

**Long term operation of a thermophilic anaerobic reactor:
process stability and efficiency at decreasing sludge retention time**

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Abstract

33

34 The aim of this study was to evaluate the performance of thermophilic sludge
35 digestion at decreasing sludge retention time (SRT) and increasing organic loading rate
36 (OLR), in terms of methane production, effluent stabilisation, hygienisation and
37 dewaterability. Focus was put on determining indicators to help prevent process failure.
38 To this end, a lab-scale reactor was operated for nearly two years at 55 °C. Methane
39 production rate was increased (from 0.2 to 0.4-0.6 m³ CH₄ m⁻³ reactor d⁻¹) by decreasing the
40 SRT from 30 to 15-10 days, while increasing the OLR from 0.5 to 2.5-3.5 kg VS m⁻³
41 reactor d⁻¹. Sludge dewaterability was worsened at SRT below 15 days; while pathogen
42 destruction was always successful. The following concentrations might be used to
43 prevent process failure: VFA C2-C5 (3.7 g COD L⁻¹), acetate (0.6 g L⁻¹),
44 acetate/propionate (0.5), intermediate alkalinity (1.8 g CaCO₃ L⁻¹), intermediate/partial
45 alkalinity (0.9), intermediate /total alkalinity (0.5), CH₄ in biogas (55 %).

46

47 **Keywords:** Biogas; Biosolids; Dewaterability; Hygienisation; Wastewater

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49

50 **1. Introduction**

51

52 In anaerobic digesters, biogas production depends on the amount of organic
53 matter biodegraded by anaerobic microorganisms. Thus, it depends on the composition
54 of the substrate, and presence and equilibrium between anaerobic consortia in the
55 reactor. Design and operation parameters of the process include sludge retention time
56 (SRT), organic loading rate (OLR), temperature and reactor flow, amongst others.

57 Sludge hydrolysis is often regarded as the rate limiting stage of the overall
58 process (Vavilin *et al.*, 2007); it affects the total amount of solids converted into soluble
59 compounds and ultimately to biogas. However, soluble substrates utilization rates for
60 fermentation and methanogenesis play a key role on process stability. The concentration
61 of intermediate products like volatile fatty acids (VFA) is a common indicator of
62 process unbalance (Marchaim and Krause, 1993; Pind *et al.* 2002). An accumulation of
63 VFA in the digester may result from problems in the syntrophic bacterial relationships
64 between the H₂-producing and the H₂-consuming bacteria; insufficient methanogenic
65 population to utilize all VFA produced or insufficient retention time for this process to
66 take place.

67 Since growth rates of methanogenic archaea are lower than those of fermentative
68 bacteria, they determine the minimum (or washout) SRT for the process. At 20, 25 and
69 35 °C the washout SRT are 7.8, 5.9 and 3.2 days, respectively, which turn into 40, 30
70 and 15 days design values by taking a safety factor of 5 for suspended growth processes
71 (Metcalf and Eddy, 2003). Because growth rates of thermophilic methanogens are 2-3
72 times higher than those of mesophilic homologues (Van Lier *et al.*, 1993), the minimum
73 and design SRT would be in the range of 1-2 and 5-8 days, respectively.

74 Process temperature not only affects the reaction rate and required SRT to
75 achieve a certain process efficiency (i.e. solids removal and methane production), but
76 also plays a key role in the stability of the process. Methanogenic archaea are especially
77 sensitive to temperature fluctuations, even to changes around 1 °C d⁻¹ (Metcalf and
78 Eddy, 2003). This can be particularly critical for thermophilic processes, since they are
79 reported to be less stable than mesophilic ones (Buhr and Andrews, 1977). For this
80 reason, a number of studies have focused on the effect of temperature fluctuations on
81 thermophilic anaerobic digestion (Van Lier *et al.*, 1993; Ahring *et al.*, 2001; El-Mashad
82 *et al.*, 2004).

83 Both temperature and SRT have direct influence on treatment costs, with respect
84 to initial capital investment (i.e. digester volume depends on the SRT), as well as
85 operation and maintenance costs (i.e. digester heating, mixing and pumping). Hence,
86 interest has also been put on studying the effect of the SRT on process performance (Lin
87 *et al.*, 1986; Zhang and Noike, 1994; Miron *et al.*, 2000; De la Rubia *et al.*, 2006a; De la
88 Rubia *et al.*, 2006b; Ponsá *et al.*, 2008). From an economical point of view, it would be
89 most effective to operate at a minimum SRT allowing optimising methane production
90 and solids removal, whilst assuring process stability.

91 Considering the whole sludge treatment line in wastewater treatment plants
92 (WWTP), sludge stabilisation in anaerobic digesters is followed by sludge conditioning
93 and dewatering steps. Since solids dewatering may account for some 7 % of the energy
94 requirements in conventional activated sludge WWTP (Metcalf and Eddy, 2003), the
95 reduction of dewatering costs by enhancing sludge dewaterability is of major
96 importance. However, from the literature it is not clear whether the anaerobic process
97 improves or degrades sludge dewaterability; and how mesophilic and thermophilic

98 effluents compare in terms of dewaterability is not clear either (Houghton *et al.*, 2000;
99 Houghton and Stephenson, 2002; Neyens and Baeyens, 2002; Novak *et al.*, 2003).

100 According to the 3rd Draft EU Working Document on Sludge (Environment DG,
101 EU, 2000), thermophilic digestion should enable effluent hygienisation for its use on
102 land, which is strongly recommended whenever it is possible to recycle the nutrients
103 contained in the sludge, improving soil fertility and minimising the amount of waste
104 going to incineration or landfill. Consequently, there has been a growing interest upon
105 this technology.

106 The aim of this study was to assess the impact of SRT and OLR on the anaerobic
107 thermophilic digestion of sewage sludge. Process performance was monitored at
108 decreasing SRT, while the influence of the solid content in the feed sludge, hence the
109 OLR and its variability, were evaluated. The combined effect of all these process
110 parameters on biogas and methane production, as well as effluent stabilisation,
111 hygienisation and dewaterability, were assessed. Focus was put on seeking alert values
112 for parameters which may be used to prevent process failure.

113

114 **2. Materials and methods**

115

116 ***2.1 Sewage sludge***

117

118 The sludge used for this work was obtained from two municipal WWTP near
119 Barcelona (Spain), which serve an equivalent population of 130,000 equivalent
120 inhabitants (EI) each. The conventional wastewater treatment used in these plants
121 consists of preliminary and primary treatment and secondary treatment in the activated
122 sludge unit. Primary sludge (PS) and secondary waste activated sludge (WAS) are

123 thickened and mixed (sampling point), before undergoing mesophilic (38 °C) anaerobic
124 digestion at 40 days SRT. Finally, digested sludge is dewatered in a centrifuge.

125 The inoculum used to seed the digester was mesophilic digested sludge (since no
126 thermophilic sludge digestion plant operates in the Barcelona area). The substrate was
127 the mixture of thickened PS and WAS (75 / 25 % v/v), which was collected weekly and
128 stored at 4 °C until use. Low-solids sludge (total solids (TS) < 40 g L⁻¹) was used for the
129 first 14 months; whereas high-solids sludge (TS > 40 g L⁻¹) was used thereafter. Volatile
130 solids (VS) contents were 14-24 g L⁻¹ (68-77 % VS/TS) and 30-35 g L⁻¹ (58-75 %
131 VS/TS) in the low-solids and high-solids sludge, respectively. In general, the values are
132 typical of sludge from conventional activated sludge WWTP entering digestion, with
133 TS below 50 g L⁻¹ and VS/TS around 70 % (Speece, 1988).

134

135 ***2.2 Experimental set-up***

136

137 The experimental set-up used in this work consists of a 5 L continuous stirred
138 tank reactor (CSTR) with automated semi-continuous feeding, temperature control (35-
139 55 °C) and on-line biogas measurement. It is described in detail elsewhere (Ferrer *et al.*,
140 2008).

141

142 ***2.3 Experimental procedures***

143

144 The reactor was seeded with 5 L of mesophilic digested sludge from a full-scale
145 reactor. The temperature was switched from 38 to 55 °C in a single step, while stopping
146 the organic loading for a few days. The OLR was thereafter increased by decreasing the
147 SRT to 30, 25, 20, 15, 12.5, 10, 8, 7 and 6 days. The OLR was also increased by

148 changing from low-solids to high-solids sludge. Each subsequent SRT decrease was
149 made once the digester had reached stable operation (i.e. fairly constant performance in
150 terms of biogas production, VFA concentration and pH in the reactor) as proposed by
151 other authors (Angelidaki and Ahring, 1994; El-Mashad *et al.*, 2004). This digester was
152 operated for 21 months, under the conditions summarised in Table 1.

153

154 **2.4 Analytical methods**

155

156 The solids content of sludge was determined according to the Standard Methods
157 procedure 2540G (APHA, 1999). TS and VS were determined directly from sludge
158 samples, whereas total dissolved solids (TDS) and volatile dissolved solids (VDS) were
159 determined from the supernatant of samples centrifuged at 7000 rpm. Supernatants
160 underwent filtration through 1.2 µm nominal pore size glass fiber filters (Albet
161 FVC047, Spain). The particulate fractions, total suspended solids (TSS) and volatile
162 suspended solids (VSS) were subsequently deduced. pH, alkalinity and VFA (acetic,
163 propionic, iso-butyric, n-butyric, iso-valeric and n-valeric acids) were also analysed
164 from the filtrate supernatant. Samples for VFA analysis were further filtered through a
165 0.45 µm nylon syringe filter. VFA and biogas composition were determined by gas
166 chromatography (Perkin-Elmer AutoSystem XL Gas Chromatograph), as described in
167 Ferrer *et al.* (2008).

168 Total, partial and intermediate alkalinities were determined as proposed by
169 Ripley *et al.* (1986). The method consists of a two step titration: a first one down to pH
170 5.75, which is due to HCO_3^- species and is known as partial alkalinity (PA); and a
171 second one down to pH 4.3, which corresponds to the total alkalinity (TA). The
172 intermediate alkalinity (IA), which is related to VFA concentration, is then estimated as
173 the difference between TA and PA. It can be used as an indirect measurement of VFA

174 concentration. The alkalinity ratio (AR), defined as the ratio between intermediate and
175 total alkalinity (IA/TA), or between intermediate and partial alkalinity (IA/PA); may
176 also be a useful indicator of the concentration of VFA in the sample.

177 Sludge dewaterability was determined using the Capillary Suction Time (CST)
178 test, according to the Standard Methods procedure 2710G (APHA, 1999). The CST
179 used was a Triton CST filterability tester, model 200, Triton Electronics Ltd., Essex,
180 UK. Standard filter papers (Part No. 815095) were supplied by Triton Electronics.

181 Sludge hygienisation was evaluated by the concentration of *Escherichia coli* and
182 *Salmonella sp.* in digested effluents. *E. coli* were quantified by the methodology ISO
183 16649:2000 and the results were expressed as colony forming units per mL (CFU·mL⁻¹).
184 ¹). In the case of *Salmonella sp.*, only presence or absence was determined by the
185 methodology NF-V08-052 and the results were presence or absence per 50 mL of
186 sample.

188 **3. Results and discussion**

190 ***3.1 Thermophilic anaerobic sludge digestion at decreasing SRT***

192 Process performance during the long term operation of the reactor (654 days) is
193 illustrated in Figures 1 and 2. Mean values of operating and efficiency parameters
194 during stable periods under each condition assayed are summarised in Table 2.

195 The process was start-up by seeding the digester with mesophilic sludge and
196 rising process temperature from 38 to 55° C in a single-step. Working at 30 days SRT,
197 the OLR reached 0.69 kg VS m⁻³_{reactor} d⁻¹, leading to methane production rates around
198 0.22 m³_{CH4} m⁻³_{reactor} d⁻¹ and 40-50 % VS destruction (Table 2, period II). Such values are

199 in the range of those reported in the literature for thermophilic digestion of sewage
200 sludge at high SRT. For instance, De la Rubia *et al.* (2006a) obtained around $0.19 \text{ m}^3_{\text{CH}_4}$
201 $\text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$ and 53 % VS removal working at 27 days SRT.

202 The SRT was subsequently reduced to 25 and 20 days ($\text{OLR} \sim 1 \text{ kg VS m}^{-3}_{\text{reactor}}$
203 d^{-1}), leading to biogas production rates between $0.35\text{-}0.42 \text{ m}^3_{\text{biogas}} \text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$, with 62-
204 68 % CH_4 in biogas (Table 2, periods III and IV). VS destruction was lower at 20 days
205 SRT (40 % vs. 53 %) due to fluctuations in influent VS concentration. Other authors
206 have obtained similar results at 20 days SRT ($\sim 0.4 \text{ m}^3_{\text{biogas}} \text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$, 60-65 % CH_4 in
207 biogas, ~ 53 % VS destruction) (De la Rubia *et al.*, 2002; Gavala *et al.*, 2003).

208 The best results were obtained at the lowest SRTs (15 and 10 days), with OLR
209 of 1-1.6 and 1.5-2 $\text{kg VS m}^{-3}_{\text{reactor}} \text{d}^{-1}$, respectively. In particular, the highest biogas and
210 methane production rates (up to 0.56 and $0.36 \text{ m}^3 \text{m}^{-3} \text{d}^{-1}$, respectively) correspond to 10
211 days SRT (Table 2, period VI).

212 After switching from low-solids to high-solids sludge ($40\text{-}60 \text{ g TS L}^{-1}$; $30\text{-}35 \text{ g}$
213 VS L^{-1}), OLR as high as $3\text{-}4 \text{ kg VS m}^{-3}_{\text{reactor}} \text{d}^{-1}$ were maintained (Figure 1). Biogas
214 production rate was almost doubled from 0.5 to $1 \text{ m}^3_{\text{biogas}} \text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$ at 10 days SRT
215 feeding low- and high-solids sludge, respectively (Table 2, periods VI and VII).
216 However, higher effluent VFA ($> 4 \text{ g COD L}^{-1}$) were detected (Figure 3(a)).

217 The SRT was gradually decreased to 6 days with OLR ranging from 4.5 to 6.5
218 $\text{kg VS m}^{-3}_{\text{reactor}} \text{d}^{-1}$ (Figure 1), which are amongst the highest OLR and lowest SRT
219 reported for single stage sludge digestion (Buhr and Andrews, 1977; Speece, 1988; De
220 la Rubia *et al.*, 2006a; De la Rubia *et al.*, 2006b). Initially, biogas production reached its
221 highest rates ($\sim 1.5 \text{ m}^3_{\text{biogas}} \text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$), with 58-69 % CH_4 in biogas. However, these
222 operating conditions prompted VFA accumulation (VFA C2-C5 increase from 4 to 10 g

223 COD L⁻¹), as shown in Figure 3(a). Methane content in biogas drop below 50 % (Figure
224 2) and VS removal to 13 %. To avoid digester failure, the SRT was set back to 10 days.

225

226 **3.2 Process stability**

227

228 During almost two years of experimental work, process stability was disturbed
229 whenever the OLR increased, either as a result of decreasing the SRT or due to
230 fluctuations in the solids content of feed sludge. Additionally, the process was unsteady
231 after temperature fluctuations episodes (caused by occasional operating problems),
232 especially when they happened together with organic overloading. In all cases, the
233 immediate response of the system was a decrease in methane content in biogas from
234 around 60 % to below 50 % and VFA accumulation (Figures 2-3) as a result of
235 decreased methanogenic activity.

236 Based on this study, limit concentrations to detect and prevent digester failure
237 during thermophilic sludge digestion are proposed (Table 3) and discussed a follows.

238

239 **3.2.1 Volatile fatty acids**

240

241 Although the concentration of all VFA increased during the instability episodes,
242 the rise in acetate concentration was perhaps the most accentuated. Throughout the
243 whole experimental period, acetate fluctuated within a wider range of concentrations,
244 compared to other major VFA like propionate, iso-butyrate and iso-valerate. Figure 3(b)
245 shows that these three VFA followed parallel trends, propionate concentration always
246 being the highest. On the other hand, acetate concentration ranged from almost 0 to
247 nearly 1 g L⁻¹. This clearly indicates that occasional temperature fluctuations and

248 organic overloading affected methanogens to a higher extent than acidogens, with
249 subsequent accumulations of acetate in the liquor. Since changes in propionate
250 concentration were less pronounced, the trend followed by the acetate to propionate
251 ratio (A/P ratio) was similar to that of acetate, as can be seen from Figure 3(b).

252 As well as individual and total VFA, some authors have proposed acetate
253 concentration and A/P ratio as valuable indicators to predict process failure (Marchaim
254 and Krause, 1993; Pind *et al.*, 2002). For manure, an acetic acid concentration of 0.8 g
255 L⁻¹ and an A/P ratio of 1.4 have been proposed as limit values (Hill *et al.*, 1987; cited in
256 Marchaim and Krause, 1993). To our knowledge, such limit values for thermophilic
257 sewage sludge digestion have not yet been proposed. In the present study, acetate
258 concentration was usually below 0.6 g L⁻¹ (Table 2, all periods) and only in cases of
259 organic overloading or temperature fluctuations (due to operating problems) did this
260 value rise above 0.6 g L⁻¹ and up to 2 g L⁻¹. Furthermore, concentrations above 1 g L⁻¹
261 were only reached when the SRT was reduced to 6 days, with OLR greater than 5 kg
262 VS m⁻³_{reactor} d⁻¹, as shown in Figure 3. Therefore, a limit concentration of 0.6 g L⁻¹ of
263 acetic acid would seem more appropriate to predict digester failure in the case of
264 thermophilic sludge digestion. Similarly, during stability periods the A/P ratio was
265 below 0.5 (Table 2, all periods); hence the limit A/P ratio to predict digester failure
266 ought to be reduced to around 0.5. From our experimental results, it might be
267 hypothesized that the total VFA (C2-C5) concentration corresponding to these values
268 would be around 3.7 g COD L⁻¹, depending on the individual VFA concentration. Such
269 a high concentration would be detrimental to the quality of the effluent sludge, meaning
270 that subsequent post-treatments ought to be considered.

271

272 3.2.2 Alkalinity

273

274 According to Ripley *et al.* (1986), the total alkalinity (TA) of a sample is a result
275 of HCO_3^- species, which is known as partial alkalinity (PA); and VFA, which is known
276 as intermediate alkalinity (IA). The latter is estimated as the difference between the TA
277 and PA. For this reason, the IA consists of an indirect measurement of VFA, and the
278 alkalinity ratios between intermediate and total (IA/TA) or partial (IA/PA) alkalinities
279 are alternative process indicators. In the present study, the profile of the IA/PA ratio was
280 indeed very similar to that of total VFA, acetate concentration and A/P ratio in Figure 3;
281 while variations in the IA/TA ratio were less pronounced. In general, the IA/PA ratio
282 was more sensible to variations in the VFA concentration than the IA/TA.

283 The correlation between total VFA (C2-C5) concentration, acetate concentration
284 or A/P ratio; and alkalinity ratios or intermediate alkalinity was further analysed (Figure
285 4). Obviously, the best correlated parameter was intermediate alkalinity, followed by
286 IA/PA and IA/TA ratios. The best correlations were obtained with respect to total VFA
287 concentration ($R^2 \leq 0.79$); while the correlations with acetate concentration were very
288 poor ($R^2 \leq 0.65$) and no correlations were found with the A/P ratio ($R^2 \sim 0$).

289 If threshold values were to be set in order to predict process failure based on
290 alkalinity measurements (which is common practise at industrial scale); the values
291 corresponding to the aforementioned VFA C2-C5 concentration of 3.7 g COD L^{-1}
292 would be around 0.9 for IA/PA ratio, 0.5 for IA/TA ratio and $1.8 \text{ g CaCO}_3 \text{ L}^{-1}$ for
293 intermediate alkalinity.

294

295 3.2.3 Methane content in biogas

296

297 With regards to the methane content in biogas, during stable periods this value
298 always ranged between 60-70 % (Table 2, all periods), which is typically reported in the
299 literature for thermophilic sludge digestion (Krugel *et al.* 1998; Záborská *et al.* 2000;
300 De la Rubia *et al.* 2006a; De la Rubia *et al.* 2006b; Ferrer *et al.*, 2008; Palatsi *et al.*,
301 2009). It only fell below 55 % in cases of organic overloading or temperature
302 fluctuation, which suggests a warning concentration of 55 % for thermophilic sludge
303 digestion. It should be noticed that such a value would be within the common range for
304 other processes; for instance in digesters treating the organic fraction of municipal solid
305 wastes methane content in biogas ranges from 50-60 %.

306

307 3.2.4. pH

308

309 In terms of pH, this parameter was fairly constant and remarkably high (around
310 8). Even working at 6 days SRT, with the highest OLR ($> 5 \text{ kg VS m}^{-3}_{\text{reactor}} \text{ d}^{-1}$), when
311 all other indicator parameters were above the limit values proposed, the pH was still 8.
312 The reason for this is that the alkalinity of the system was also the highest; hence the
313 buffer capacity of the system prevented from pH drop resulting from VFA
314 accumulation. In sewage sludge digesters, sufficient alkalinity is generally found (3-5 g
315 $\text{CaCO}_3 \text{ L}^{-1}$) to prevent the pH from falling below the limit for methanogenesis inhibition
316 (Metcalf and Eddy, 2003). Studies with high-solids sludge (4-10 % TS) have shown that
317 the optimum pH range for high rate digestion is 6.6-7.8, while the acceptable pH range
318 is 6.1-8.3; meaning that below 6.1 the process may fail due to an excessively low
319 methanogenesis rate compared to acidogenesis rate, while above 8.3 the process might
320 be inhibited by free ammonia (Lay *et al.*, 1997). Ammonia inhibition is favoured by

321 high process temperature (Angelidaki and Ahring, 1994) and is pointed out as a major
322 cause for low biogas production treating pig slurries (Bonmatí and Flotats, 2003).

323

324 ***3.3. Effect of SRT and OLR on process efficiency and stability***

325

326 The main objective of decreasing the SRT was to determine the minimum SRT
327 allowing a stable anaerobic process performance at 55 °C. Bearing in mind that the
328 minimum design SRT is around 15 days at 35 °C (Metcalf and Eddy, 2003), and that the
329 growth rates of thermophilic methanogens are 2-3 times higher than those of mesophilic
330 homologues, (Van Lier *et al.*, 1993), the theoretical SRT may be reduced to 5-8 days at
331 55 °C. However, such a reduction is likely to deteriorate process efficiency, especially
332 regarding the quality of the effluent which is generally poorer in thermophilic digesters
333 (Buhr and Andrews, 1977). Digested sludge dewaterability might consequently be
334 degraded.

335 For the purposes of this study, the SRT was gradually reduced from 30 to 6 days.
336 However, because the feeding sludge was collected weekly from the WWTP, seasonal
337 variations and operational changes affected its composition and organic content.
338 Furthermore, low-solids and high-solids sludge were used. Whilst operating under a
339 fixed SRT, the OLR was affected by the sludge organic content; thus it was also
340 necessary to assess the effect of OLR on the thermophilic sludge digestion.

341 Figure 5 shows methane production rate, effluent VFA and effluent VS as a
342 function of the OLR. In general, high correlations were obtained for methane production
343 rate and VFA ($R^2=0.96$). This means that daily methane production, hence
344 methanogenic activity, was very much dependant on the OLR, regardless of the SRT.
345 Similarly, acidogenesis increased with the OLR (Figure 5), but short SRT were not

346 enough to convert all VFA to methane, which means that a portion of hydrolysed
347 organic compounds did not end up yielding methane.

348 De la Rubia *et al.* (2006a) found a similar dependence of methane production
349 rate on OLR and SRT over the range of 15-75 days during thermophilic anaerobic
350 digestion of PS and WAS. COD mass balances indicated that the amount of COD used
351 for methane generation increased at decreasing SRT or increasing OLR. The results
352 obtained by these authors suggest that higher OLR ($> 2.2 \text{ kg VS m}^{-3} \text{ d}^{-1}$) or lower SRT
353 (< 15 days) might have resulted in further methane production improvement (> 0.4
354 $\text{m}^3_{\text{CH}_4} \text{m}^{-3} \text{d}^{-1}$).

355 Miron *et al.* (2000) reported that, during psychrophilic digestion of PS, SRT of
356 10 days were enough to obtain methanogenic conditions in the reactor, while lower SRT
357 (8 days) resulted in acidogenic conditions. Taking into account that reaction rates are
358 higher under thermophilic conditions, it might be speculated that the homologous SRT
359 for a thermophilic process would be lower.

360 In the present study, the minimum SRT assayed was 6 days, but the minimum
361 SRT ensuring a stable performance was also 10 days. Methane production under
362 thermophilic conditions was improved by decreasing the SRT from 30 to 10 days. It
363 was further enhanced at 6 days SRT with an OLR higher than $5 \text{ kg VS m}^{-3} \text{ d}^{-1}$, feeding
364 high-solids sludge. However, when the OLR eventually increased ($> 6 \text{ kg VS m}^{-3} \text{ d}^{-1}$) as
365 a result of fluctuations in the solids content of the feed sludge, methanogenic activity
366 was severely affected; as indicated by decreased biogas production, with methane
367 content below 50 %, and a sudden accumulation of VFA, with a total concentration
368 higher than 6 g L^{-1} . Furthermore, the quality of the effluent in terms of VS content was
369 worsened.

370 On the other hand, working at SRT of 10 days still with high OLR (3-4 kg VS
371 $\text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$), the process was more stable. Biogas and methane production rates (0.55-
372 0.6 and 0.35-0.4 $\text{m}^3 \text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$) were increased by 50 % compared previous results at
373 higher SRT. Gas production at 10 days SRT was within the range obtained by other
374 authors at 15 days SRT (De la Rubia *et al.*, 2006a; Benabdallah *et al.*, 2006); but clearly
375 higher than that obtained at 20 days SRT (De la Rubia *et al.*, 2002; Gavala *et al.*, 2003).
376 In practise, this means that the sludge daily flow rate could be doubled or the digester
377 volume reduced, while producing the same amount of methane (i.e. energy). However,
378 higher effluent VS and especially higher VFA, ought to be expected at this reduced
379 SRT; which might deteriorate subsequent sludge dewatering.

380

381 **3.4 Sludge dewaterability**

382

383 Sludge dewaterability was measured by determining the capillary suction time
384 (CST) of digested sludge samples obtained during each stability period. Figure 6(a)
385 shows that CST values increased proportionally to the OLR ($R^2=0.92$). The trends are
386 similar when the CST is expressed per g TS or g VS.

387 A clear dependence of CST on the solids concentration in the sludge is shown in
388 Figure 6(b): the higher the solids concentration, the higher the CST. Hence, it may be
389 speculated that any increase in effluent VS and TS resulting from changing the OLR
390 and/or SRT may ultimately affect digested sludge dewaterability. From the results of
391 this study, it seems that digested sludge dewaterability was deteriorated with TS higher
392 than 26 g L^{-1} and VS higher than 17 g L^{-1} ; which corresponded to OLR above 3 kg VS
393 $\text{m}^{-3}_{\text{reactor}} \text{d}^{-1}$ and SRT below 10 days.

394 According to the work by Miron *et al.* (2000), the dewaterability of PS worsened
395 under acidogenic conditions ($SRT \leq 8$ days), while it improved under methanogenic
396 conditions ($SRT \geq 10$ days). This was related to a decrease in the mean particle size,
397 thus an increase in the total surface area, under acidogenic conditions. Moreover, only at
398 high SRT of 15 days was digested sludge dewaterability improved compared to that of
399 influent sludge. The results of the present study are quite consistent with those findings,
400 since only at SRT above 15 days was the CST value (60-160 s) below that of influent
401 sludge (437 s). Sludge dewaterability was worsened (CST ~ 630-1370 s) at shorter SRT
402 (10-6 days), which were typically associated to higher effluent VFA, thus higher soluble
403 VS. Indeed, an increasing trend was followed by CST with respect to effluent VFA
404 (Figure 6(c)).

405 Some controversy exists in the literature regarding the effect of anaerobic
406 digestion on sludge dewaterability, and it is still not clear whether mesophilic and
407 thermophilic digestion has any effect in sludge dewaterability. It has been shown that
408 sludge dewaterability, as well as the amount of chemicals required for sludge
409 conditioning, are directly dependant on the concentration of biopolymer in the solution
410 (Novak *et al.*, 2003). Houghton *et al.* (2000) and Houghton and Stephenson (2002)
411 reported that the composition of microbial extracellular polymer (ECP) varied after
412 sludge digestion and was also affected by the feed composition; attributing excess ECP
413 production to acidogenic bacteria. This might also explain higher CST values obtained
414 in the present study in samples with higher VFA concentration, in which the presence of
415 acidogenic bacteria should be higher.

416

417 **3.5. Effluent hygienisation**

418

419 Sludge hygienisation was assessed by quantifying pathogen indicators
420 *Escherichia coli* and *Salmonella* spp. from digested sludge samples obtained during
421 each stability period, and comparing them to the values obtained from influent sludge
422 samples. While *Salmonella* spp. was never detected; the concentration of *E. coli* in the
423 influent sludge was in the range of 10^6 CFU mL⁻¹. A complete destruction of *E. coli* was
424 achieved at SRT higher than 20 days, but concentrations in the range of 10^1 and 10^2
425 CFU mL⁻¹ were found at SRT of 10-15 days and 6 days, respectively (Table 4). *E. coli*
426 concentration in the effluent seemed to be depended on the OLR hence, on the influent
427 characteristics.

428 Hygienisation of thermophilic effluent sludge in laboratory and full-scale
429 reactors working at a range of SRT is reported in the literature (Zábranská *et al.*, 2000;
430 Laffite-Trouqué and Forster, 2002; Lu *et al.*, 2007). It is in fact a major advantage of
431 thermophilic anaerobic digestion, compared to mesophilic operation. In this study, *E.*
432 *coli* and *Salmonella* spp. concentrations in all effluent samples were below the limits
433 proposed in the 3rd Draft EU Working Document on Sludge (Environment DG, EU,
434 2000) for unrestricted land application of digested sludge; which suggests that a
435 minimum SRT of 6 days at 55 °C might be sufficient to prevent the spread of pathogens
436 in the environment upon land application of digestates.

437

438 **4. Conclusions**

439

440 This long term study showed that the minimum SRT for a stable thermophilic sludge
441 digestion was 10 days. Methane production was increased to $0.4-0.6 \text{ m}^3_{\text{CH}_4} \text{ m}^{-3}_{\text{reactor}} \text{ d}^{-1}$
442 by decreasing the SRT to 15-10 days, but VS removal and sludge dewaterability were
443 worsened below 15 days SRT, with high effluent VFA. Besides, the concentrations of

444 pathogens were always below the limits proposed for unrestricted land application. The
445 following indicators may be useful to prevent digester failure: VFA C2-C5 (3.7 g COD
446 L⁻¹), acetate (0.6 g L⁻¹), A/P (0.5), IA (1.8 g CaCO₃ L⁻¹), IA/PA (0.9), IA/TA (0.5), CH₄
447 in biogas (55 %).

448

449 **Acknowledgements**

450

451 The authors wish to thank the financial support provided by the Spanish
452 Ministry of Science and Technology and FEDER (REN2002-00926/TECNO). Eva
453 Romero from GIRO Technological Centre is kindly acknowledged for her contribution.

454

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Table 1. Operating conditions

Period	Time (days)	Temperature (°C)	SRT (d)	Solids content in feed sludge *
I	1-79	55	> 30	low-solids
II	80-161	55	30	low-solids
III	162-203	55	25	low-solids
IV	204-256	55	20	low-solids
V	257-331	55	15	low-solids
VI	332-437	55	10	low-solids
VII	484-529	55	10	high-solids
VIII	569-606	55	6	high-solids
IX	607-653	55	10	high-solids

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* low-solids: total solids < 4 %; high-solids: total solids > 4 %

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Note: Transition periods have not been included

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Table 2. Average feed and digested sludge characteristics and operational parameters during anaerobic digestion of low-solids (periods I-VI) and high-solids (periods VII-IX) sludge

Parameter	Period					
	I	II	III	IV	V	VI
Working conditions						
T (°C)	55.3 ± 1.2	55.4 ± 1.3	55.4 ± 0.5	55.3 ± 0.2	54.7 ± 0.4	54.2 ± 1.7
SRT (d)	29.1 ± 1.5	30.3 ± 3.3	25.4 ± 4.4	20.4 ± 2.8	16.0 ± 1.7	10.4 ± 0.5
OLR (kg VS m ⁻³ reactor d ⁻¹)	0.47 ± 0.1	0.69 ± 0.1	0.97 ± 0.5	1.05 ± 0.2	1.38 ± 0.3	1.65 ± 0.3
Feed composition						
TS (g L ⁻¹)	19.63 ± 1.67	32.77 ± 8.04	31.48 ± 10.84	30.34 ± 7.38	28.86 ± 6.86	23.22 ± 5.17
VS (g L ⁻¹)	13.30 ± 0.85	22.16 ± 4.91	23.25 ± 7.70	21.34 ± 4.12	21.01 ± 5.14	17.93 ± 3.85
VS/TS	68.90 ± 4.67	68.21 ± 0.74	74.23 ± 1.79	70.59 ± 2.20	74.78 ± 1.80	77.52 ± 2.00
VFA C2-C5 (g COD L ⁻¹)	1.68 ± 0.32	4.30 ± 0.69	3.59 ± 0.55	2.72 ± 0.55	4.51 ± 0.79	3.68 ± 0.76
pH	6.97 ± 0.57	6.04 ± 0.11	5.75 ± 0.18	6.25 ± 0.12	5.92 ± 0.07	6.13 ± 0.29
Effluent composition						
TS (g L ⁻¹)	13.09 ± 1.74	17.60 ± 1.58	14.92 ± 1.15	20.11 ± 2.80	17.59 ± 0.94	18.90 ± 4.63
VS (g L ⁻¹)	7.90 ± 0.92	11.15 ± 1.18	9.55 ± 0.87	13.50 ± 0.78	11.62 ± 0.68	14.00 ± 2.31
VS/TS	61.76 ± 0.98	63.19 ± 1.68	63.94 ± 1.14	64.81 ± 1.27	66.39 ± 2.34	70.06 ± 0.86
VFA C2-C5 (g COD L ⁻¹)	0.94 ± 0.53	2.16 ± 0.52	1.60 ± 0.81	2.49 ± 0.48	2.60 ± 0.22	2.31 ± 0.63
Acetate (g L ⁻¹)	0.12 ± 0.17	0.31 ± 0.13	0.17 ± 0.15	0.17 ± 0.05	0.03 ± 0.04	0.22 ± 0.12
Propionate (g L ⁻¹)	0.29 ± 0.12	0.69 ± 0.13	0.51 ± 0.24	0.79 ± 0.15	0.92 ± 0.07	0.99 ± 0.10
iso-Butyrate (g L ⁻¹)	0.07 ± 0.05	0.19 ± 0.04	0.12 ± 0.09	0.24 ± 0.06	0.27 ± 0.02	0.29 ± 0.03
Butyrate (g L ⁻¹)	0.01 ± 0.02	0.00	0.00	0.00	0.34 ± 0.03	0.08 ± 0.10
iso-Valerate (g L ⁻¹)	0.12 ± 0.06	0.21 ± 0.08	0.20 ± 0.10	0.34 ± 0.07	0.34 ± 0.03	0.42 ± 0.07
Valerate (g L ⁻¹)	0.00	0.00	0.00	0.00	0.00	0.03 ± 0.04
A/P ratio	0.46 ± 0.39	0.44 ± 0.17	0.39 ± 0.33	0.21 ± 0.07	0.08 ± 0.03	0.22 ± 0.09
IA (g CaCO ₃ L ⁻¹)	0.88 ± 0.08	1.26 ± 0.18	1.09 ± 0.23	1.32 ± 0.13	1.40 ± 0.12	1.47 ± 0.19
IA/TA ratio	0.31 ± 0.03	0.43 ± 0.04	0.37 ± 0.06	0.39 ± 0.04	0.41 ± 0.02	0.46 ± 0.03
IA/PA ratio	0.45 ± 0.07	0.75 ± 0.12	0.59 ± 0.16	0.65 ± 0.09	0.71 ± 0.07	0.86 ± 0.12
pH	8.18 ± 0.11	8.03 ± 0.09	8.15 ± 0.17	8.08 ± 0.11	7.86 ± 0.12	7.91 ± 0.09
Removal efficiency						
TS removal (%)	30.7 ± 10.9	39.7 ± 15.9	50.1 ± 14.2	36.1 ± 17.1	35.0 ± 17.7	27.5 ± 20.9
VS removal (%)	42.2 ± 5.9	44.1 ± 5.9	53.4 ± 3.0	40.5 ± 9.1	43.2 ± 4.	22.7 ± 4.5
Biogas characteristics						
Biogas prod. rate (m ³ m ⁻³ reactor d ⁻¹)	0.18 ± 0.06	0.28 ± 0.07	0.35 ± 0.12	0.41 ± 0.14	0.36 ± 0.11	0.56 ± 0.14
Specific biogas prod. (m ³ kg VS _{fed} ⁻¹)	0.37 ± 0.11	0.36 ± 0.07	0.42 ± 0.12	0.43 ± 0.08	0.29 ± 0.10	0.37 ± 0.10
Biogas yield (m ³ kg VS _{removed} ⁻¹)	0.63 ± 0.09	0.70 ± 0.10	0.90 ± 0.43	0.99 ± 0.47	0.81 ± 0.68	1.15 ± 0.20
Methane prod. rate (m ³ m ⁻³ reactor d ⁻¹)	0.08 ± 0.02	0.22 ± 0.04	0.20 ± 0.04	0.30 ± 0.07	0.24 ± 0.03	0.36 ± 0.11
Specific methane prod. (m ³ kg VS _{fed} ⁻¹)	0.17 ± 0.03	0.26 ± 0.03	0.28 ± 0.08	0.29 ± 0.08	0.19 ± 0.04	0.23 ± 0.06
Methane yield (m ³ kg VS _{removed} ⁻¹)	0.40 ± 0.05	0.47 ± 0.05	0.61 ± 0.29	0.70 ± 0.31	0.59 ± 0.43	0.71 ± 0.13
Methane content (%)	63.64 ± 3.03	64.57 ± 4.86	65.07 ± 2.58	66.21 ± 1.20	64.02 ± 1.37	61.78 ± 1.49

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566 **Table 2 (cont).** Average feed and digested sludge characteristics and operational parameters
 567 during anaerobic digestion of low-solids (periods I-VI) and high-solids (periods VII-IX) sludge

Parameter	Period		
	VII	VIII	IX
Working conditions			
T (°C)	53.2 ± 0.3	53.6 ± 1.1	52.3 ± 1.5
SRT (d)	9.4 ± 0.8	6.2 ± 1.3	10.1 ± 1.1
OLR (kg VS m ⁻³ reactor d ⁻¹)	3.7 ± 0.4	5.2 ± 0.5	2.4 ± 0.3
Feed composition			
TS (g L ⁻¹)	45.39 ± 3.52	54.61 ± 7.65	40.60 ± 10.93
VS (g L ⁻¹)	34.86 ± 2.34	31.21 ± 3.60	24.23 ± 2.70
VS/TS	75.71 ± 0.59	58.08 ± 10.29	62.02 ± 9.11
VFA C2-C5 (g COD L ⁻¹)	4.37 ± 1.83	2.49 ± 0.99	1.40 ± 0.22
pH	6.61 ± 0.12	6.81 ± 0.31	7.05 ± 0.25
Effluent composition			
TS (g L ⁻¹)	21.91 ± 2.34	37.97 ± 9.69	24.33 ± 6.40
VS (g L ⁻¹)	14.94 ± 1.72	18.49 ± 4.02	14.39 ± 2.76
VS/TS	68.08 ± 0.79	49.07 ± 2.82	60.18 ± 4.78
VFA C2-C5 (g COD L ⁻¹)	5.48 ± 0.74	2.62 ± 0.87	3.48 ± 0.70
Acetate (g L ⁻¹)	0.58 ± 0.18	0.18 ± 0.27	0.52 ± 0.20
Propionate (g L ⁻¹)	1.43 ± 0.07	1.03 ± 0.12	1.17 ± 0.15
iso-Butyrate (g L ⁻¹)	0.52 ± 0.03	0.10 ± 0.09	0.18 ± 0.10
Butyrate (g L ⁻¹)	0.06 ± 0.08	0.01 ± 0.03	0.01 ± 0.01
iso-Valerate (g L ⁻¹)	0.78 ± 0.10	0.33 ± 0.16	0.40 ± 0.13
Valerate (g L ⁻¹)	0.02 ± 0.03	0.00	0.00
A/P ratio	0.40 ± 0.11	0.16 ± 0.22	0.45 ± 0.19
IA (g CaCO ₃ L ⁻¹)	2.09 ± 0.17	1.64 ± 0.30	2.18 ± 0.13
IA/TA ratio	0.44 ± 0.03	0.39 ± 0.03	0.40 ± 0.02
IA/PA ratio	0.79 ± 0.11	0.63 ± 0.07	0.66 ± 0.07
pH	8.03 ± 0.11	8.13 ± 0.04	8.18 ± 0.07
Removal efficiency			
TS removal (%)	50.2 ± 7.5	39.8 ± 11.1	37.2 ± 19.0
VS removal (%)	57.3 ± 4.2	40.6 ± 10.1	38.6 ± 10.6
Biogas characteristics			
Biogas prod. rate (m ³ m ⁻³ reactor d ⁻¹)	1.07 ± 0.15	1.46 ± 0.14	0.61 ± 0.14
Biogas yield (m ³ kg VS _{fed} ⁻¹)	0.30 ± 0.03	0.28 ± 0.03	0.27 ± 0.04
Specific biogas prod. (m ³ kg VS _{removed} ⁻¹)	0.51 ± 0.20	0.71 ± 0.21	0.59 ± 0.14
Methane prod. rate (m ³ m ⁻³ reactor d ⁻¹)	0.62 ± 0.13	0.86 ± 0.12	0.38 ± 0.08
Methane yield (m ³ kg VS _{fed} ⁻¹)	0.18 ± 0.04	0.17 ± 0.03	0.16 ± 0.03
Specific methane prod. (m ³ kg VS _{removed} ⁻¹)	0.35 ± 0.11	0.43 ± 0.11	0.38 ± 0.09
Methane content (%)	62.13 ± 3.46	64.33 ± 7.50	63.81 ± 3.75

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569

570 **Table 3.** Limit values proposed to prevent process failure during the anaerobic thermophilic
571 digestion of sludge

Parameter	Limit value
Acetate concentration (g L ⁻¹)	0.6
A/P ratio	0.5
VFA C2-C5 (g COD L ⁻¹)	3.7
Intermediate alkalinity (g CaCO ₃ L ⁻¹)	1.8
IA/PA ratio	0.9
IA/TA ratio	0.5
Methane content in biogas (% CH ₄)	55

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Table 4. Microbiological analyses of influent and effluent sludge samples

Pathogens	Influent (PS+WAS)	Effluent (SRT)					
		30 d	25 d	20 d	15 d	10 d	6 d
<i>E. coli</i> (CFU mL ⁻¹)	1.0×10^6	Absence	Absence	Absence	1.0×10^1	1.0×10^1	1.1×10^2
<i>Salmonella</i> spp. (in 50 mL)	Absence	Absence	Absence	Absence	Absence	Absence	Absence

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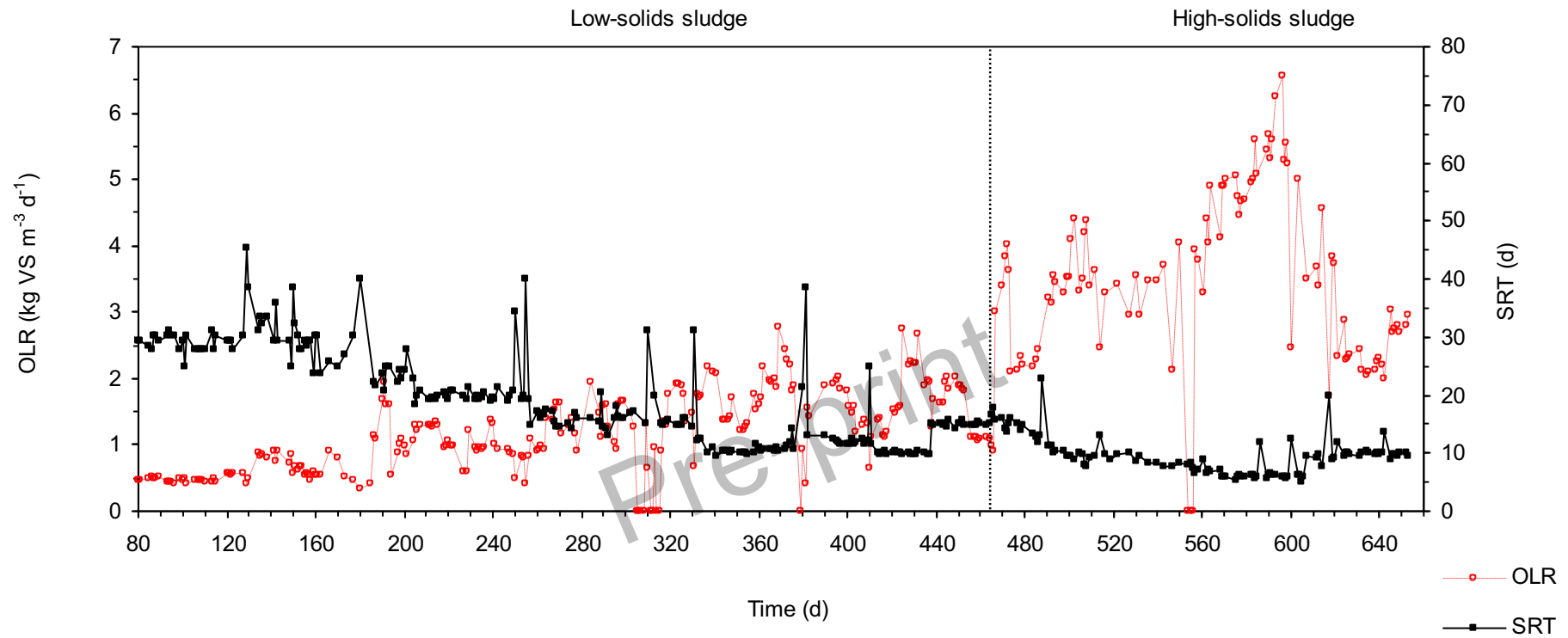


Figure 1. Sludge retention time (SRT) and organic loading rate (OLR) and during thermophilic (55 °C) sludge digestion

Note: The start-up period has not been included

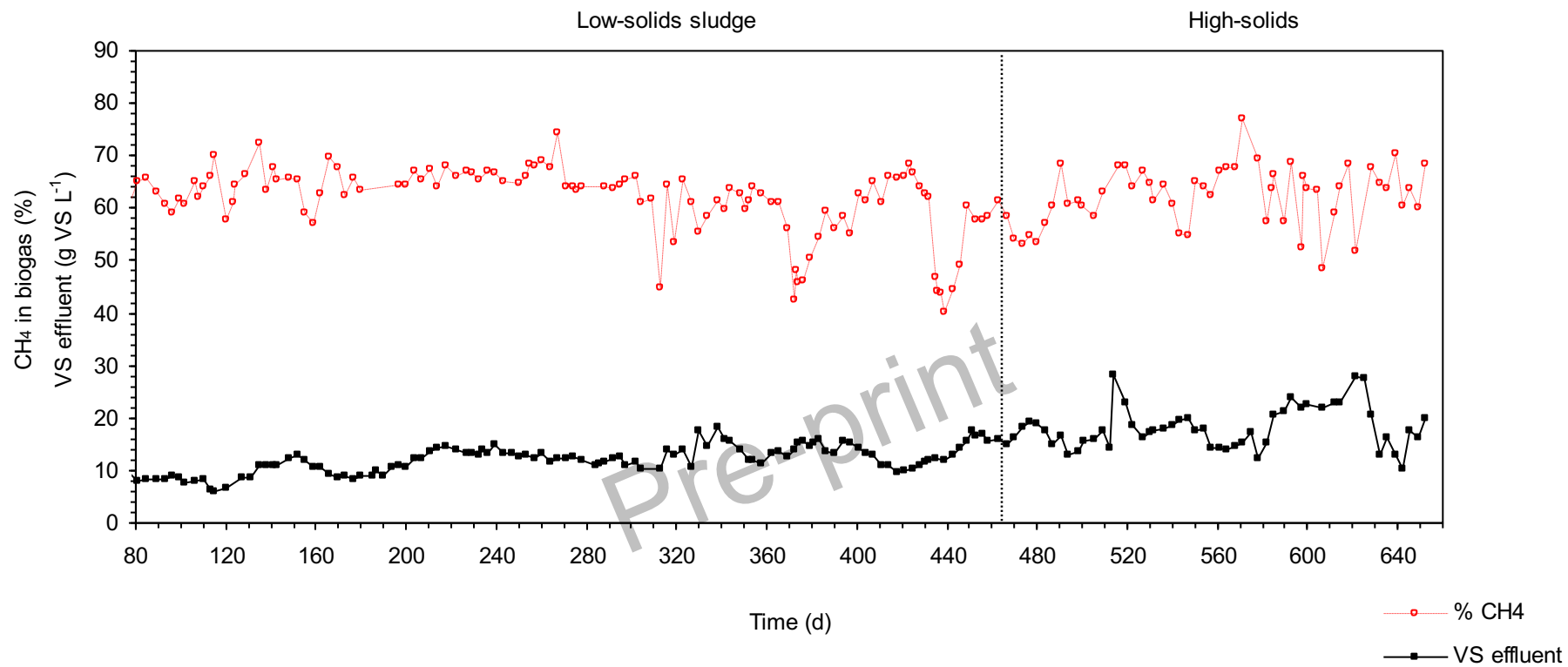


Figure 2. Methane content in biogas (% CH₄) and effluent VS during thermophilic (55 °C) sludge digestion

Note: The start-up period has not been included

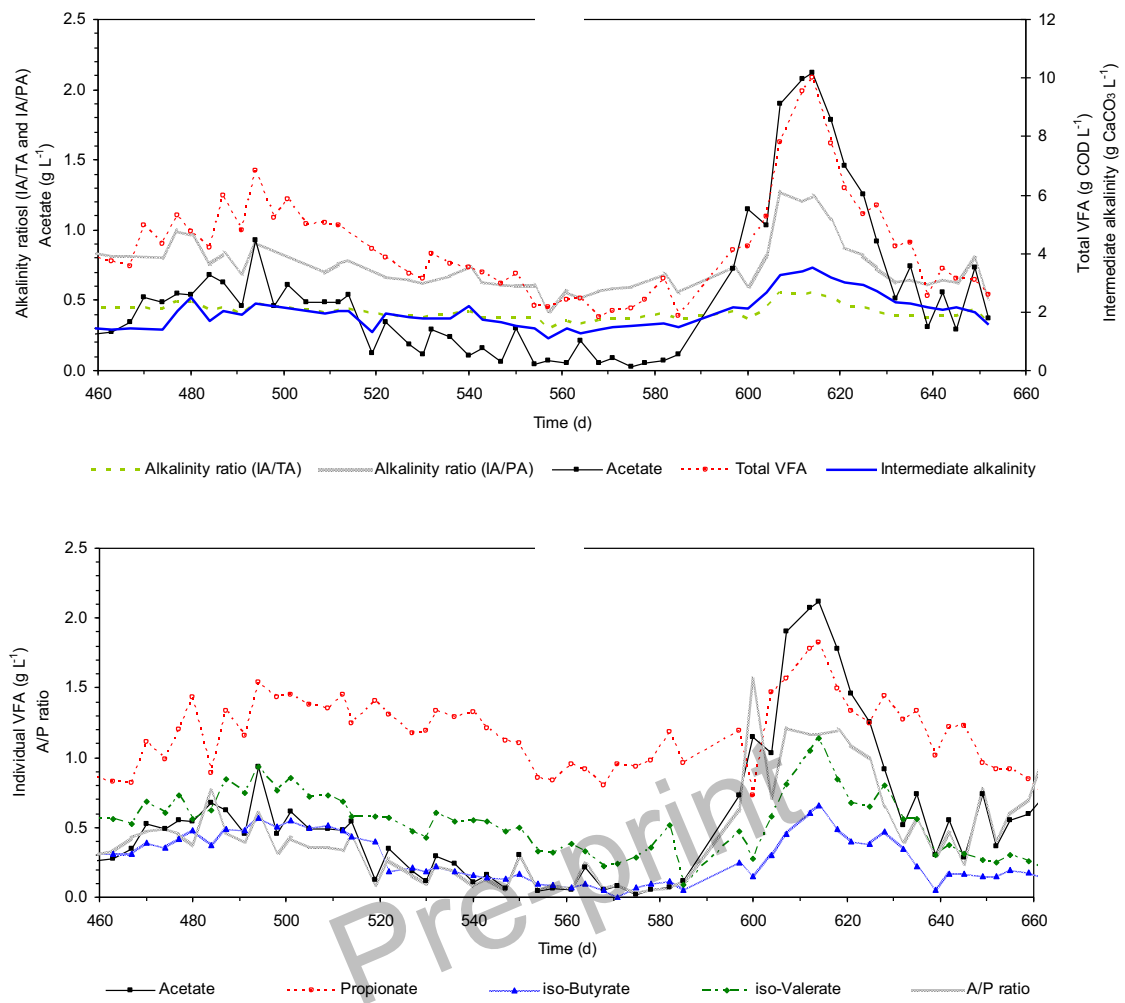


Figure 3. Volatile fatty acids (VFA) and alkalinity during thermophilic digestion of high-solids sludge: (a) Total VFA (VFA C2-C5), acetate concentration, intermediate alkalinity (IA), intermediate to total alkalinity ratio (IA/TA) and intermediate to partial alkalinity ratio (IA/PA); (b) individual VFA concentration and acetate to propionate ratio (A/P)

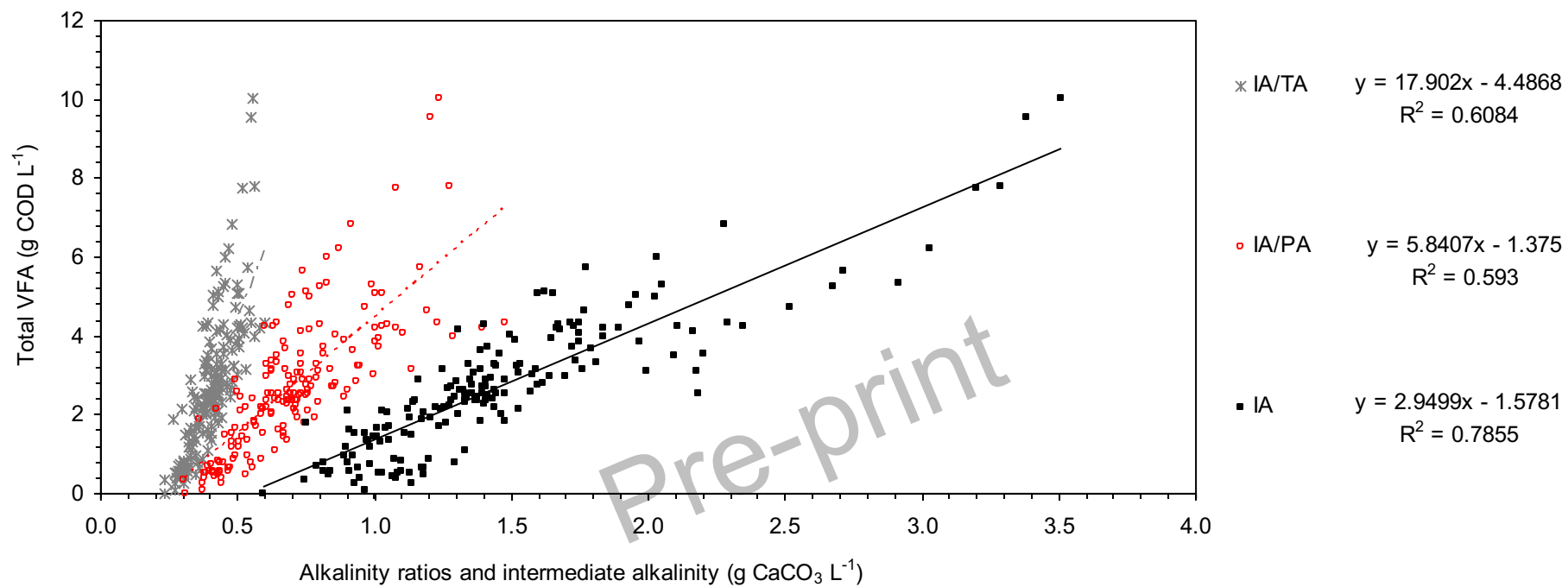


Figure 4. Correlation between total volatile fatty acids (VFA C2-C5) concentration and intermediate alkalinity (IA), IA to total alkalinity (IA/TA) and IA to partial alkalinity (IA/PA) ratios

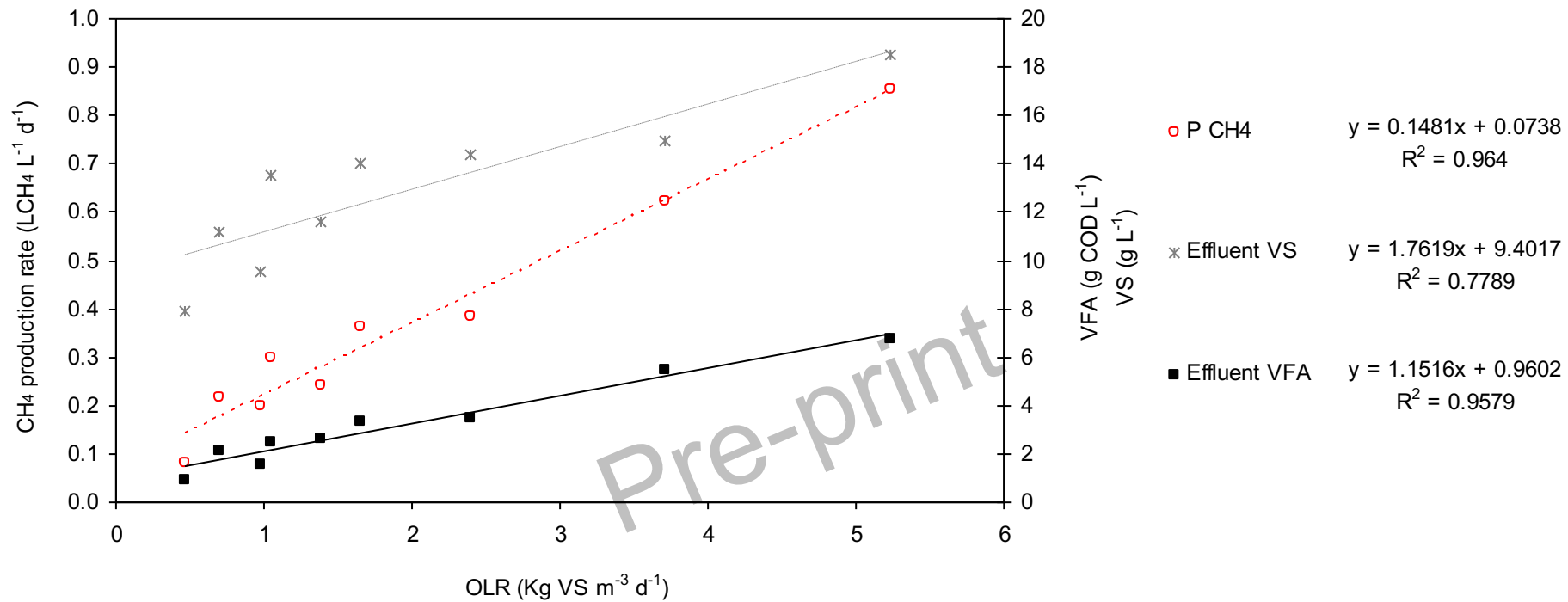


Figure 5. Methane production rate (P_{CH_4}), effluent volatile fatty acids (VFA C2-C5) and volatile solids (VS) as a function of the organic loading rate (OLR), during thermophilic sludge digestion

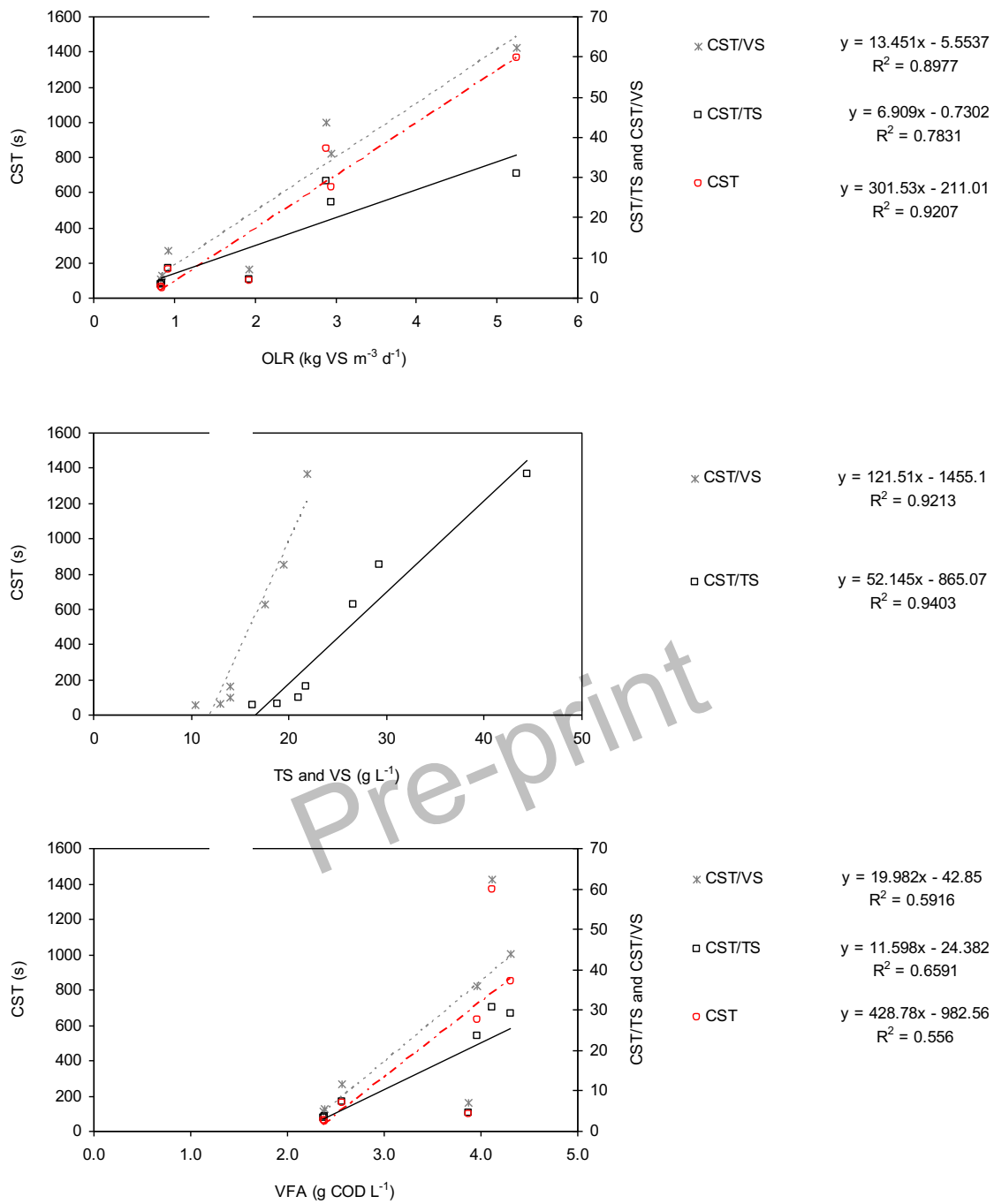


Figure 6. Capillary suction time (CST) of thermophilic digested sludge: (a) CST, CST per total solids (CST/TS) and CST per volatile solids (CST/VS) vs. organic loading rate (OLR); (b) CST vs. TS and VS; (c) CST, CST/TS and CST/VS vs. total volatile fatty acids (VFA C2-C5)

APPENDIX:

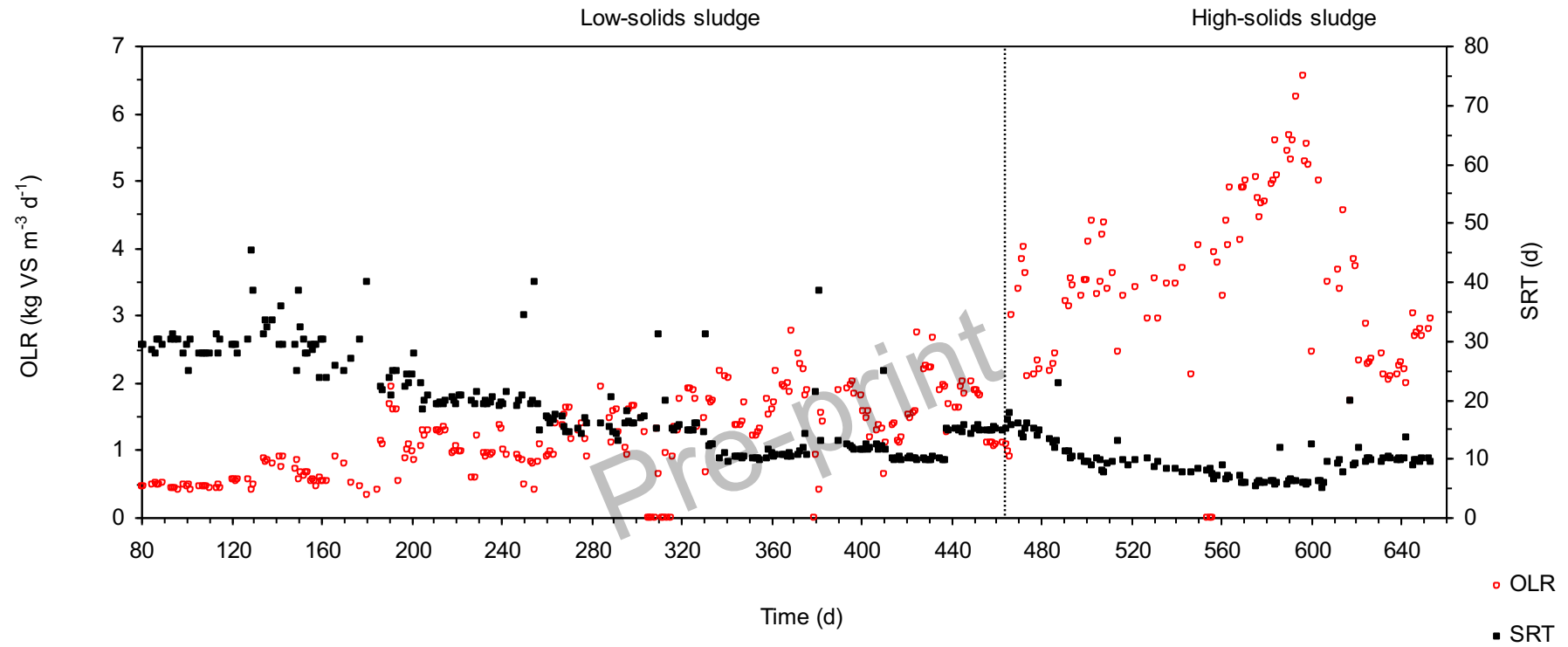


Figure 1. Sludge retention time (SRT) and organic loading rate (OLR) and during thermophilic (55 °C) sludge digestion

Note: The start-up period has not been included