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Comparison of pre- and inter-stage aerobic treatment of wastewater sludge: effects on biogas production and COD removal

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Abstract

The aim of this study was to investigate thermophilic (55 C) aerobic digestion (TAD) as pre- and inter-stage treatment of sludge anaerobic digestion and to analyse the change in organic matter accessibility and complexity. Pre-treatment decreased methane yield (up to -70%), due to oxidation losses whereas inter-stage treatment slightly improved overall methane yield (+2.6%) and total COD removal (+5%) compared to control. Anaerobic degradability and COD removal in the second anaerobic stage significantly increased, by 13 to 40%. Organic matter fractionation showed that TAD led to an increase in sludge organic matter accessibility in all cases. Organic matter complexity, measured by fluorimetry, increased after TAD pre-treatment whereas it remained constant after inter-stage treatment. TAD was shown to

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be more efficient if applied to a more recalcitrant substrate and should thus be used as inter-stage treatment to avoid decreasing methane production.

Keywords: recalcitrance, accessibility, complexity, organic matter loss, methane

1. Introduction

Anaerobic digestion is a proven technology for energy recovery and sludge stabilisation (Pèrez-Elvira et al., 2006). Most substrates—but especially lignocellulosic and bacterial cell biomass—are only partially degraded during anaerobic digestion, and various treatments to increase anaerobic conversion of recalcitrant organic matter have been developed (Carrère et al., 2010; Monlau et al., 2013). Chemical and physical treatments led to increase conversion efficiency but are often energy-intensive and expensive, and chemical treatments can harm downstream biological processes (Pèrez-Elvira et al., 2006). To avoid those drawbacks, biological treatments can be used.

Combined aerobic-anaerobic biological treatments more completely degrade sludge and other organic wastes than either does alone. Despite some comparison, exactly how organic matter utilisation differs between aerobic and anaerobic communities is not clear (Burton, 1992; Kumar et al., 2006; Dumas et al., 2010; Tomei et al., 2011; Monlau et al., 2013; Braguglia et al., 2014; Cheng et al., 2015).

Among aerobic treatments, thermophilic aerobic digestion (TAD) has been combined with anaerobic digestion to increase biogas production and organic matter destruction of municipal wastewater sludge. From literature, effect of TAD pre-treatment on COD and VS reduction are unanimous but

21 effects on biogas production are inconsistent (Jang et al., 2014; Dumas et al.,
22 2010; Hasegawa et al., 2000; Pagilla et al., 2000; Ward et al., 1998). One
23 study reported an increase in biogas production from swine manure (Pagilla
24 et al., 2000) and another from wastewater sludge (Jang et al., 2014) but in
25 the latter case it is not clear whether TAD really increased overall methane
26 production as COD mass balance and methane production were not consis-
27 tent. In other studies aerobic pre-treatment did not affect or even decreased
28 biogas production despite an increase in substrate destruction and anaero-
29 bic degradability (Ward et al., 1998; Hasegawa et al., 2000). Co-treatment,
30 where some of the digestate recirculated to the digester is treated in a TAD
31 reactor (65°C), led to similar results (Dumas et al., 2010). In general, TAD
32 as a pre-treatment for biogas production has not been popular because it
33 oxidises organic matter, leaving much less substrate available for anaerobic
34 conversion (Le, 2006).

35 Substrates of biological origin contain a mix of materials with a wide
36 range in degradability (Rittmann and McCarty, 2001), and it is the most
37 degradable of these that is oxidised to the greatest extent during aerobic
38 biological treatment. The place of the biological treatment in a production
39 chain influences the success of the process. We hypothesised that inter-stage
40 TAD can increase anaerobic conversion and reduce oxidation loss by ensuring
41 that the most degradable substrate is converted to methane prior to aerobic
42 treatment. This study compared the use of TAD as a pre- and inter-stage
43 treatment in terms of biogas production and organic matter removal dur-
44 ing anaerobic digestion of municipal wastewater sludge. Changes in organic
45 matter accessibility and complexity for both configurations were also investi-

46 gated. Furthermore, we proposed a simple framework for understanding and
47 evaluating aerobic biological treatments.

48 **2. Materials and Methods**

49 Four experiments were carried out: two with pre-treatments (P1 and P2),
50 and two with inter-stage treatments (I1 and I2) (Fig. 1). They provided
51 data to evaluate anaerobic degradability and methane production from TAD
52 effluent, to characterise the effects of the TAD treatment and to measure
53 the overall effect of the treatment chain on methane production and COD
54 removal.

55 *2.1. Substrates*

56 Original substrates were raw municipal wastewater sludges and digestates
57 from a wastewater treatment plant producing biogas (VCS, Ejby Mølle, Den-
58 mark; treating capacity 385 000 person equivalents) (Table 1). The digesters
59 at the plant are fed a mixture of primary (60%), dewatered secondary sludge
60 (40%) and highly degradable organic waste (depending on availability). Sec-
61 ondary sludge dewatered by centrifugation (including polymer addition) was
62 the substrate in P1, P2, and I2. Secondary sludge alone was used because it
63 is generally to be more recalcitrant to biogas conversion than primary sludge.
64 For I1, original substrate was the full-scale original feed in order to better
65 assess the effect of treatment under the plant conditions.

66 *2.2. Batch thermophilic aerobic digestion*

67 The TAD reactor was 3 L, aerated with compressed air, heated to 55°C
68 by a heating plate and stirred by three flat blade impellers. TAD feed was de-

69 watered secondary sludge for pre-treatment experiments and digested sludge
70 for inter-stage treatment experiments.

71 TAD inoculum was collected from a semi-continuous TAD reactor that
72 had been running for at least two weeks, and was fed secondary dewatered
73 sludge every 4 to 5 days. Inoculum was taken before feeding to ensure that
74 its COD was low. Reacting mass was about 1.5 kg to provide sufficient
75 headspace in case of foaming. Inoculum-to-substrate ratio was 1:4 based on
76 wet mass. Mixing rate was $> 1150 \text{ rev}\cdot\text{min}^{-1}$ to break up foam. Aeration rate
77 was $0.25 \text{ L}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (L air per kg reacting mass) at the start of TAD and
78 remained constant for P1 and I2. It was not adjusted after each sampling
79 for P2 and I1 and aeration rate was 0.36 and $0.4 \text{ L}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ at the end
80 of P2 and I1 respectively. Duration of P1 was 5 days with sampling every
81 24 h. The other experiments lasted 24 h with 3-4 intermediate samplings
82 (data not shown). Initial samples taken after mixing of TAD inoculum and
83 substrate but before aeration served as controls to evaluate the effect of TAD
84 treatment. For P2, I1 and I2, heat-only samples (55°C , no aeration) were
85 included to assess the heat effect. Following TAD or heat treatment, all
86 samples were subjected to anaerobic digestion.

87 *2.3. Anaerobic digestion*

88 First stage anaerobic digestion for I1 took place at the full-scale digester
89 of the wastewater treatment plant (37°C , average HRT of 28.6 days in 2015).

90 For I2, first stage anaerobic digestion was conducted at 37°C for 25.5 days
91 in (20 L) stirred reactor in batch mode. Anaerobic inoculum was digestate
92 from the same wastewater treatment plant in all cases. Inoculum-to-substrate
93 ratio was 1:1 based on wet mass (COD ratio ca. 0.5:1).

94 Post-TAD anaerobic digestion was carried in batch in the laboratory for
95 20 days at 37°C. Reacting mass (50-100 g) was put into 0.1-0.5 L glass serum
96 bottles, sealed with butyl septa and screw caps, and flushed with N₂. Target
97 substrate mass was 10 to 16 g of effluent from the TAD reactor. Inoculum-
98 to-substrate ratio was relatively high (2.5:1 based on COD) to avoid any
99 inoculum limitation. Quality of inoculum was checked according to VDI
100 (2006). Contribution of the anaerobic inoculum to the methane volume was
101 measured in inoculum-only bottles and subtracted. All conditions were run
102 in triplicate.

103 Biogas volume was measured every five days or more frequently using
104 syringes. Measurements were checked using a gravimetric approach (Hafner
105 et al., 2015). Gas samples were collected at each volume measurement in
106 10 mL vacuum vials and analysed for methane and carbon dioxide using a
107 gas chromatograph equipped with a thermal conductivity detector (Agilent
108 7890A, column: J&W 113-4332GS – GASPRO, oven temperature 250°C).

109 *2.4. Sample handling and analysis*

110 COD was measured in triplicate using Hach COD vials (Hach Company,
111 Loveland, CO, USA) based on sample mass. If necessary, samples were stored
112 at 4°C before analysis for a maximum of 2 days.

113 Evaluation of accessibility and complexity of the organic matter before
114 and after TAD treatment was done on frozen samples from P1 and I2 follow-
115 ing Jimenez et al. (2014, 2015). Bioaccessibility was quantified based on COD
116 solubilisation after extractions with successively stronger chemicals (Jimenez
117 et al., 2015). This approach is based on the assumption that bioaccessibility
118 follows chemical accessibility, as it has been shown for wastewater sludge by

119 Jimenez et al. (2014). Fractionation resulted in six fractions as defined in
120 Fig. 2. The more COD is found in the top fractions (DOM, SPOM, REOM),
121 the more the substrate is considered to be accessible (Fig. 2). Fractionation
122 was done in duplicate. Successive extractions were done on 1.5-3 g pellets
123 using around 10-30 mL of extractant (8 mL for 1 g of pellet).

124 Complexity was quantified based on 3D fluorescence spectroscopy (Perkin
125 Elmer LS55) for one replicate per extracted fraction. Excitation wavelengths
126 ranged from 200 to 600 nm with an increment of 10 nm. Based on coordinates
127 of excitation-emission wavelengths, resulting spectra were divided in seven
128 zones corresponding to biochemical family-like fluorescence. The simplest
129 molecules (e.g. amino acids) are located in the zones 1 to 3 and the more
130 complex molecules are located in the zones 4 to 7. Finally, the proportion of
131 total fluorescence in each zone was calculated by integrating the fluorescence
132 intensity and zone area (Jimenez et al., 2015).

133 Complexity characterisation is a qualitative tool as only aromatic molecules
134 can be quantified by fluorimetry.

135 To characterise the changes, complexity was related to the abundance of
136 each fraction obtained in the accessibility analysis.

137 2.5. Data treatment

138 Data processing and statistical analysis was done in R (R Core Team,
139 2017). Cumulative methane production was calculated using the biogas pack-
140 age (v. 1.6) (Hafner and Rennuit, 2015) and statistical analysis using the
141 stats package (R Core Team, 2017). Effect of treatment was evaluated using
142 a two-factor (factors were experiment and a binary factor for TAD treatment)
143 analysis of variance (ANOVA) at $\alpha = 0.05$.

144 Anaerobic degradability was calculated by dividing the methane produc-
145 tion by the theoretical methane production expected from 1 g of COD (350
146 $\text{mL}\cdot\text{g}^{-1}$) (Rittmann and McCarty, 2001). To evaluate anaerobic degradabil-
147 ity of TAD effluent, methane production was normalised by the COD of the
148 TAD effluent. To calculate anaerobic degradability of initial sludge methane
149 production at the different stages was normalised by the COD of the ini-
150 tial sludge (TAD inoculum contribution subtracted). Characterisation of the
151 TAD treatment was done by analysing changes in accessibility and complex-
152 ity, as well as by COD mass balance. COD mass balance was conducted
153 by normalising the residual COD, oxidised COD and COD converted into
154 methane by the COD of the TAD influent (containing the TAD inoculum).
155 COD conversion to methane (g COD per g substrate wet mass) during the
156 anaerobic stage was calculated by dividing cumulative methane production
157 after 20 days (normalised by substrate wet mass) by $350 \text{ mL}\cdot\text{g}^{-1}$ (mL CH_4
158 per g COD) (Rittmann and McCarty, 2001). Overall performance of the
159 treatment chain was calculated by normalising the methane production and
160 COD removal by the COD of the initial sludge and TAD inoculum contribu-
161 tion was subtracted. Normalisation of methane production were made using
162 the COD concentration of the substrate.

163 2.6. *Oxidation losses and anaerobic degradability*

164 We assumed that aerobic biological treatment consists of two processes
165 with opposing effects on methane production: 1) increase in substrate anaer-
166 obic degradability and 2) loss of substrate through oxidation by the microor-
167 ganisms carrying out the treatment, and based on the oxidation losses, we
168 quantified the minimal increase in anaerobic degradability required to in-

169 crease methane production. This implies that a successful treatment would
170 increase methane production (overall methane production) only when in-
171 creased anaerobic degradability (after treatment) is higher than the loss due
172 to substrate consumption and oxidation. Hence, the mass of COD converted
173 into methane from the treatment process must be greater than the mass of
174 COD converted into methane into the control process, as shown in Eq. 1.

$$d_2 > d_1 / (1 - l) \quad (1)$$

175 where d_2 and d_1 are the fractional conversion of COD to methane after the
176 treatment and for the control control and l is the fraction of initial substrate
177 COD lost to oxidation during treatment (in $\text{g} \cdot \text{g}^{-1}$ (g COD per g total COD)).

178 If the potential fractions anaerobically converted to methane before and
179 after the treatment (d_1 and d_2) are known, maximum loss of substrate by
180 oxidation could be found by solving Eq. (1) for l .

181 3. Results and discussion

182 3.1. Anaerobic degradability and methane production after TAD treatment

183 Contrary to TAD treatment of raw sludge (P1 and P2), anaerobic degrad-
184 ability and methane production increased with the treatment of digested
185 sludge (I1 and I2) (Fig. 4). Anaerobic degradability of TAD effluent was
186 reduced by more than half after 1 day of pre-treatment (from 0.43 to 0.19 in
187 P1 and 0.67 to 0.19 in P2).

188 It increased by 13 to 40% with inter-stage treatment (from 0.19 to to
189 0.22 after 1 d for I1, and from 0.14 to 0.20 after 1 d for I2, maximal increase
190 for I1 was after 4.6 h, where anaerobic degradability increased from 0.19 to

191 0.24). In I1, a maximum increase in methane production from stage 2 of
192 around 25% was obtained at 4.6 h in TAD and around 40% more methane
193 was obtained after 24 h in TAD in I2 (Fig. 4) Heat control reactors yielded
194 in average increases of 10% and 10-20% of second stage methane production
195 for P2 and I1 respectively while a decrease of around 9% was observed in
196 P2. A COD loss of around 5% was observed after the heat control in P2
197 (from 64.3 ± 0.34 to 61.3 ± 0.65 g·kg⁻¹). No changes in COD after the heat
198 treatment in I1 and I2 were observed (data not shown). The increase observed
199 after inter-stage treatment was close to the +50% increase by micro-aeration
200 found by Hasegawa et al. (2000) after pre-treatment of secondary sludge and
201 considering the low anaerobic degradability of the digested sludge compared
202 to the one of raw sludge (0.19 vs 0.64 in I1, and 0.14 vs 0.76 in I2) this
203 percentage of increase was quite large.

204 Treating digested sludge, as done with inter-stage treatment, reduced ox-
205 idation losses and resulted in a larger increase in anaerobic degradability of
206 remaining substrate than did pre-treatment of raw sludge. For a successful
207 pre-treatment, calculations with eq. 1 show that with the pre-treatment as
208 it was done on raw sludge it was impossible to increase methane production
209 (from the calculations anaerobic degradability d_2 should increase by 85%
210 and more than 100% (P1 and P2)). This theory was confirmed by the ex-
211 perimental results. For treatment of digested sludge, increase in anaerobic
212 degradability should be at least 18 to 33% (Eq. 1), which could be achieved
213 in I1 and I2.

214 *3.2. Characteristics of TAD influent and effluent*

215 *3.2.1. COD mass balance and accessibility*

216 For all experiments, COD removal in TAD increased monotonically with
217 treatment time (intermediates times not shown) but treatments effects on
218 the raw and digested sludge were different.

219 Treatment of digested sludge by TAD resulted in much less COD removal
220 in TAD than treatment of raw sludge (COD was reduced by $17 \pm 1\%$ and
221 $6.8 \pm 0.8\%$ after 24 h for I1 and I2 respectively vs. 31 ± 3 and $32 \pm 2\%$ for
222 P1 and P2, Fig. 3).

223 The relative increase in DOM (compared to total COD after TAD) was
224 around 10% for both treatments (Fig. 5) showing that accessibility was
225 improved after TAD.

226 Those results are in accordance with Ward et al. (1998) and Hasegawa
227 et al. (2000) who found an increase in solubilisation after TAD.

228 However, differences were observed in the the pool size of the DOM frac-
229 tion: it was not affected by pre-treatment and increased with interstage
230 ($+0.8\%$ vs $+76\%$ increase in pool size) (Fig. 5). All other fractions decreased
231 with pre-treatment (from -24 to -83% for PEOM and REOM). During inter-
232 stage treatment PEOM and NEOM fractions remained stable while REOM
233 and SEOM significantly decreased (-50 and -43%) and SPOM was reduced
234 by 18%.

235 Accessibility after inter-stage TAD was most likely improved by the mean
236 of solubilisation of hydrolysis products while the increase after pre-treatment
237 is most probably explained by the large reduction in total COD observed after
238 pre-treatment.

239 The additional DOM fraction observed after the inter-stage treatment
240 may have been derived from solubilisation of the SPOM and REOM com-
241 partment as they were reduced by inter-stage treatment (SPOM and REOM
242 together decreased by $2.4 \text{ g}\cdot\text{kg}^{-1}$ while DOM increased by $2.5 \text{ g}\cdot\text{kg}^{-1}$ (g COD
243 per kg wet mass)). The real mechanism might be a more complex transfer
244 between compartments more than a direct solubilisation from less accessi-
245 ble layers to DOM. As the effect measured in heat controls was much less
246 than half of the observed effect on solubilisation in TAD (the heat control in-
247 creased the DOM size by +24%), it can be concluded that solubilisation was
248 due to microbial activity under aeration and was enhanced by thermophilic
249 temperature.

250 Can solubilisation alone explain the changes in methane production ob-
251 served? From a COD mass balance, DOM could not be the only source of
252 CH_4 , since the CH_4 produced was greater than the sample DOM both before
253 and after TAD treatment (7.9 vs 5 and 6.9 vs $5.8 \text{ g}\cdot\text{kg}^{-1}$ g COD per kg wet,
254 for P1 and I2 after treatment). Complexity changes in other less accessible
255 compartments due to aerobic treatment could have also influenced methane
256 production (as for instance, it occurred for the REOM after inter-stage TAD
257 (Fig. 6)).

258 Moreover, not all the DOM produced was converted into CH_4 . The in-
259 crease in DOM fraction was greater than the COD converted into methane:
260 compared to the reference $2 \text{ g}\cdot\text{kg}^{-1}$ was converted to methane while $2.5 \text{ g}\cdot\text{kg}^{-1}$
261 (g COD per kg wet mass) was solubilised in the DOM during the inter-stage
262 treatment. This was also true for the heat control. Hence, in accordance
263 with (Kim et al., 2013), it cannot be assumed that there was a simple re-

264 lationship between increased COD fraction and higher methane production
265 found in this study.

266 3.2.2. Complexity

267 Total fluorescence percentage of complex zones (4 to 7) was higher af-
268 ter both treatments (except for REOM after inter-stage) and compared to
269 control the proportion of complex zones was greater after pre-treatment than
270 after inter-stage (Fig. 6). These trends were also observed for the most repre-
271 sented organic fractions (DOM, SEOM and PEOM). When relating changes
272 in complexity to the abundance of each fraction it was found that complex-
273 ity clearly increased after pre-treatment while it remained in the same range
274 after inter-stage.

275 In general the complexity of digestate was greater for all fractions as
276 compared to secondary sludge except for the PEOM fraction.

277 The most accessible organic matter fractions (DOM, SPOM, REOM)
278 constituted a smaller part of the overall COD in digested sludge as compared
279 to secondary raw sludge ($16 \pm 0.5\%$ versus $12 \pm 0.9\%$). Digestate was less
280 accessible and more complex than the raw secondary sludge, as found by
281 Aemig et al. (2016). This is also supported by the much lower anaerobic
282 degradability measured in digestate.

283 As a large COD reduction and an increase in DOM complexity was ob-
284 served after pre-treatment, it seems that most of the COD solubilised was
285 directly oxidized during pre-treatment and that hydrolysis products were
286 less oxidised during inter-stage treatment (less COD was removed and DOM
287 complexity remained similar). Kinetics of hydrolysis and uptake of soluble
288 products for oxidation may play a role.

289 During TAD, soluble products from recalcitrant substrates would be more
290 slowly oxidised than the ones from less recalcitrant substrates. Slower oxida-
291 tion would give the opportunity to use the hydrolysis products for anaerobic
292 digestion where a longer retention time would facilitate their degradation and
293 conversion into methane. Results suggest that the more recalcitrant (less ac-
294 cessible and more complex) the substrate, the less its hydrolysis products are
295 oxidised during TAD, leaving more soluble organic matter for conversion to
296 methane.

297 Hence the positive effect of TAD might be related to a more efficient
298 hydrolysis in aerobic conditions (compared to anaerobic) and a slower uptake
299 rate of hydrolysis products for oxidation in TAD. Further, it seems that TAD
300 as a pre-treatment for anaerobic digestion is effective only if applied to a
301 complex-like substrate with low anaerobic degradability. This difference in
302 recalcitrance of the substrate could explain the difference in relative effects
303 of TAD observed here and in previous studies. The few results in literature
304 showing a positive effect on methane production (Jang et al., 2014; Pagilla
305 et al., 2000) may be due to a 'sufficient' recalcitrance of the initial substrate
306 used (mix wastewater sludge and swine manure).

307 3.3. Process performances

308 3.3.1. Overall methane production and COD removal

309 Inter-stage treatment slightly increased overall methane production. An
310 increase of 1.8 and 2.6% of total methane production was found for I1 and
311 I2 (from 20.9 to 22.3 and 21.8 to 22.4 LCH₄·kg⁻¹) (Table 2). Inter-stage
312 treatment increased total COD removal by 5 to 8% compared to the control
313 (from 69.1 to 72.3 and 74.8% for I1 with 4.6 and 24 h in TAD, and from 81.6 to

314 86.3% for I2 with 24 h in TAD) (Table 2). Total COD removal was >70% in
315 both inter-stage experiments. Stage 1 accounted for >90% of total methane
316 production and 64 to 76% of total COD removal (Table 2). Relative to
317 other non-biological treatments, the increase found in this inter-stage study is
318 small. With physical or chemical inter-stage treatments, increases of overall
319 yields from 14 to 33% have been found (acidic and alkaline co-treatment
320 (referred to as post-treatment by the authors) studied by Takashima and
321 Tanaka (2014) and Li et al. (2013) or thermal inter-stage with CO₂ stripping
322 proposed by Nielsen et al. (2011)). Inter-stage treatment of swine manure
323 with TAD of 1 day SRT increased overall methane production by 25% (Pagilla
324 et al., 2000). The small extent of the increase in total methane production
325 observed with inter-stage treatment can be partly explained by the high
326 production of methane during the first stage (>90% of methane production
327 and >70% of COD removal) but also by the low anaerobic degradability of
328 the substrate entering the second stage of anaerobic digestion. The estimate
329 of methane production from the first stage for I1 was probably overestimated,
330 since highly degradable organic wastes were included as digester feed at this
331 plant, increasing first-stage methane production.

332 Anaerobic degradability of substrates in their first digestion ranged from
333 0.44 to 0.76 but it was only 0.19 and 0.14 in stage 2 for the control reactors
334 in I1 and I2. A shorter first stage digestion might have led to different results
335 because degradability of digested sludge could have been higher.

336 TAD pre-treatment decreased total methane production from 55 to 70%
337 in P1 and P2 (from 4.7 to 2.1 and 13.9 to 4.1 LCH₄·kg⁻¹ (Table 2). Interme-
338 diate samples collected in P2 showed that COD reduction in TAD increased

339 and anaerobic conversion decreased monotonically with TAD retention time
340 (data not shown). In P1, $31 \pm 3\%$ (\pm standard error) of the initial COD was
341 converted into methane in the control condition and more COD was degraded
342 during the pre-treatment ($37 \pm 3\%$) than during anaerobic digestion (Table
343 2). A similar trend was found in P2: pre-treatment resulted in total COD
344 reduction of $41 \pm 11\%$ after 24 h (Table 2). Compared to control (anaerobic
345 digestion only), total COD removal was improved by 20% in P1 (from 31 to
346 37%) but decreased by 20% in P2 (from 52 to 40%)(Table 2). Contrary to
347 inter-stage TAD, total COD removal was not systematically increased with
348 TAD pre-treatment time (intermediates times not shown). While globally
349 more COD was removed after 24 h in TAD than for the control in P1, best
350 removal was achieved for the control sample for P2, meaning that the anaer-
351 obic digestion following the TAD treatment in P2 was less efficient to remove
352 COD than was anaerobic digestion of raw sludge. This difference might be
353 linked to the composition of the sludge which was different even though it
354 came from the same waste water plant. COD from raw sludge in P2 was
355 76.3 compared to 44 $\text{g}\cdot\text{kg}^{-1}$, it contained also more DM and VS than the
356 sludge from P1 (Table 1). The TAD might have converted some of the more
357 readily accessible and simple organic matter from the high COD sludge into
358 less accessible and more complex organic matter, hindering the subsequent
359 anaerobic digestion.

360 Reported effect of non-biological pre-treatment on overall methane pro-
361 duction from sludge ranges from 11% for low temperature treatment (50°C ,
362 48 h) to 88% for acidic pre-treatment or ultra-sonication (Tyagi and Lo, 2011)
363 (increases in anaerobic degradability and in total methane production were

364 assumed to be identical as no COD losses generally occur during chemical or
365 physical treatment).

366 It ranges from none to 40% for biological pre-treatments (Carrère et al.,
367 2010; Jang et al., 2014) but the later case the real effect on overall methane
368 production is not clear as the increase in methane production needs to ac-
369 count for VS and COD losses during the pre-treatment.

370 In P1 and I1, best COD removal was achieved for the longest time in
371 TAD and did not correspond to the optimal treatment time for methane
372 production (no treatment for P1 and 4.6 h for I1). Thus it is difficult to
373 propose general treatment conditions that could apply for sludge in general
374 and treatment optimisation would need to be adapted to the sludge and in
375 some cases a compromise between COD removal and CH₄ production.

376 3.3.2. Viability of TAD pre- and inter-stage treatments

377 Improved destruction of COD by the use of TAD treatment could reduce
378 sludge production and associated disposal costs. Compared to non-biological
379 treatments, TAD has the advantage of avoiding any input or disposal of chem-
380 icals and is effective for sludge hygienisation and does not require external
381 heat at full scale (Ward et al., 1998; Layden et al., 2007). Any potential
382 increase in methane and COD destruction must be compared to the cost of
383 aeration and of a more complex system to evaluate full-scale feasibility. Ef-
384 fectiveness of TAD treatment could almost certainly be improved through se-
385 lection of optimal operating conditions (including reducing the retention time
386 of the first-stage anaerobic digestion and optimising retention time and aer-
387 ation in TAD). An advantage of inter-stage configuration over pre-treatment
388 is that treated material has a much lower dry matter concentration than with

389 pre-treatment, possibly reducing aeration costs and equipment wear and tear.
390 An inter-stage configuration for sludge thermal treatment was recommended
391 by Nielsen et al. (2011) and Ortega-Martinez et al. (2016) using batch assays
392 and full scale data.

393 However, inter-stage treatment requires the investment in an additional
394 reactor which might not pay off. In this way, pre-treatment might be more
395 profitable. If the choice of pre-treatment is made, it should be applied to
396 a sufficiently complex substrate to benefit methane production. Another
397 possibility to minimise the reactors requirements is the use of co-treatment
398 where TAD effluent is recirculated back to the initial digester as proposed by
399 Dumas et al. (2010). However, this configuration did not increase methane
400 production. In co-treatment, the anaerobic digester cannot be run in batch
401 and ensure that all the material is degraded to a sufficient extent, which
402 seems to be one of the important parameter for the success of the treatment.
403 Moreover, the use of only one anaerobic reactor might hinder the possibility
404 for the micro-organism community to adapt to the quality of the substrate
405 treated.

406 In order to increase methane production by exploiting the complementar-
407 ity of anaerobic and aerobic biodegradation, it is necessary to minimise the
408 loss of organic matter to oxidation while increasing anaerobic degradabil-
409 ity. This work was based on thermophilic aerobic digestion of wastewater
410 sludge but this approach may be effective for other substrates and biological
411 treatments. Understanding how and why hydrolysis and subsequent uptake
412 and metabolism of hydrolysis products differs between aerobic and anaero-
413 bic conditions, and degradable and recalcitrant material, will be essential for

414 optimising aerobic treatment for biogas production.

415 **4. Conclusions**

416 TAD used as inter-stage treatment successfully reduced oxidation losses
417 and did not decrease total methane production. Overall increase in methane
418 production for TAD inter-stage treatment was low (1.8 to 2.6%) but optimi-
419 sation of treatment conditions could improve it. TAD proved to be a useful
420 pre-treatment for complex substrates as it could increase anaerobic degrad-
421 ability of digested sludge (>40%). Adding a short aerobic stage to anaerobic
422 digestion can substantially increase COD removal (up to 2-fold change in
423 COD removal for treatment of digested sludge). More work is needed to
424 understand how TAD increases anaerobic degradability of poorly accessible
425 and complex substrates.

426 E-supplementary data for this work with details on derivation for eq. 1,
427 results for CH₄, COD for all times, calculated oxidation losses and accessi-
428 bility and complexity of initial substrates can be found in e-version of this
429 paper online.

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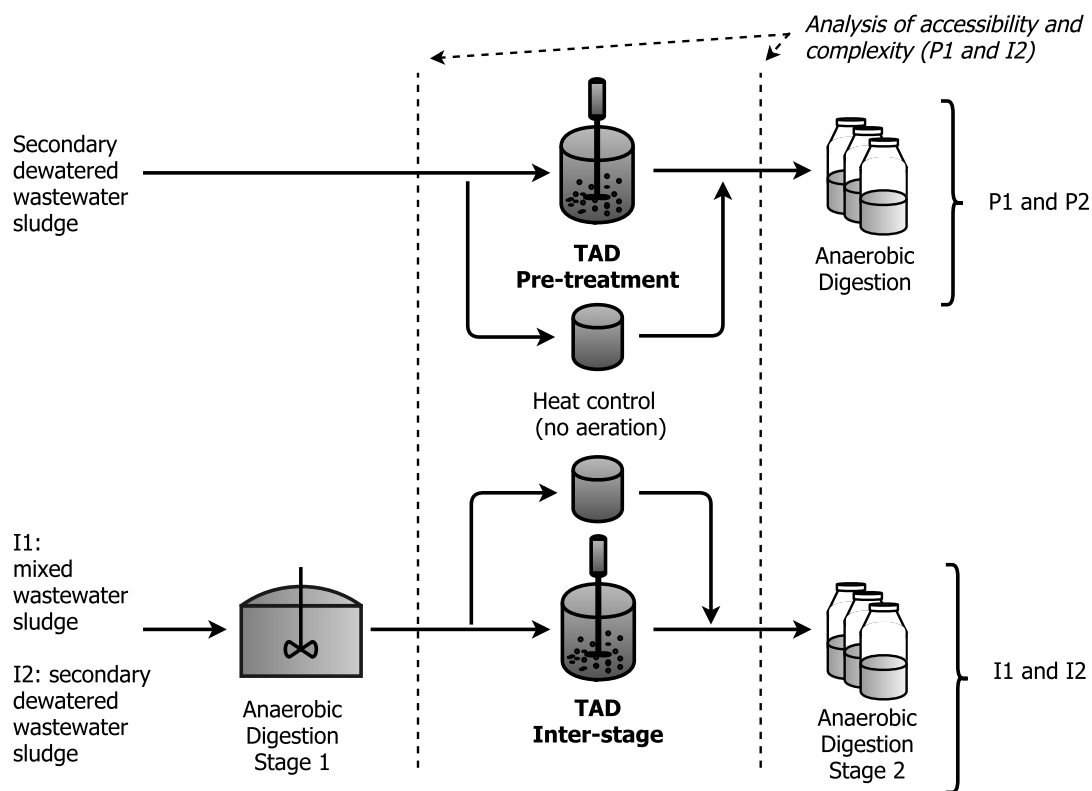


Figure 1: Treatment sequence used in P1, P2, I1 and I2. The first stage of anaerobic digestion was done either in a full scale biogas plant or in a laboratory continuously-stirred reactor. Post-TAD anaerobic digestion was done in batch reactors in the laboratory. Heat control was for P2, I2 and I2.

Table 1: Characteristics of the municipal wastewater sludge used as substrate.

Experiment	Pre-treatment		Inter-stage treatment			
	P1	P2	I1		I2	
Substrate	Secondary sludge	Secondary sludge	Mixed sludge (original) ¹	Digested mixed sludge	Secondary sludge (original)	Digested secondary sludge
COD (g·kg ⁻¹)	44.0 (0.97)	76.3 (0.91)	86.3 ²	31.2 (3.24)	76.3 (0.91)	36.5 (1.98)
DM (g·kg ⁻¹)	39.9 (0.20)	66.0 (0.02)	-	27.2 (1.11)	69.1 (4.42)	40.3 (0.01)
VS (g·kg ⁻¹)	28.6 (0.06)	48.0 (0.09)	-	15.0 (0.55)	50.3 (3.25)	23.0 (0.09)

Figures presented in parenthesis correspond to the standard deviation ($n = 3$ for COD, $n = 2$ for DM and VS).

¹ Feed to first stage anaerobic digestion as described in section 2.1.

² COD estimated from COD mass balance as the sum of the COD in digestate and the COD converted into methane during stage 1. Calculation was based on CH₄ production from full scale (19.3 L·kg⁻¹ (L CH₄ per kg wet mass)), measurement of COD in digestate and conversion of COD in stage 1 as in Rittmann and McCarty (2001) (1 g COD yields 350 mL CH₄).

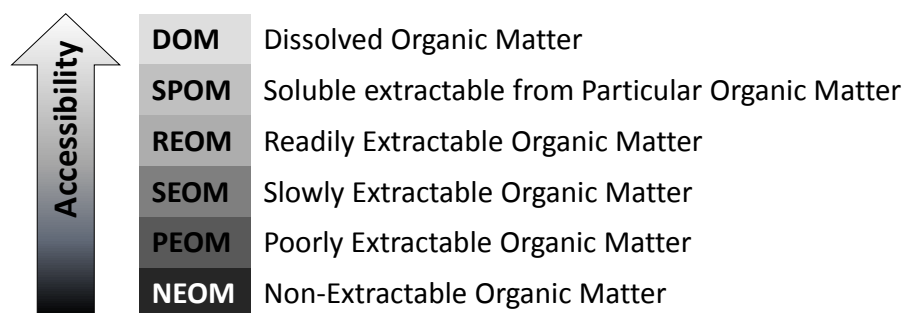


Figure 2: Definition and accessibility of the different organic matter fractions obtained by the organic matter fractionation.

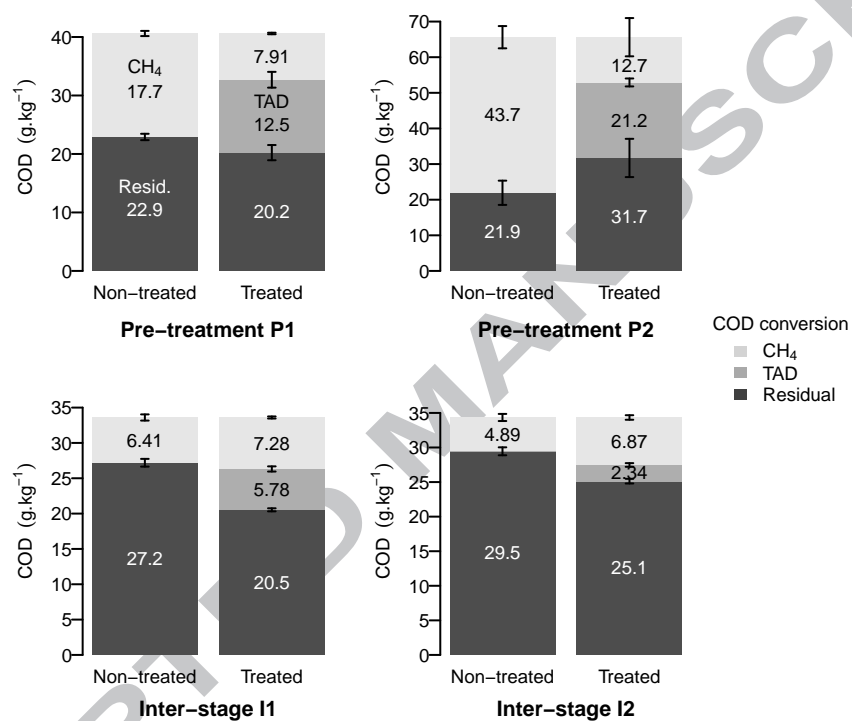


Figure 3: Conversion of wastewater sludge COD during pre- and inter-stage thermophilic aerobic digestion (TAD) and subsequent anaerobic digestion in four experiments. COD is expressed per mass of initial wet sludge. TAD treatment time was 24 h and methane production was evaluated after 20 days.

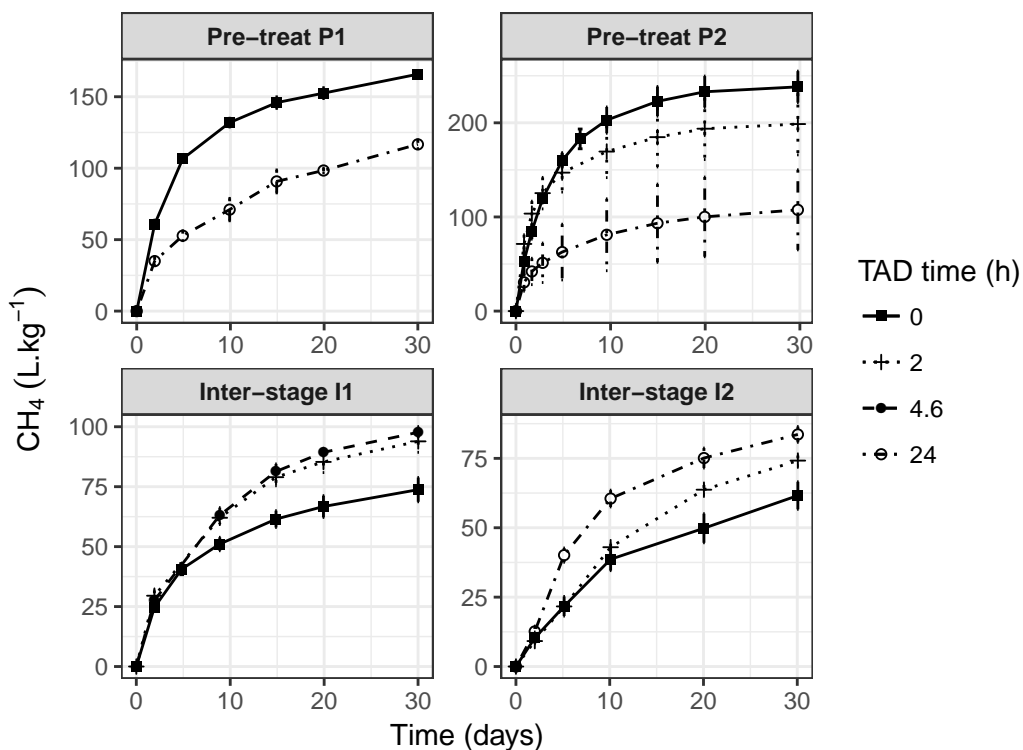


Figure 4: Cumulative methane production normalised per mass of COD in TAD effluent. Times in TAD showing the largest effect compared to the control are presented as well as an intermediate one.

Table 2: Effect of the different treatment steps on COD removal and methane production

Experiment	TAD time (h)	COD mass balance				COD removal				
		COD initial ¹ (g·kg ⁻¹)	CH ₄ stage 1 ² (L·kg ⁻¹)	COD lost TAD (g·kg ⁻¹)	CH ₄ stage 2 (L·kg ⁻¹)	Stage 1 (%)	TAD (%)	Stage2 (%)	Total (%)	
Pre-treatment	P1	0	44 (0.10)	-	-	4.7 (0.52)	-	-	30.7 (3.38)	30.7 (0.00)
	24	44 (0.10)	-	10.2 (1.58)	2.1 (0.39)	-	23.2 (3.58)	13.7 (2.50)	-	37 (4.37)
Inter-stage treatment	P2	0	76.3 (0.91)	-	-	13.9 (1.57)	-	-	52.2 (5.91)	52.2 (0.00)
	24	76.3 (0.91)	-	19.7 (1.90)	4.1 (2.97)	-	25.8 (2.51)	15.2 (11.13)	-	41 (11.41)
Inter-stage treatment	I1	0	86.3 (1.00)	19.3 (1.00)	-	1.6 (0.25)	63.9 (3.39)	-	5.2 (0.84)	69.1 (0.00)
	4.6	86.3 (1.00)	19.3 (1.00)	1.6 (0.58)	2 (0.24)	63.9 (3.39)	1.9 (0.67)	6.5 (0.80)	-	72.3 (3.57)
	24	86.3 (1.00)	19.3 (1.00)	4.3 (0.66)	1.8 (0.24)	63.9 (3.39)	5 (0.76)	5.9 (0.80)	-	74.8 (3.59)
Inter-stage treatment	I2	0	76.3 (0.91)	20.4 (1.00)	-	1.4 (0.27)	76.2 (3.85)	-	5.3 (1.02)	81.6 (0.00)
	24	76.3 (0.91)	20.4 (1.00)	2 (0.40)	2 (0.21)	76.2 (3.85)	2.6 (0.52)	7.5 (0.77)	-	86.3 (3.99)

¹ COD of original substrate (before any treatment) in g COD per kg wet mass. See Table 1 for more details on initial substrates.

² in L CH₄ per kg wet mass. Can be divided by 0.35 to get the COD value.

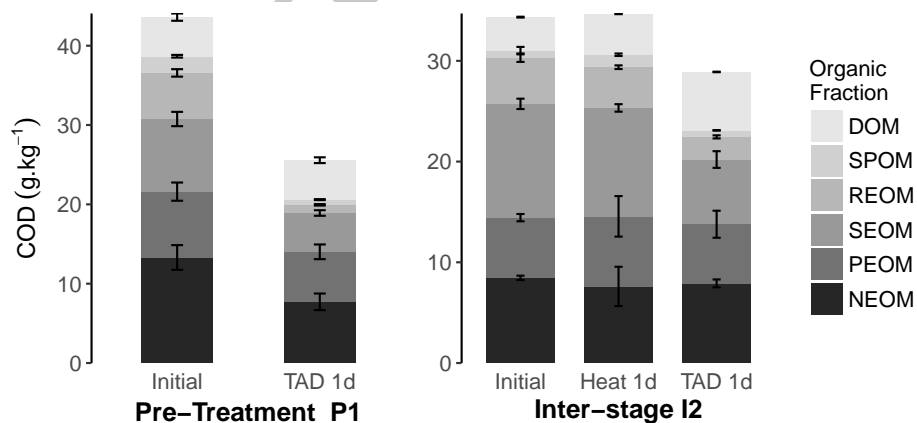


Figure 5: COD of the different organic fractions, based on the fractionation of organic described in Section 2.4 and Figure 2.

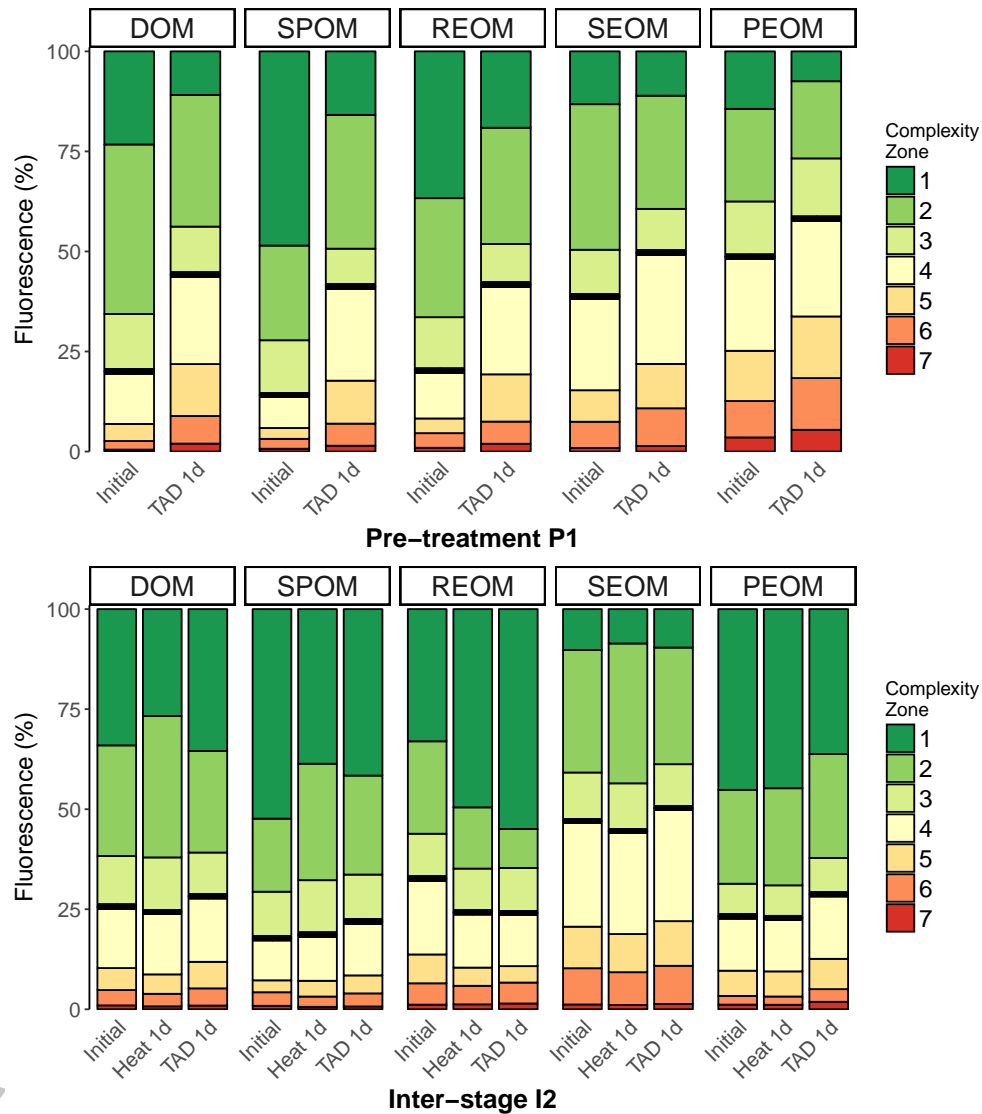


Figure 6: Complexity as a percentage of total fluorescence of each organic fraction obtained by the fractionation of organic matter (Section 2.4 and Figs. 2 and 5). Complexity increases from zone 1 to 7. The bold between zones 3 and 4 indicates the boundary between complex and simple organic matter.