and

463   the TAN buildup [Figures 2 and 4]. As an example, in co-digestion reactor A, pH

464   increased from 7.0 to 7.9 from day 37 to 41, while acetic acid decreased from 4.30 to

465 2.40 g/kg, triggering a biogas production of 0.6 L/kg reactor content and a methane

466 content increase from 59 to 70 % [Figure 4 and 5].

467

468 **3.4.2 Biogas Production and Composition**

469 Mono-digestion of OFMSW resulted in a cumulative biogas production of 65 and 66

470 L/kg reactor content in reactor A and B, respectively [Figure 2e]. Biogas production

471 was mainly correlated to the acetic acid consumption [Figure 3a], as mentioned in the

472 previous subsection. For example, 21 L/kg reactor content of biogas were measured

473 during the initial 20 days of reactor A, before acetic acid accumulated and biogas

474 production slowed down.

475

476 Biogas composition measurements started on day 60 showing an average of 63 % CH_4

477 in both mono-digestion reactors [Figure 2f], which subsequently fluctuated showing a

478 downward trend alongside the VFA accumulation. The CH_4 content dropped below 40

479 % in both reactors right after biogas production definitely ceased on days 78-79. The

480 reduction of CH_4 content in the biogas is also an indicator of AD imbalance, though it

481 might be inappropriate to assess rapid changes in the reactor performance (Drosg,

482 2013). The highest H_2 concentration (data not shown) was 1.8 and 1.1 % on day 59 in

483 reactors A and B, respectively, while H_2 remained below 0.8 % in both reactors during

484 the rest of the experiment. The presence of H_2 indicated that the hydrogenotrophic

485 methanogens were unable to cope with the rapid H_2 production from acidogenesis, since

486 H_2 higher than 1-2 % in the gas phase is normally associated with AD overloading

487 (Drosg, 2013; Molina et al., 2009).

488

489 Co-digestion in reactor A led to a cumulative biogas production of 48 L/kg reactor

490 content, while 49 and 27 L/kg reactor content were observed in reactors B and C,

491 respectively [Figure 4e]. In spite of the higher OLR used in co-digestion, the biogas

492 production was considerably lower than that obtained with mono-digestion (i.e. 65 L/kg

493 reactor content). Thus, the specific biogas production was 229 ± 20 L/kg VS added in

494 mono-digestion and 86 ± 18 L/kg VS added in co-digestion (i.e. 62 % lower), due to the

495 reduced biodegradability of sawdust.

496

497 The CH_4 content [Figure 4f] reached a peak of 75 % during the first two weeks of

498 operation in the three co-digestion reactors, but it decreased subsequently as VFA

499 accumulated [Figure 5]. A minimum 43 % CH_4 was detected in reactor A associated

500 with the last biogas production observed (day 82), while a sharp drop from 60 to 29 %

501 CH_4 was observed in reactor C right after day 60. H_2 was detected at 0.3 % in the three

502 co-digestion reactors on day 23 (data not shown). Thereafter, H_2 was not detected in

503 reactor A, while reactor B showed a single H_2 peak of 1.5 % on day 70, right after the

504 reactor was accidentally opened to the atmosphere. In reactor C, H_2 peaks of 1.7, 1.2

505 and 1.6 % were observed on days 41, 47 and 58, respectively, supporting the occurrence

506 of a more extensive overload in this reactor.

507

508 **3.5 Testing Recovering Strategies**

509 Once acidification occurred, feeding was stopped and some recovering strategies were

510 tested to resume methanogenesis. In mono-digestion reactor A, a 3 M $NaHCO_3$ buffer

511 solution was added on days 83 and 84 to raise the pH (i.e. from 6.2 to 6.8) within a

512 suitable range for methanogens (i.e. 6.5-7.0). Adding $NaHCO_3$ is normally used to

513 counteract acidification when digesters show a reduced ALK_P (Chen et al., 2008;

514 Holliger et al., 2016). However, methanogenesis did not recover after more than 20

515 days.

516

517 On day 76, $FeCl_2$ was supplemented to mono-digestion reactor B in a higher amount

518 than the stoichiometric, to precipitate the total H_2S in the system (i.e. 30 mg H_2S/kg,

519 data not shown). However, $FeCl_2$ overdosing resulted in a pH drop from 6.3 to 5.7 (days

520 76-77). Thus, 2 M $NaHCO_3$ solution was rapidly added to recover the pH to 6.6 (day

521 77). Both Fe^{2+} and/or Fe^{3+} can be used to precipitate sulfide in AD, but Fe^{2+} was

522 preferred in this study to avoid the inclusion of a strong electron acceptor (i.e. Fe^{3+}) that

523 could react with organic compounds in the anaerobic digester (i.e. Fe^{3+} + 1/2 H_2 –> Fe^{2+}

524 + H^+, $\Delta G^{o\prime} << 0$) (Fermoso et al., 2015; Rittman & McCarty, 2001). After 2 weeks of

525 methanogenic inhibition (day 90), 200 g of 'wet' AD inoculum from the source reactor

526 were added to reactor B, allowing a gradual methanogenic recovery, associated with an

527 increase of pH from 6.9 to 7.3 [Figure 3b] and CH_4 content from 20 to 52 % [Figure 2f],

528 until the end of the reactor operation.

529

530 Aiming to recover methanogenesis in all co-digestion reactors, water was progressively

531 added to dilute the effect of potential methanogenic inhibitor(s). The progressive

532 addition of low amounts of water in co-digestion reactors permitted to maintain HS-AD

533 conditions (i.e. TS $\geq$ 10 %), thanks to the elevated TS content reached before reactors

534 acidified (i.e. TS $\geq$ 30 %). Dilution was performed in reactors A and B from day 79 and

535 in reactor C from day 62. In reactor A and B an average of 180 and 170 mL of water

536 was used, respectively, on days 79, 82, 84, 91 and 98, while an average of 160 mL of

537 water was added to reactor C on days 62, 63, 68 and 91.

538

539 In conclusion, neither water, nor buffer addition permitted to recover

540 acidified/acidifying HS-AD reactors, probably because of the important imbalance

541 between methanogens and acid-producers in the system (Gerardi, 2003). In these

542 conditions, inoculum addition might be the only way to recover an acidified HS-AD

543 reactor, though emptying and re-inoculating the reactor might be necessary (Fricke et

544 al., 2007). Another strategy to prevent reactor acidification and/or enhance the digester

545 performance is trace element (i.e. Se, Ni, Co, Mo, W) addition (Fermoso et al., 2015;

546 Mancini et al., 2018b). However, this was out of the scope of this manuscript.

547

548 **3.6 Ammonia Buildup**

549 In this study, overloading was associated with the high OLR used, but also with the NH_3

550 buildup, in the semi-continuous reactors. The high OLR and the degradation of the

551 protein content of OFMSW increased the TAN content in both mono-digestion reactors

552 [Figure 2d]. TAN ranged from 3.4 g N/kg (day 0) to a maximum of 4.8 and 4.9 g N/kg

553 in reactors A and B (day 104), respectively, with both reactors showing a minimum

554 TAN of 3.0 g N/kg around day 20. The initial NH_3 was 1.1 and 1.7 g N/kg in reactors A

555 and B, respectively. Subsequently, NH_3 fluctuated with an overall decreasing trend

556 along the pH modification in both reactors, showing peaks higher than 1.0 g N/kg

557 mainly when pH was relatively high (i.e. ≥ 8.0) [Figure 3]. In reactor A, NH_3 reached

558 peaks of 1.4 (day 7) and 1.5 g N/kg (day 34), while NH_3 higher than 1.5 g N/kg was

559 repeatedly observed in reactor B (i.e. days 20, 27, 34 and 41).

560

561 In co-digestion, the initial TAN was 3.0 g N/kg and slightly increased to a maximum of

562 3.3, 3.6 and 3.3 g N/kg (day 61) in reactors A, B and C, respectively [Figure 4d]. TAN

563 subsequently decreased due to the reduced OFMSW feeding and the progressive

564 dilution used for HS-AD recovering, until a minimum of 1.9, 2.3 and 2.8 g N/kg was

565 reached in reactors A, B and C, respectively (day 112). The initial NH_3 was 2.0 g N/kg

566 and progressively decreased in the three reactors alongside pH. NH_3 peaked at 1.5 g

567 N/kg (day 12) and 1.2-1.7 g N/kg (day 19), rapidly decreasing to ≤ 0.1 g N/kg (day 23),

568 similarly in all reactors. From this point, NH_3 was maintained below 1.0 g N/kg in the

569 three reactors. Thus, NH_3 was considerably reduced during co-digestion alongside the

570 reduction of OFMSW in the feed, since peaks higher than 1.0 g N/kg were not observed

571 from day 20 onwards, in contrast to mono-digestion reactors.

572

573 NH_3 inhibition was likely one of the main triggers of overloading in this study, since the

574 high NH_3 levels observed (i.e. ≥ 1.0 g N/kg) are normally associated with methanogenic

575 inhibition and VFA accumulation in AD (Drosg, 2013; Rajagopal et al., 2013). Thus,

576 despite each AD system might show particular NH_3 inhibition thresholds depending on

577 the anaerobic consortia (Fricke et al., 2007; Westerholm et al., 2016), a gradual

578 methanogenic adaptation to high levels of TAN (i.e. ≥ 4.0 g N/kg) might be crucial to

579 increase OLR in semi-continuous HS-AD of OFMSW (Hartmann & Ahring, 2006;

580 Rajagopal et al., 2013).

581

582 In this study, a tradeoff was needed between the 'undesired' TAN buildup and the rapid

583 TS removal observed, to reach HS-AD conditions (i.e. TS ≥ 10 %) with mono-digestion

584 of OFMSW. For example, the different TS and TAN dynamics can be appreciated in

585 mono-digestion reactor A from day 30, when TS fluctuated while TAN steadily

586 increased [Figure 2]. Potential ammonia contingency strategies in AD, as increasing the

587 substrate dilution, reducing the OLR, and/or increasing the MRT (Kayhanian, 1999;

588 Rajagopal et al., 2013), would have lengthened considerably the experimental time, or

589 even prevented to achieve HS-AD conditions (i.e. TS $\geq$ 10 %) with mono-digestion of

590 OFMSW.

591

592

4 CONCLUSIONS

594 In this study, reducing the effluent compared to the influent mass (i.e. 18 %) permitted

595 to extend the MRT in semi-continuous mono-digestion of OFMSW, and obtain a

596 specific biogas production of 229 L/kg VS added, due to the high biodegradability of

597 OFMSW. However, the sole implementation of influent/effluent uncoupling was not

598 sufficient to avoid reactor overload and acidification when reaching HS-AD conditions

599 (i.e. TS $\geq$ 10 %). The average OLR was 4.5 g VS/kg·d, whereas a maximum 11.5 % TS

600 was reached. In contrast, the addition of beech sawdust to OFMSW allowed to operate

601 co-digestion reactors with an average OLR of 8.3 g VS/kg·d, and reach a maximum

602 29.0 % TS. Co-digestion lowered by 22 % the TAN content, though an average 186

603 L/kg VS added of biogas was obtained. Therefore, the addition of sawdust, as an

604 example of lignocellulosic substrate, to OFMSW (i.e. 1-2 g VS-Sawdust/g VS-

605 OFMSW) is an adequate strategy to stabilize HS-AD at very high TS contents (i.e. 20-

606 30 %). Nonetheless, a compromise must be found between increasing the TS content

607 and reducing the specific biogas production by co-digestion, since both aspects strongly

608 determine the HS-AD economy for OFMSW treatment.

609

610

611 **Acknowledgements**

615

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712

713

714

TABLE AND FIGURE CAPTIONS

716 **Table 1**: Bio-physical-chemical characterization of substrates.
717
718 **Table 2**: Physical-chemical characterization of inoculums.
719
720
721 **Figure 1**: Experimental setup. 1) Reactor body; 2) reactor head; 3) feeding port; 4) gas
722 output; 5) gas measuring port; and 6) opening valves.
723
724 **Figure 2**: Mono-digestion of OFMSW: a) Organic loading rate; b) mass retention time;
725 c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas
726 production; and f) methane content. Black arrows represent the $NaHCO_3$ addition in
727 reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B.
728
729 **Figure 3**: Mono-digestion of OFMSW: Volatile fatty acids and pH in a) reactor A; and
730 b) reactor B. Black arrows represent the $NaHCO_3$ addition in reactor A, while dotted
731 arrows represent the $FeCl_2$ or inoculum addition in reactor B.
732
733 **Figure 4**: Co-digestion of OFMSW and sawdust: a) Organic loading rate – parentheses
734 indicate the sole addition of OFMSW; b) mass retention time; c) total solids; d) total
735 and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane
736 content.
737
738 **Figure 5**: Co-digestion of OFMSW and sawdust: Volatile fatty acids and pH for a)
739 reactor A; b) reactor B; and c) reactor C.
740

1. Reactor Body
2. Reactor Head
3. Feeding Port
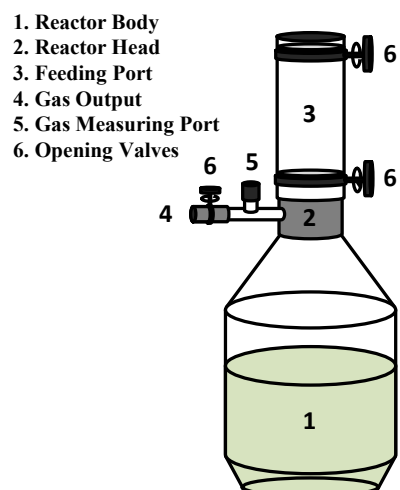4. Gas Output
5. Gas Measuring Port
6. Opening Valves

Figure 1: Experimental setup. 1) Reactor body; 2) reactor head; 3) feeding port; 4) gas output; 5) gas measuring port; and 6) opening valves.
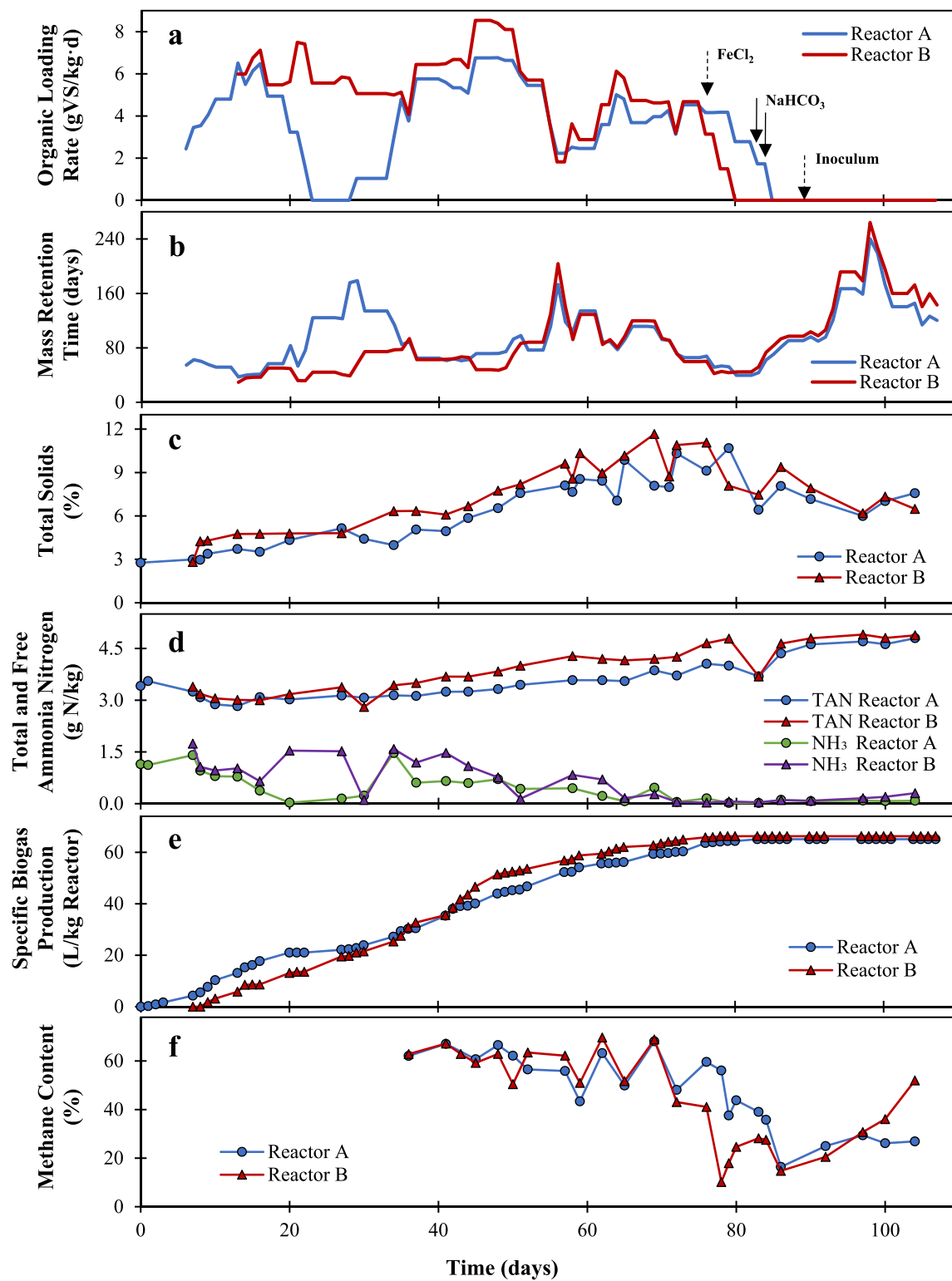
Figure 2
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Figure 2: Mono-digestion of OFMSW: a) Organic loading rate; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content. Black arrows represent the $NaHCO_3$ addition in reactor A, while dotted arrows represent the $FeCl_2$ or inoculum addition in reactor B.
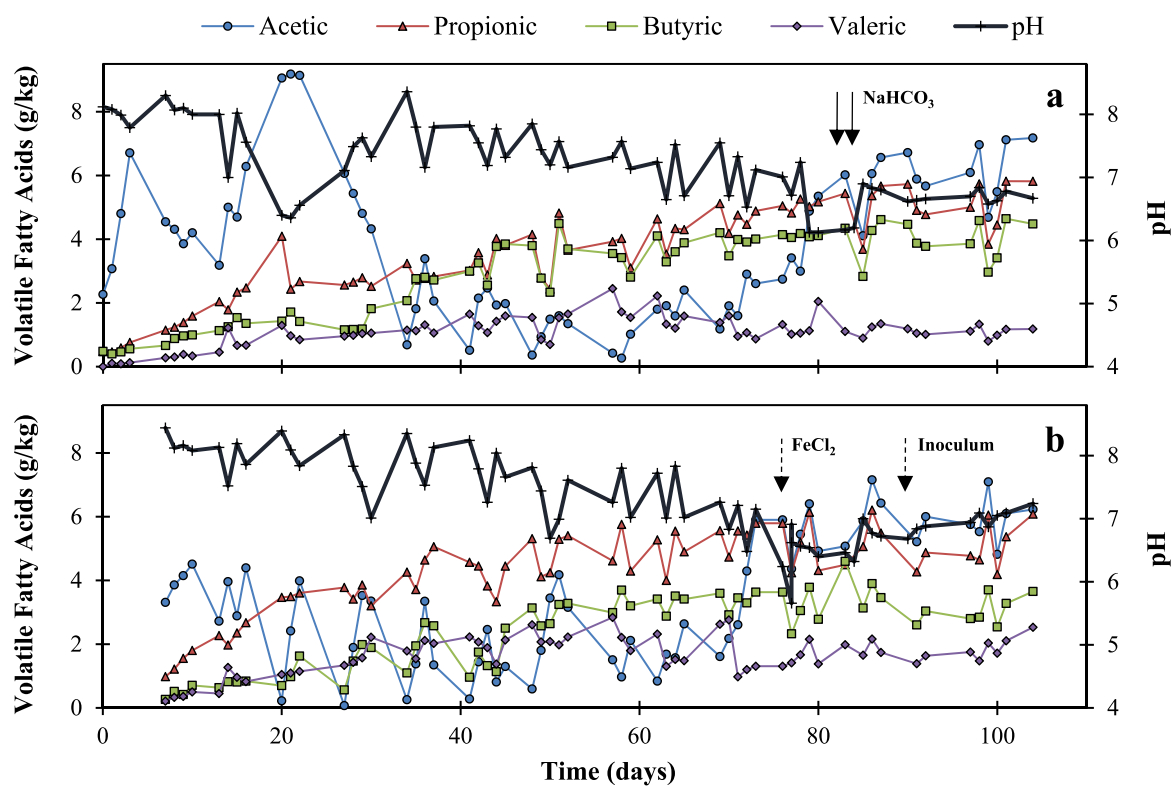
Figure 3: Mono-digestion of OFMSW: Volatile fatty acids and pH in a) reactor A; and b) reactor B. Black arrows represent the NaHCO$_3$ addition in reactor A, while dotted arrows represent the FeCl$_2$ or inoculum addition in reactor B.
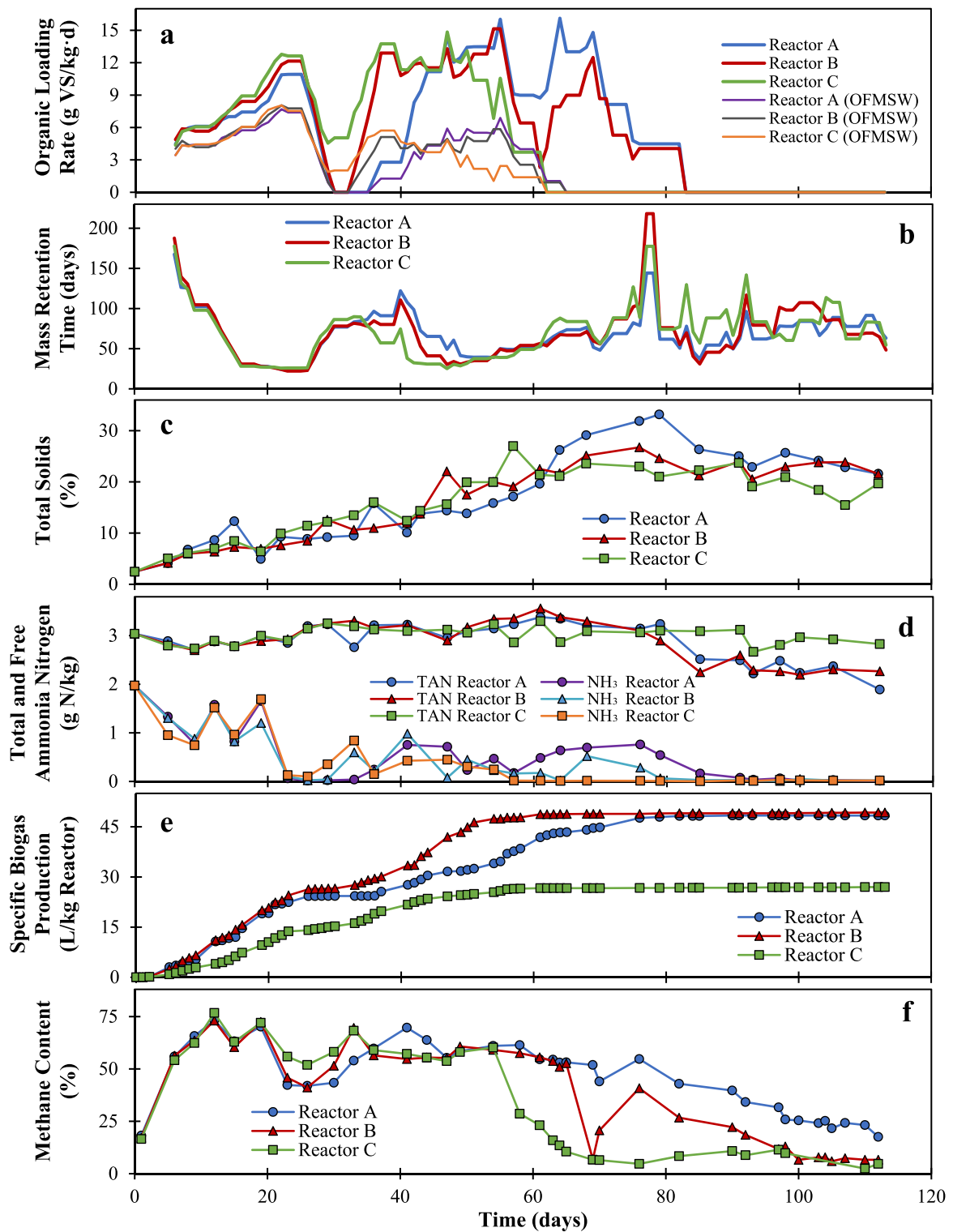
Figure 4
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Figure 4: Co-digestion of OFMSW and sawdust: a) Organic loading rate – parentheses indicate the sole addition of OFMSW; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH_3); e) cumulative biogas production; and f) methane content.
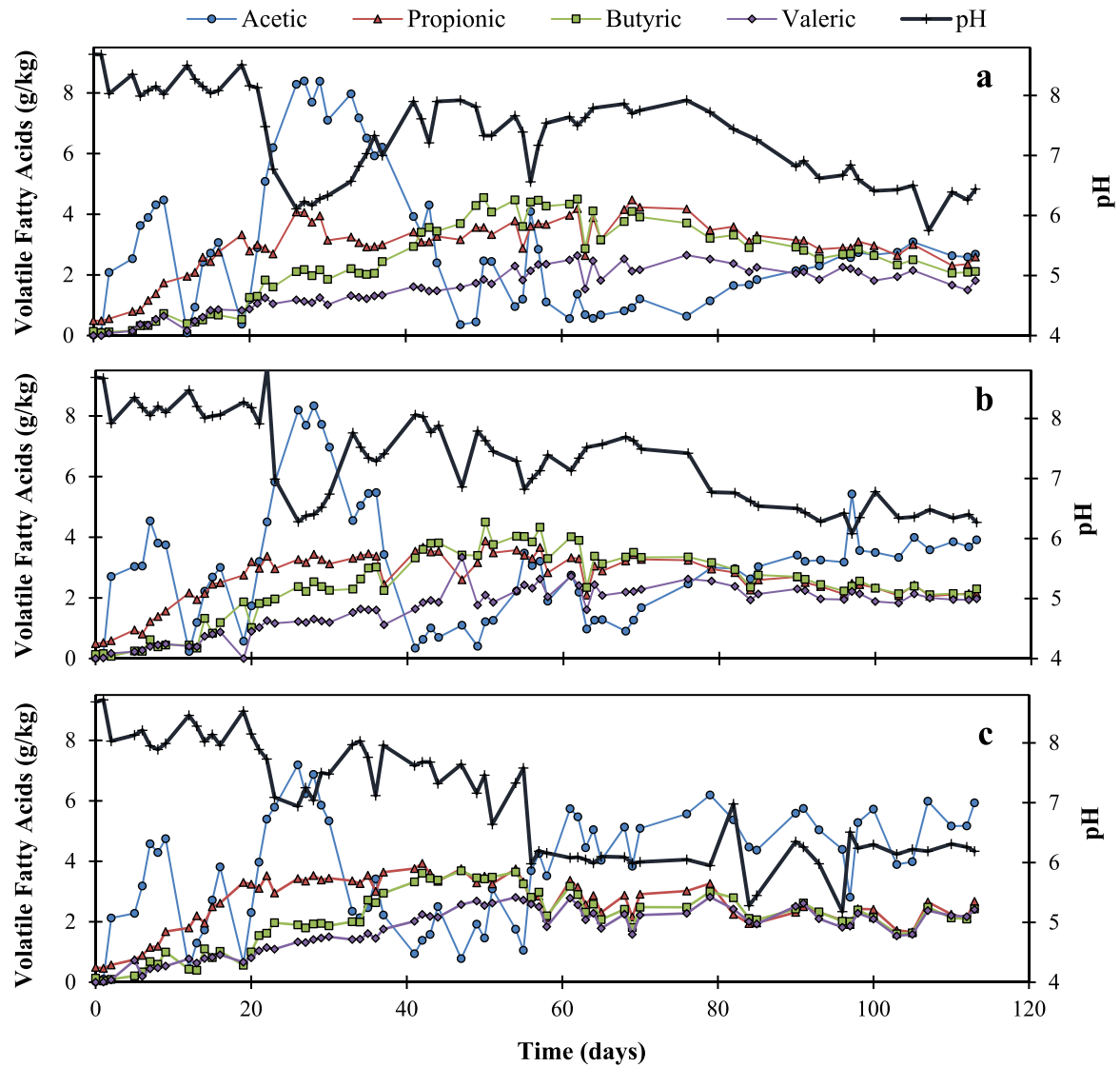
Figure 5
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Figure 5: Co-digestion of OFMSW and sawdust: Volatile fatty acids and pH for a) reactor A; b) reactor B; and c) reactor C.

Table 1: Bio-physical-chemical characterization of substrates.

	OFMSW	Sawdust
TS (%)	26.52 ± 1.35	93.69 ± 0.42
VS (%)	24.62 ± 1.27	92.64 ± 0.70
VS/TS	0.93 ± 0.02	0.99 ± 0.01
TKN (g N/kg TS)	24.78 ± 1.50	0.98 ± 0.17
TAN (g N/kg TS)	4.92 ± 0.06	0.12 ± 0.01
pH	4.40 ± 0.14	5.65 ± 0.06
ALK$_I$ (g Acetic/kg)	1.17 ± 0.82	1.50 ± 0.26
BMP (NmL CH$_4$/g VS)	497 ± 58	161 ± 12

Table 2
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Table 2: Physical-chemical characterization of inoculums.

	Mono-digestion		Co-digestion
	Reactor A	**Reactor B**	**Reactors A, B & C**
TS (%)	2.8	2.8	2.5
VS (%)	1.9	2	1.6
VS/TS	0.69	0.70	0.64
TKN (g N/kg TS)	161	161	139
TAN (g N/kg TS)	122	121	122
pH	8.12	8.44	8.69
ALK_P (g $CaCO_3$/kg)	9.6	9.6	9.3
ALK_I (g Acetic/kg)	5.3	6.3	3.2
Acetic (mg/kg)	2260	3310	20
Propionic (mg/kg)	470	980	490
Butyric (mg/kg)	480	260	140
Valeric (mg/kg)	0	210	0