

Comparative analysis between a conventional and a temperature-phased anaerobic digestion system:
Monitoring of the process, resources transformation and energy balance

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1 **Comparative analysis between a conventional and a temperature-phased**
2 **anaerobic digestion system: monitoring of the process, resources**
3 **transformation and energy balance**

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23 **Abstract**

24 This study was carried out with the principal aim of obtaining reliable outcomes for the future
25 implementation of a temperature-phased anaerobic digestion (TPAD) process in a large (2M population
26 equivalent, p.e.) WWTP. With the aid of pilot-scale (10 L) reactors fed by pure primary sludge (PS), a
27 TPAD process, where the first and the second reactor were operated at 50 °C and 38°C, respectively, was
28 compared with a conventional mesophilic (38 °C) anaerobic digestion (AD) process. The initial hydraulic
29 retention time (HRT) of the first, acidogenic, reactor of the TPAD was reduced from 3 to 2 days in the
30 second part of the test.

31 The results demonstrated that the TPAD system had been stable for all the duration of the test (approx.
32 100 days), as testified by the steady values of pH and tVFAs/TA ratio, notwithstanding the decrease in
33 the HRT. The TPAD proved to be more efficient in volatile solid (VS) reduction and methane generation,
34 compared to the conventional mesophilic AD process. In fact, the VS reduction increased from 42% to
35 approx. 55% and the specific methane potential (SMP) from 280 to 332 NL/kg VS added. An excellent
36 phase separation was observed between the two acidogenic and methanogenic reactors, as demonstrated
37 by the low SMP (only 3% of the overall production) recorded from the first reactor of the TPAD system.
38 However, the energy analysis demonstrated that the higher SMP obtained in the TPAD was not sufficient
39 to compensate the higher amounts of heat required for sludge heating and heat loss compensation. Only
40 a process of heat recovery could make the TPAD system really profitable, thus increasing the aliquot of
41 energy in the form of methane, available for users external to the WWTP, by 20%. This result represents
42 a step in the evolution of traditional WWTPs towards more energy efficient and sustainable facilities.

43

44 **Keywords:** biological hydrolysis; primary sludge; sludge pre-treatment; solids reduction; thermophilic-
45 mesophilic phase; energy analysis

46 **Highlights**

- 47 • A thermophilic – mesophilic TPAD was compared with a conventional process
- 48 • Values of pH and tVFAs/TA ratio in both TPAD stages were steady for the whole test
- 49 • An excellent phase separation between the first and the second reactor was obtained
- 50 • The TPAD proved to be more efficient in VS reduction and methane generation
- 51 • Only a process of heat recovery made the TPAD system really energy profitable

52 **1. Introduction**

53 The treatment of municipal or industrial wastewaters generates large amounts of sewage sludge, that
54 normally include primary and secondary sludge. Sewage sludge management is a major issue, because it
55 accounts by approx. 50% of the operating costs of the wastewater treatment plant (WWTP)
56 (Collivignarelli et al., 2019; Przydatek and Wota, 2020). Furthermore, in the view of effectively
57 implementing circular economy objectives, a special attention must be devoted to sewage sludge
58 management, because of the possibility of recovering energy, nutrients and valuable raw materials
59 (Kiselev et al., 2019; Shaddel et al., 2019). In fact, sewage sludge produced in medium or large WWTPs
60 are usually stabilized by means of an anaerobic digestion (AD) process, that is of great benefit because
61 it leads to the production of biomethane, a source of renewable energy, and fertilizers. Moreover, in the
62 last years a growing interest has been emerged to use sewage sludge as a feedstock in other added value
63 processes, such as the production volatile fatty acids (VFAs) (Crutchik et al., 2018; Yuan et al., 2019).
64 In sludge AD processes, hydrolysis has been recognized as the limiting phase; in this phase organic
65 particulates, soluble macromolecules, extracellular polymeric substances and soluble microbial products
66 are hydrolyzed to low molecular weight dimmers or monomers (<1 kDa) before they can be assimilated
67 for cell metabolism (Teo, 2016). A lot of efforts have been made to fasten the rate of the hydrolysis
68 process and, consequently, to enhance the overall AD by using several types of pre-treatments
69 (mechanical, chemical, thermal, biological or a combination of them). These pre-treatments have been
70 tested at a lab, pilot and, in some cases, at a full scale, as extensively reviewed by recent review papers
71 (Carrère et al., 2016; Elalami et al., 2019; Kor-Bicakci and Eskicioglu, 2019; Zhen et al., 2017). Among
72 the various pre-treatments, biological pre-treatments aim at enhancing the hydrolysis process in an
73 additional stage prior to the main digestion process. The most common type of biological pre-treatment
74 is the two-phase anaerobic digestion (2PAD), which was first developed in 1971. It takes separated the
75 acidogenic and methanogenic phase, thus permitting the selection and enrichment of different bacteria

76 in each digester by independently controlling the digester operating conditions (Qin et al., 2017).
77 Acidogenesis typically operates at a short hydraulic retention time (HRT, 1–5 days) while the
78 methanogenic phase requires longer HRTs (>7 days) (Fu et al., 2014). In 2PADs the first phase is usually
79 carried out in either thermophilic (50-55 °C) or hyper-thermophilic (between 60 °C and 70 °C) conditions
80 (Carrère et al., 2010), from that the name of temperature-phased anaerobic digestion (TPAD). The
81 thermophilic hydrolytic step is mediated by hydrolytic and fermentative bacteria, whereas the second
82 stage of digestion is driven by a mixture of acetogenic bacteria and a methanogenic archaeal population
83 (Lin and Li, 2018; Hameed et al., 2019). The TPAD technology makes use of thermophilic or hyper-
84 thermophilic systems not only to accelerate the hydrolysis process but also for pathogen control and VS
85 reduction. What is more, the majority of the digestion takes place in the mesophilic stage, with an evident
86 advantage in terms of energy balance (Grübel and Suschka, 2015).

87 In the last decade several studies have investigated the advantages offered by the application of a TPAD
88 system. Hameed et al. (2019) used two systems made of two semi-continuous reactors each to study the
89 effect of the temperature of the first digestion phase. They found that the main AD reactor, that had
90 received the pretreated sludge, generated approximately the same specific methane potential (SMP, ca.
91 $0.89 \text{ m}^3 \text{ CH}_4/\text{kg VS removed}$), irrespective of the temperature at which the pre-treatment was performed
92 (45 or 55 °C). Zamanzadeh and Parker (2018) carried out several tests in single and dual batch reactors
93 to study the kinetic of the hydrolysis process in traditional and TPAD systems where mesophilic (M) and
94 thermophilic (T) phases had been combined in all the possible ways (M/M, T/T, M/T, T/M). Martín-
95 Pascual et al. (2017), by using a pilot-scale test, compared the efficiency of a conventional mesophilic
96 (33-34 °C) AD reactor with a two-stage system, where the first reactor was kept at the ambient
97 temperature (18-22 °C). No significant differences were found concerning VS and COD reduction,
98 conversely, the specific biogas and methane productions (as L/L treated sludge) seemed to be higher in

99 the conventional reactor. The increase of the temperature in the first reactor, from the first to the last
100 cycle of test, in combination with the reduction of HRT positively affected the production of methane.
101 Other studies were aimed to find the best pre-treatment conditions to optimize both the extraction of
102 VFAs and the production of methane and, eventually, to promote the inactivation of pathogens. They
103 used batch tests to reproduce the pre-fermentation phase and BMP tests to quantify the substrate
104 biodegradability after the pre-treatment (Ding et al., 2017; Peces et al., 2016; Riau et al, 2010a). Lin and
105 Li (2018) applied the acidogenic fermentation process in a TPAD system to treat a primary sludge
106 obtained from the FeCl₃-based chemical enhanced primary sludge process to convert organic substances
107 of the sludge to VFAs.

108 Finally, some studies combined a TPAD system with an abiotic pre-treatment. Grübel and Suschka
109 (2015) placed a hybrid alkali-hydrodynamic treatment before a TPAD system to obtain a higher COD
110 solubilization and a better hygienization of the substrate in the view of a further utilization of the digestate
111 in agricultural applications. Low energy-input microwave irradiation and ultrasonication were used to
112 pretreat pure WAS or a mixed sludge before a TPAD so as to achieve higher net energy along with
113 improved digestate for agricultural applications (Akgul et al., 2017; Riau et al., 2015). Sarwar et al.
114 (2018) treated a waste activated sludge (WAS) with a high pressure thermal hydrolysis before co-
115 digesting the pre-treated WAS with a PS in a TPAD system.

116 The implementation of a TPAD scheme in the existing sludge line of a WWTP requires economic efforts
117 for the modification of the present reactors' configuration. This intervention can only be justified if it
118 can be demonstrated that the new configuration produces more energy, to be used by the WWPT itself
119 and external users, than the present. Even though the energy aspect is of capital importance for the full-
120 scale implementation of TPAD systems, very few papers have dealt with this issue with, in some cases,
121 discordant results (Fu et al., 2014; Wahidunnabi and Eskicioglu, 2014; Wu et al., 2015). It was not
122 uniquely proved that a TPAD system had been superior than a traditional system. The disagreement

123 among the results of the different studies was due, on the one hand, to different values of the data used
124 for the energy balance (i.e. reference volume of the reactors, heat transfer coefficient of the materials of
125 the walls, geometry of the digester, options of heat recovery, that, sometimes, are missing from the
126 methods' description) and, on the other hand, to the obtained values of SMP, that are affected not only
127 by the nature of the substrate (pure primary or WAS vs. mixed sludge), but also by the operating mode
128 (batch vs. semi-continuous) and scale of the reactors used for the tests.

129 The aim of this study was to obtain reliable SMP data, from a TPAD realized with a combination of pilot
130 scale (10 L) reactors fed by pure primary sludge, to be used for an energy assessment of the process, in
131 the view of its implementation in a WWTP serving approx. 2M equivalent inhabitants. A comprehensive
132 comparison of a conventional mesophilic (38 °C) AD process with a TPAD system, in which the first
133 digester was kept at 50 °C, with HRTs of 3 and 2 days, was carried out for what concerned VS reduction,
134 COD solubilization, process stability and biogas production. An energy balance completed the study,
135 with the aim of firstly verifying the self-sustainability of the process and, subsequently, quantifying the
136 amount of energy that could be exploited by users external to the WWTP. The outcomes of this study
137 can provide basic and essential information for the future implementation of a TPAD system in the sludge
138 line of a large (2M population equivalent, p.e.) WWTP.

139

140 **2. Materials and Methods**

141 **2.1 Substrate**

142 The substrate used in this study was the PS obtained from the SMAT WWTP located in Castiglione
143 Torinese (NW Italy). A detailed description of the WWTP water and sludge lines was provided in a
144 previous paper (Ruffino et al., 2014). Shortly, the WWTP has a standard configuration that includes
145 preliminary treatments (screening and sand/oil removal), primary settling, pre-denitrification, biological

146 oxidation, with a sludge retention time in the order of approx. 25 days, secondary settling and final
147 filtration on a gravel and anthracite bed.

148 The substrate was prepared weekly and stored at 4 °C until use. The sludge presented the characteristics
149 and fluctuations of a PS extracted from a real WWTP. Regular analyses were performed to determine the
150 characteristics of the feed material in terms of total solids (TS), volatile solids (VS), pH, total volatile
151 fatty acids (tVFAs), total alkalinity (TA), soluble COD (sCOD) and C, H, N content.

152 Table 1 shows the average elemental composition of the PS used in the tests carried out in this study (see
153 Section 2.2), to which corresponded the raw formula: C_{10.7}H_{18.4}O_{9.0}N. From this information the specific
154 tCOD value (as g O₂/g TS) of the PS was evaluated as in Equation 1 (van Lier et al., 2008).

155

156
$$tCOD = \frac{8(4n+a-2b-3d)}{(12n+a+16b+14d)} \text{ as } \left(\frac{g \text{ COD}}{g \text{ C}_n\text{H}_a\text{O}_b\text{N}_d} \right) \quad (1)$$

157

158 Table 1. Average elemental composition of the PS used in the study

	N (%)	C (%)	H (%)	O (%)
TS	4.568	41.819	6.048	46.994 (*)
FS	< DL	0.546	0.253	ND

159 FS, fixed solids (TS – VS); DL, detection limit; ND, not determined

160 (*) The oxygen amount was calculated as 100 minus the sum of the amounts of C, N, H.

161

162 Details of the substrate used in each of the tests are provided in Section 2.2. The analytical methods used
163 for substrate characterization are described in Section 2.3.

164 **2.2 Reactor set up and operations**

165 This study included two tests. Both tests were carried out in continuous stirred tank reactors (CSTRs)
166 with a working volume of 10 liters. The 10 L reactors were made of a stainless steel tank where the heat
167 was provided through a coil wrapped around each tank. The mixing inside the reactors was guaranteed
168 through biogas recirculation for 15 min every hour. Each reactor was equipped with gasometers and
169 systems for on-line monitoring of biogas volume and composition.

170 The first test was a traditional, semi-continuous, mesophilic (38 °C) digestion test, with an HRT of 20
171 days; it lasted approximately 3 months. Fresh substrate was fed five times per week, from Monday to
172 Friday, and digestate was extracted with the same frequency. A new sample of PS was collected,
173 characterized and used as a substrate for the AD process every week of the test. Table 2 shows the average
174 characteristics of the PS fed to the reactor, based on 11 feed collections over three months, and the organic
175 loading rate (OLR) of the system.

176 Table 2. Average characteristics of the PS fed to the one-stage mesophilic reactor (test 1) and to the first
 177 stage of the TPAD (test 2)

test	TS (%)	VS/TS (%)	pH	tVFAs/TA	OLR (kg VS/m ³ ·d)
One stage mesophilic	2.82 ± 0.50	76.6 ± 2.4	6.08 ± 0.28	2.24 ± 0.69	1.12 ± 0.19
TPAD (I stage)	2.84 ± 1.28	72.8 ± 5.9	6.07 ± 0.57	2.01 ± 2.03	see Table 3

178

179 The second test was a TPAD, two-stage test, in which the main AD process was preceded by a BH pre-
 180 treatment. The test apparatus included two CSTRs with the same characteristics of the digester used in
 181 the first test. Fresh substrate was fed to the first reactor (i.e. the acidogenic reactor, AR) five times per
 182 week, from Monday to Friday, and the pre-treated sludge was extracted with the same frequency. The
 183 AR was operated at 50 °C, while the HRT was changed during the test as shown in Table 3. For the AR
 184 it was possible to identify four running phases: from day 0 to day 12th, start-up; from day 13th to day 60th,
 185 first phase; from day 61st to 70th, transitional phase in the correspondence of HRT decrease; from day
 186 71st to the end, second phase. The OLR was quite variable and depended on the characteristics of the fed
 187 PS, especially on its thickening degree. Table 2 shows the average characteristics of the PS fed to the
 188 AR, based on 12 feed collections over three months.

189 The pre-treated sludge was used as a feedstock for the main digester. The methanogenic reactor (MR)
 190 was kept in mesophilic conditions (38 °C), with an HRT of 20 days. The MR was fed with the pre-treated
 191 sludge starting from day 13th from the beginning of the test, that is at the end of the start-up phase of the
 192 AR.

193 Table 3. Main parameters of the second test (TPAD)

Reactor	Time (d)	Phase	HRT (d)	Temperature (°C)	OLR (kg VS/m ³ ·d)
AR	0 – 12	Start up	3	50	5.78 ± 0.17
AR	13 – 60	Phase 1	3	50	6.07 ± 2.89
AR	61 – 70	Transition	2	50	15.4 ± 2.5
AR	71 - end	Phase 2	2	50	14.6 ± 1.3
MR	0 – 12	Start up	20	38	NA
MR	13 – 60	Phase 1	20	38	0.76 ± 0.19
MR	61 - end	Phase 2	20	38	1.40 ± 0.08

194 NA, not available

195

196 2.3 Analytical methods

197 TS, VS and pH were determined according to the Standard Methods (APHA, AWWA, WEF, 2012). The
 198 tVFAs/TA parameter is the ratio between the tVFAs, which stands for volatile fatty acids, expressed in
 199 equivalent milligrams of acetic acid per liter, and TA, which stands for Total Alkalinity, expressed in mg
 200 equivalent of calcium carbonate per liter. It was obtained by a potentiometric titration, according to the
 201 Nordmann method (Nordmann, 1977), by using a SI Analytics automatic titrator. Specifically, a sample
 202 of 20 mL of fermentation substrate is titrated by 0.1 N of sulfuric acid solution (H₂SO₄) up to pH 5.0 to
 203 calculate the TA value, expressed in mg/L of calcium carbonate (CaCO₃). Then the VFA value is
 204 obtained after a second titration step between pH 5.0 and pH 4.4. It is expressed in mg/L of acetic acid
 205 (CH₃COOH).

206 Soluble COD, sCOD, is the fraction of COD separated after an initial centrifugation at 15,000 rpm for
 207 10 min and a subsequent filtration of the supernatant on a 0.45 mm nylon membrane filter, as
 208 recommended by Roeleveld and van Loosdrecht (2002). The elemental composition analysis was carried

209 out on samples of PS dried at 105 °C and on the residual ashes after combustion at 600 °C. A Flash 2000
210 ThermoFisher Scientific CHNS analyzer was used for the elemental analysis.

211

212 **2.4 Calculations**

213 The capacity of the hydrolytic / fermentative process, that develops in the first reactor, in COD
214 solubilization was quantified by using two parameters that were analogous to the disintegration rate (DR)
215 used for batch tests (Ruffino et al., 2016; Campo et al., 2017) The first of these two parameters was the
216 COD solubilization (Sarwar et al., 2018), as in Eq. 2.

217

$$218 \text{ COD solubilization} = \frac{(sCOD_f - sCOD_i)}{pCOD_i} \quad (2)$$

219 Where sCOD_f and sCOD_i were the outlet and inlet concentrations of soluble COD from and to the AR
220 respectively, and pCOD_i was the inlet concentration of particulate COD (that is tCOD minus sCOD) of
221 the substrate.

222 The second parameter was the extent of solubilization (Ge et al., 2011b), as in Eq. 3.

223

$$224 \text{ Extent of solubilization} = \frac{COD_{CH_4} + sCOD_f - sCOD_i}{tCOD_i - sCOD_i} \quad (3)$$

225 where COD_{CH₄} was the methane production as mg COD from the AR; sCOD_f and sCOD_i were the outlet
226 and inlet concentrations of soluble COD respectively; and tCOD_i was the concentration of total COD at
227 the inlet of the AR.

228 **3. Results and Discussion**

229 **3.1 Effect of the TPAD on VS reduction and COD solubilization**

230 This section analyzes the effects of the biological pre-treatment, carried out through the TPAD system,
231 on VS reduction and COD solubilization of the substrate, compared with a conventional, one-stage,
232 mesophilic (38 °C) AD process. Similarly to previous studies, the VS content was used as an indicator
233 of the amount of organic matter contained into the sludge (Arnaiz et al., 2006). Figure 1 compares the
234 daily amount of VS fed to the conventional one-stage digester with the residual amount of VS daily
235 extracted with the digestate. The irregular trend of the VS fed to the reactor was smoothed by the
236 digestion process, that was able to generate a digestate with a nearly constant VS concentration. The
237 steady concentration of VS into the digestate demonstrated that the process had been correctly operated
238 and the digester was well mixed. As it can be seen from Figure 1, in the whole digestion period, lasted
239 approx. 100 days, the overall amounts of VS fed and extracted from the one-stage digester were of 534
240 g and 309 g respectively, with a consequent VS removal of 42.0%. This value was in general 10-20%
241 lower than those reported in other studies that used PS as substrate for digestion processes, carried out in
242 semi-continuous (or continuous) modality and mesophilic conditions (35-38 °C). For example, Riau et
243 al. (2010b) obtained 42% of VS reduction on a mixed sludge in a continuous digestion process, with a
244 SRT = 15 days. On a similar substrate, Martín-Pascual et al. (2017) found a value 25% higher than that
245 of this study, by carrying out analogous tests (HRT = 22 d, T = 35 °C). In a quite dated study, Ghyoot
246 and Verstraete (1997) found a VS consumption of 57% on pure PS, at an OLR of 1.36 kg VS/m³·d.
247 Finally, Ersahin (2018) measured a VS reduction of approx. 50% in a single, full-scale, anaerobic digester
248 that treated PS at a HRT of 22 days. The VS reduction is highly correlated with the methane production,
249 being the intrinsic sludge degradability and the SRT of the digestion process the two most relevant
250 parameters affecting the VS removal efficiency (Akgul et al., 2017; Athanasoulia et al., 2012).

251 One of the reasons for which an AR should be placed before a conventional, one-stage, digester would
252 be the lower residual amount of VS that remains into the digestate after the two-stage AD process. Higher
253 VS reductions are not only undoubtedly connected to a higher methane yield (see Section 3.2), but also
254 make the digestate more stable and less putrescible for agronomic uses and are beneficial for sludge
255 volume reduction after the liquid phase separation. Furthermore, if the AR operates in the thermophilic
256 range (50-55 °C), this contributes to pathogens control (Riau et al., 2010a).

257 Figure 2 compares the amount of the VS daily fed to the first digester of the TPAD system (AR) with the
258 amount of VS daily extracted with the pre-treated sludge. The trend of the inlet VS well highlighted the
259 two phases of the experimentation, in which the first-stage digester was run at HRT = 3 days for approx.
260 50 days, and, subsequently, at HRT = 2 days, for the final 30 days of the test. In the first period, that is
261 from day 12th to day 60th, a VS reduction of 14.0% was found, while in the second period, that is from
262 day 71st to the end of the test, the VS reduction decreased to the value of 11.0%. The consumption of VS
263 observed between the inlet and the outlet of the AR was a consequence of the processes that take place
264 in it. In a two-stage digestion system, the AR converts biodegradable COD to VFAs through the processes
265 of hydrolysis and fermentation (Ge et al., 2010; Ge et al., 2011a). The products of hydrolysis are typically
266 sugars, long chain fatty acids and amino acids; the subsequent process of fermentation will transform
267 some of these compounds to VFAs and CO₂ (Batstone et al., 2002). Furthermore, if the status of phase
268 separation between the two reactors is not completely achieved, some VFAs could be converted to
269 acetates and, finally, to methane (see Section 3.2). The generation of CO₂ and, potentially, of methane,
270 determines a reduction of the VS into the AR.

271 Figure 2 also compares the amount of VS daily fed to the second digester (MR) of the TPAD system
272 with the amount of VS daily extracted with the digestate. The process that takes place into the MR
273 consumed 48.5% of the VS added during the first phase of the experimentation (HRT = 3 days) and
274 57.5% of the VS added during the second phase (HRT = 2 days). Considering the VS reduction that

275 occurred in the whole system (first + second stage), it could be concluded that the overall VS reduction
276 was of 55.7% in the system with HRT = 3 days and of 62.2% in the system with HRT = 2 days.

277 The difference in VS removal between the conventional and the two-stage system (TPAD) observed in
278 this study was well evident. The reduced consumption of VS observed in the first reactor proved the
279 successful separation between the two acidogenic and methanogenic phases of the process. In some of
280 the existing literature, controversial results have been found concerning this aspect. For example, Riau
281 et al (2010b) observed a 22% VS reduction in the first reactor and an overall 85% VS reduction in a 3+15
282 days continuous TPAD system. Martín-Pascual et al. (2017) observed approximately the same VS
283 reduction, in the order of 54-56%, both in the two-stage systems and in the corresponding, one-stage,
284 control systems. It has to be underlined that in that study the AR was kept at the temperature of the
285 external environment (18-22 °C). Finally, in a very recent work, Haamed et al. (2019) found a VS
286 reduction, in the first stage of a TPAD system, 2.5 higher than that observed in the MR. The substrate of
287 the three afore-mentioned studies was, in all cases, a mixed sludge.

288 The application of Eq.1, to the results of the elemental analysis carried out on the PS used for this study
289 (see Section 2.1), returned a specific tCOD value of 1.05 g O₂/g TS or 1.65 g O₂/g VS. Consequently,
290 the ratio between soluble (sCOD) and total COD (tCOD) of the PS was in the order of 5%. Detailed data
291 of the sCOD/tCOD ratio were shown for some PS sampling dates in Figure 1. As expected, the main
292 fraction of the PS was in the particulate form, and, in line with other studies, it contributed for more than
293 90% to the tCOD (Zamanzadeh and Parker, 2018). Thus, this indicated the significance of the hydrolysis
294 step for improved biogas production.

295 The calculation of the two parameters, namely the COD solubilization and the extent of solubilization,
296 was carried out for the second phase of the experimentation (HRT = 2 days). The amount of tCOD daily
297 fed to the AR was of approx. 250 g/day for the first week and of 236 g/day for the second and the third
298 week. The COD already in the soluble form was in the order of 5%, that is 12.5 g/day for the first week

299 and approx. 12 g/day for the two subsequent weeks. The concentration of sCOD measured at the exit of
300 the reactor was reported in Figure 3, together with the inlet and outlet VSs, and was of 8,600 – 8,700
301 mg/L. Consequently, the daily load of discharged sCOD was of approx. 43 g and the COD solubilization
302 of the biological pre-treatment of 13.8%. The average daily methane production, over the period between
303 day 71st – day 90th, was in the order of 700 NmL (data not shown), that corresponds to 2 g of COD. The
304 contribution due to the methane generation allowed to calculate an extent of solubilization of 14.7%. It
305 could be seen from the values of these two parameters that only a very reduced amount (6.5%) of the
306 substances made readily degradable by the BH pre-treatment was transformed into methane already in
307 the AR. This was a demonstration that the status of phase separation between the two reactors was quite
308 successfully achieved. Ge et al (2011b) observed an extent of solubilization similar to that of this study
309 (15%) after a 2-day biological thermophilic (50 °C) pre-treatment carried out on a waste activated sludge.
310 It has to be noted that, in that study, a relevant amount (ca. 40%) of readily biodegradable organic matter
311 was transformed into methane already in the BH stage. The sCOD released in the BH stage was consumed
312 for almost 90% in the AD process carried out in the second-stage reactor, in fact the concentrations of
313 sCOD decreased from values in the order of 8,500 mg/L, at the outlet of the AR, to 600-700 mg/L, at the
314 outlet of the MR.

315

316 **3.2 Process stability and methane production**

317 The stability of the AD process was monitored through the measurement of pH, tVFAs and TA. The ratio
318 between tVFAs and TA, also known as FOS/TAC ratio in the German technical literature, is an easy-to-
319 do and reliable measure of the risk of acidification of a digester (Madsen et al., 2011; Castro et al., 2017).
320 As it can be seen from Figure 4a, the pH value of the digestate extracted from the one-stage digester had
321 been at a neutral, slightly alkali value (7.59 ± 0.24) for all the duration of the test. The ratio tVFAs/TA

322 had been at an average value of 0.10, with small variations (± 0.02). The observed tVFAs/TA value was
323 in the expected range for digestion processes of sewage sludge (Ruffino et al., 2019).

324 Figure 4b reports the time courses of the pH and tVFAs/TA ratio in the two digesters that compose the
325 TPAD system. As expected, the digestate at the outlet of the first stage was acidic, with an average pH
326 value over the whole experimentation of 6.02 (± 0.67). The pH value decreased in the first part of the
327 test from initial neutral values to values in the order of 5. From day 40th pH started rising and finally
328 stabilized on values of 6.0-6.5. An analogous trend was observed for tVFAs/TA ratio. This parameter
329 had a quite irregular trend from the beginning of the test to day 40th. From that moment it showed a more
330 regular trend, stabilizing on an average value of 2.52 (± 0.94).

331 The pH of the MR was in a range from neutral to slightly alkali, with an average value of 7.6. That was
332 an indication that the digester could receive a pre-treated, acidic substrate without showing signs of
333 inhibition. The ratio tVFAs/TA was at an average value of 0.10, the same recorded into the digestate
334 coming from the conventional one-stage digester, thus suggesting that the performance of the MR was
335 stable (Xiao et al., 2018).

336 Figure 5 shows the cumulative specific biogas production (SBP) and SMP observed from the test carried
337 out in the one-stage digester. The SBP of the PS in mesophilic conditions (38 °C) was of 511.6 ± 10.7
338 NL/kg VS added and the SMP was of 280.4 ± 6.2 NL/kg VS added, with an average methane percentage
339 into the biogas of 55.0 ± 3.1 % by volume. Figure 5 highlights a steady cumulative specific production
340 of biogas and methane from day 20th to the end of the test. Values of SBP and SMP returned by this test
341 were in the middle of a range of values found in other experimentations, thus demonstrating that the gas
342 productivity is highly dependent of the characteristics of the substrate and the operating conditions of the
343 test. For example, the batch tests carried out by Yuan and coauthors (2019) returned values of SMP in
344 the order of 180-190 NL/kg VS fed for a PS with a VS/TS ratio in the order of 75%. Conversely, Pinto
345 and coauthors (2016) observed a SMP of approx. 420 NL/kg VS fed from a PS with a VS/TS ratio of

346 68.3% digested in a semi-continuous reactor with the same total volume of that used in this study. Sarwar
347 and coauthors (2018) found an average SMP value of 237 NL/kg TCOD added (corresponding to 390
348 NL/kg VS fed for a sludge with the same characteristics of that used in this study) in BMP tests. It is
349 worthy of mentioning that the value of SMP found in this pilot-scale test for the PS was perfectly in line
350 with the production that was observed in a three-year monitoring campaign on the full scale digesters fed
351 with PS (equal to 0.280 Nm³/kg VS) located in the Castiglione Torinese WWTP (Ruffino et al., 2019).
352 A well designed TPAD system should promote the processes of hydrolysis and fermentation in the first
353 reactor and methanogenesis in the second reactor. As it can be seen from Figure 6, the SMP of the AR
354 was kept at the very low values of 10.7 ± 3.7 NL/kg VS fed and 12.8 ± 1.1 NL/kg VS fed in the first and
355 second phase of the test respectively. There are several strategies to maintain the status of phase
356 separation between the two reactors of a TPAD system. The recognized strategies to make an AC are
357 lowering the pH, dosing methanogenic inhibitors or washing out the methanogens in the first stage
358 (Kobayashi et al., 2012; Qin et al., 2017). The washing out of methanogens requires a HRT generally
359 shorter than 3 days (Metcalf et al., 2013). In this case the low SMP recorded in the first reactor proved
360 that the short HRT was able to keep under control the grow of methanogens and inhibit the methane
361 generation.

362 As it can be seen from Figure 6, the second reactor showed an apparent SMP of 388 NL/kg VS added in
363 the first phase of the test (from day 30th to day 60th) and of 372 NL/kg VS added in the second phase
364 (from day 70th to the end), represented by the upper curve. The apparent SMP was calculated by dividing
365 the cumulative production of methane by the amount of VS introduced into the second reactor (and
366 reported in Figure 2). However, the methane yield of the pre-fermented sludge had to be referred to the
367 initial organic matter content of the substrate. For that, it was necessary to take into account the losses of
368 organic substances that originated from both the processes of hydrolysis and fermentation, that take place
369 into the AR, and the analytical determinations. In fact, firstly one part of the most biodegradable organic

370 matter was converted into methane already in the AR; secondly, the method used for the determination
371 of TS and VS did not allow to preserve all the residual organic matter but the most volatile substances
372 were lost during the analytical determinations.

373 The effective methane yield, referred to the initial organic matter content of the substrate, could be
374 calculated as in Eq. 4

$$375 \quad B' = B_0 (1-\rho) \quad (4)$$

376 where B' is the overall methane yield (NLCH₄/kg VS added), B₀ is the methane yield of the sludge after
377 the pre-fermentation (NLCH₄/kg VS added), and ρ is the VS consumption from the first to the second
378 reactor (g VS final / g VS initial), as in Peces et al (2016).

379 As detailed in Section 3.1, the VS reduction observed in the first reactor was of 14.0% and 11.0% in the
380 first (HRT = 3 days) and second (HRT = 2 days) phase of the experimentation, respectively.

381 Consequently, the SMPs referred to the initial organic matter content of the substrate were of 333.7 and
382 331.0 NL/kg VS added in the phases with HRT = 3 and 2 days respectively. These SMP values were
383 very close to those observed in the study of Zamanzadeh and Parker (2018). They carried out tests in a
384 batch mode to compare several single and dual reactor systems and observed the highest methane yield
385 (approx. 0.320 NL/g VS added) for the combination of a thermophilic (HRT = 3.5 days) and a subsequent
386 mesophilic (HRT = 14 days) reactor.

387 The lower curve of Figure 6 (primary y axis) represented the trend of the cumulated SMP referred to the
388 initial amount of VS. From this curve it was not possible to distinguish the effect of the change of the
389 HRT in the first digester, in fact the SMPs over the two periods were approximately of the same extent.

390 The digestion carried out in the TPAD system allowed to produce 18.6% more methane than the
391 conventional system. In the case it was possible to recover also the methane produced in the first stage,
392 the overall methane yield would be in the order of 345 NL/kg VS added. In a similar test, carried out on
393 waste activated sludge, Qin et al. (2017) observed a SMP of 0.330 L/ g VS fed in the control reactor

394 (HRT = 30 days, T = 35 °C) and SMPs of 0.360 and 0.140 in the first (HRT = 6 days, T = 55 °C) and
 395 second (HRT = 24 days, T = 35 °C) reactors of a TPAD system, respectively. In that study the SMP
 396 increment was in the order of 50% but the methane production concentrated in the AR. In the work of
 397 Martín-Pascual et al. (2017) the specific biogas and methane productions of a mixed sludge (as L/L
 398 treated sludge) seemed to be higher in the conventional mesophilic (34 °C) control reactors than in TPAD
 399 systems where the AR was kept at the ambient temperature (18-22 °C).

400

401 3.3 Energy balance

402 The comparison between a traditional AD process carried in mesophilic conditions and a TPAD system,
 403 that included a thermophilic (50 °C) BH, was performed in terms of an energy balance. The energy
 404 balance did not include consideration of energy consumption for sludge loading/pumping/mixing. The
 405 analysis was carried out by making reference to a unit volumetric flow rate (i.e. 1 m³/h) of a PS with the
 406 same characteristics of the sludge employed in this study. The TS of the sludge was assumed to be of
 407 4%, a value that can be obtained with an efficient gravity thickening process. As in this study, the VS/TS
 408 ratio was of 0.74.

409 Table 5 resumes the main starting data and the more relevant results of the energy balance.

410 Table 5. Main starting data and the more relevant results of the energy balance

	conventional AD process	TPAD phase I	TPAD phase II
HRT (d)	20	2	20
Temperature (°C)	38	50	38
Digester, working volume (m ³)	480	48	480
Net heat from biogas combustion (kJ/h)	264,960	312,274	
Heat for sludge heating (kJ/h)	96,278	146,510	
Heat for heat loss compensation (kJ/h)	13,522	4,281	13,522

411

412 In the energy balance, the thermal energy generated from the biogas combustion was compared with the
413 thermal energy necessary to sustain the process that includes the heat for sludge heating and the heat
414 necessary to compensate the heat loss through the walls of the digesters.

415 For the calculation of the thermal energy generated from the biogas combustion, it was assumed that only
416 the biogas generated in the second reactor of the TPAD system could be collected and used for thermal
417 valorization. The lower heating value of methane was of 35,880 kJ/m³ and the boiler efficiency was fixed
418 to 0.9. The thermal energy necessary to heat the sludge was calculated by considering a specific heat
419 capacity (C, 4.18 kJ/kg/°C) and a density (ρ , 1·10³ kg/m³) of the sludge similar to those of water; the
420 ambient temperature was assumed equal to 15 °C. Finally, for the calculation of the heat loss through the
421 digester walls, the heat transfer coefficient (k) was assumed of 0.8 W/m²/°C and the surface area of the
422 AD reactor walls was calculated from the digester working volume incremented by 20%, considering a
423 radius to height ratio of 1:1 (Passos and Ferrer, 2015).

424 Figure 7 reports the main results of the energy balance. It could be seen that the both systems, that is the
425 conventional AD process and the TPAD, were completely self-sufficient from an energy point of view.
426 However, the increase in the methane generation observed in the TPAD system was not sufficient to
427 compensate the higher thermal requirements due to sludge heating (up to the temperature of 50 °C) and
428 heat loss, because of the higher temperature difference between the core of the reactor and the external
429 air. In Figure 8 the positive amount of heat that resulted from the heat balance has been accounted in
430 terms of the methane that could be transferred to the local or national gas distribution network. In the
431 case of the conventional AD system the available amount of methane was of 4.8 Nm³/h, while in the case
432 of the TPAD system it was of only 4.6 Nm³/h.

433 Because of the high temperature value of substrate into the AR, for the TPAD system, it was introduced
434 an option of thermal recovery. The extra heat of the sludge at the exit of the AR, due to the difference of
435 temperature between the first (50 °C) and the second (38 °C) reactor, was recovered with an efficiency

436 estimated at 70% to heat the sludge incoming into the AR. In this way, the heat requirement for sludge
437 heating decreased from 146.5 MJ/h to 111.3 MJ/h, with a saving of 24%. With this recovery option, the
438 TPAD offered a larger benefit in terms of the amount of methane to be transferred to the distributing
439 network, that increased from 4.6 to 5.7 Nm³/h.

440

441 **4. Conclusions**

442 This study was carried out with the principal aim of obtaining reliable outcomes to evaluate the future
443 implementation of a TPAD process in a large (2M p.e.) WWTP. With the aid of pilot scale (10 L) reactors,
444 a conventional, one-stage, mesophilic (38 °C) AD process was compared with a TPAD system, in which
445 the first digester was kept at 50 °C, with HRTs of 3 and 2 days. Primary sludge (PS) was used as a
446 substrate.

447 Based on the experimental data and assessments, the following conclusions can be drawn:

- 448 • the TPAD showed a superiority in VS reduction, in fact the overall removal of VS increased from
449 42.0%, in the one-stage reactor, to 55.7% and 62.2% for the TPAD system with a HRT of 3 and
450 2 days, respectively;
- 451 • the COD solubilization, that is the capacity of the hydrolytic / fermentative process, that takes
452 place in the AR, to release soluble substances in the form of saccharides, amino acids, and short
453 and long chain fatty acids, was of approx. 14%;
- 454 • the process developed in the two phases of the TPAD was stable for the whole period of the study,
455 as testified by the values of pH and tVFAs/TA ratio;
- 456 • the SMP observed in the AR was kept at very low values, in the order of 10-12 NL CH₄/kg VS
457 added, that is approximately 3% of the overall methane production of the TPAD; this was an
458 indication that the status of phase separation between the two acidogenic and methanogenic
459 reactors was successfully achieved;

- 460 • the higher SMP observed in the TPAD (+ 18.6%, with respect to the one-stage digester) was not
461 sufficient to balance the higher heat amounts necessary for sludge heating and heat loss
462 compensation. A process of heat recovery for the sludge between the outlet and the inlet of the
463 AR proved to be necessary to make the TPAD system really profitable;
- 464 • the TPAD system, with a section of heat recovery, produced 20% more energy, in the form of
465 methane available for users external to the WWTP, than the traditional digestion system.

466 It can be concluded that the implementation of a TPAD scheme in the sludge line of a large traditional
467 WWTP could represent a chance for its evolution towards the new concept of water resource recovery
468 facility (WRRF). In fact, the TPAD scheme could offer a substantial contribution in the production of
469 renewable energy and in the consequent reduction of the emission of greenhouse gases from fossil fuels.

470

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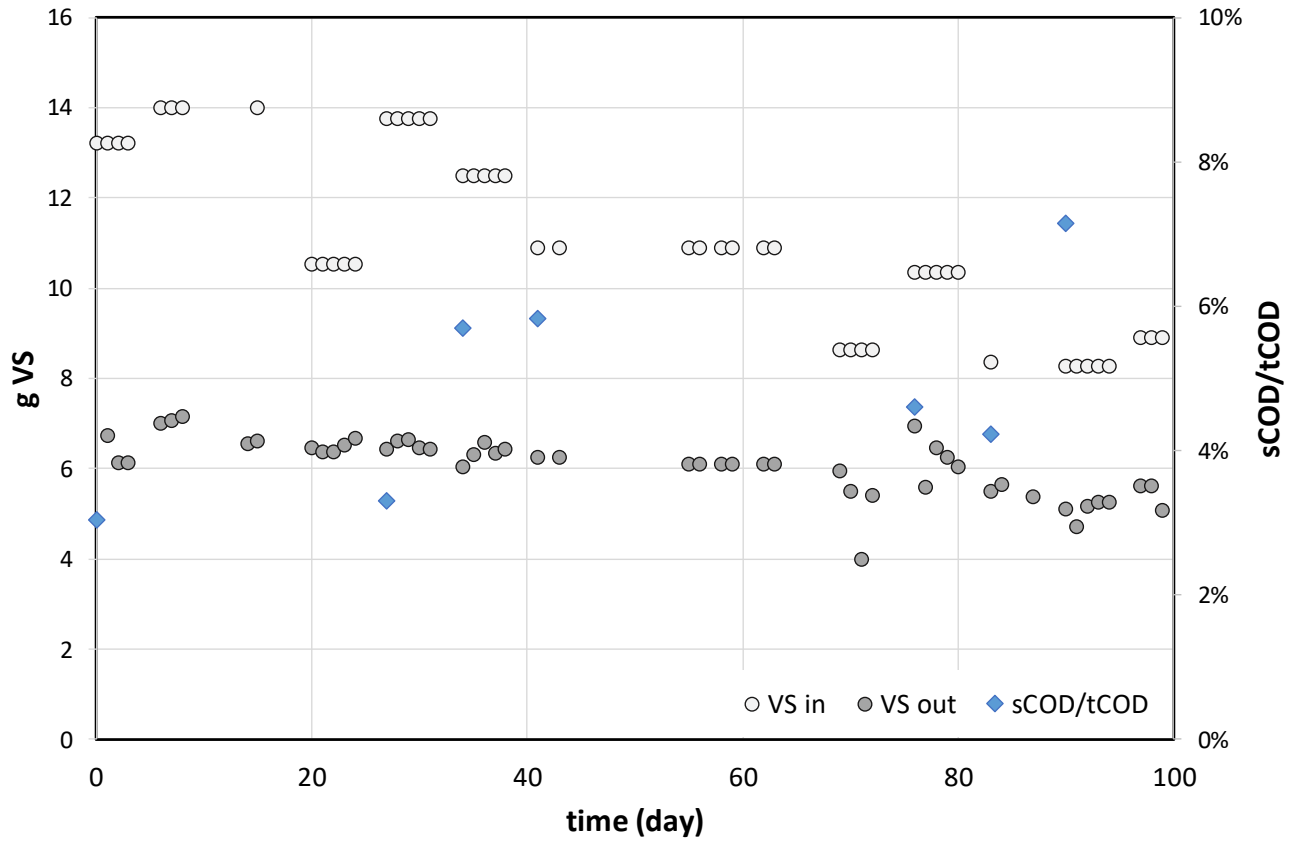
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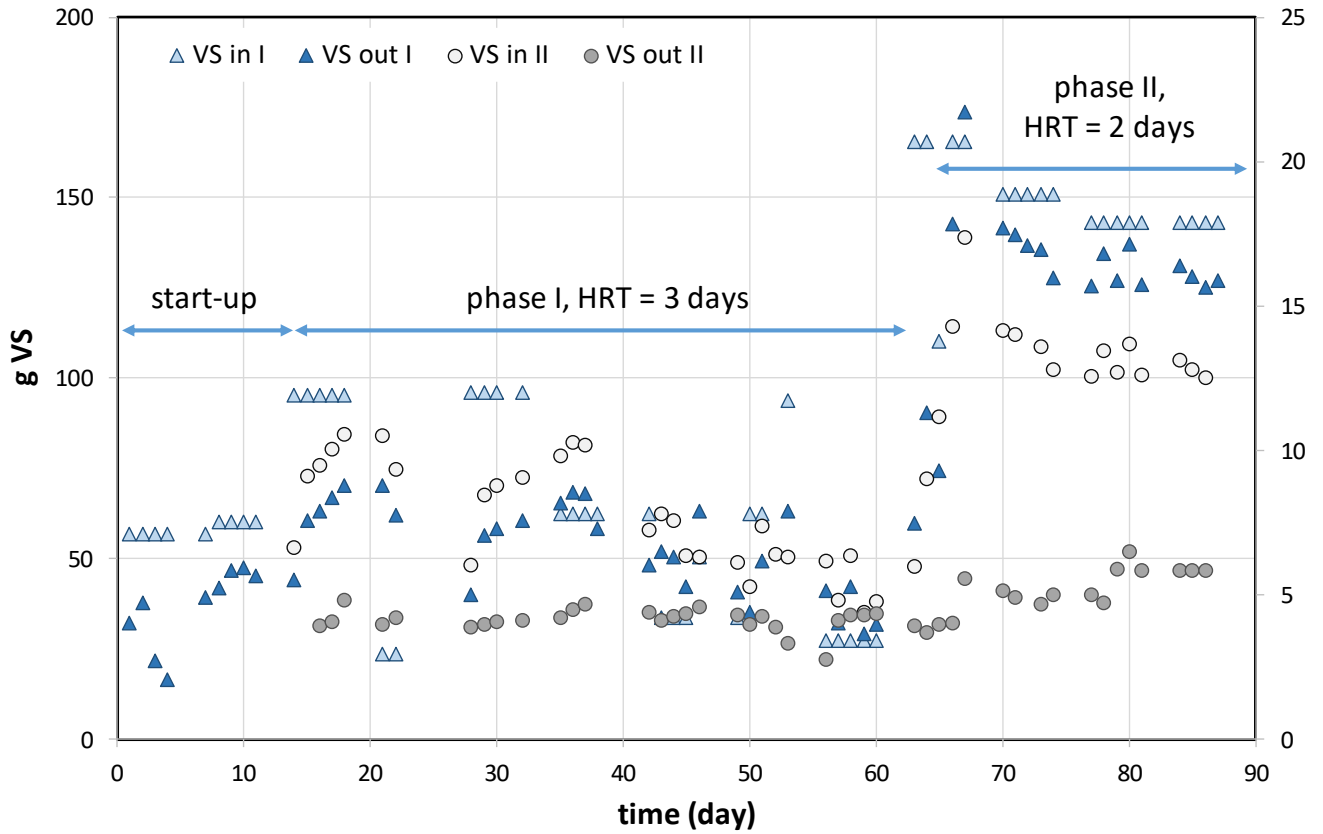
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626

627 Figure 1. Left axis: daily amount of VS fed to the conventional one-stage digester and residual amount
 628 of VS extracted with the digestate. Right axis: sCOD/tCOD ratio of the PS fed to the digester

629

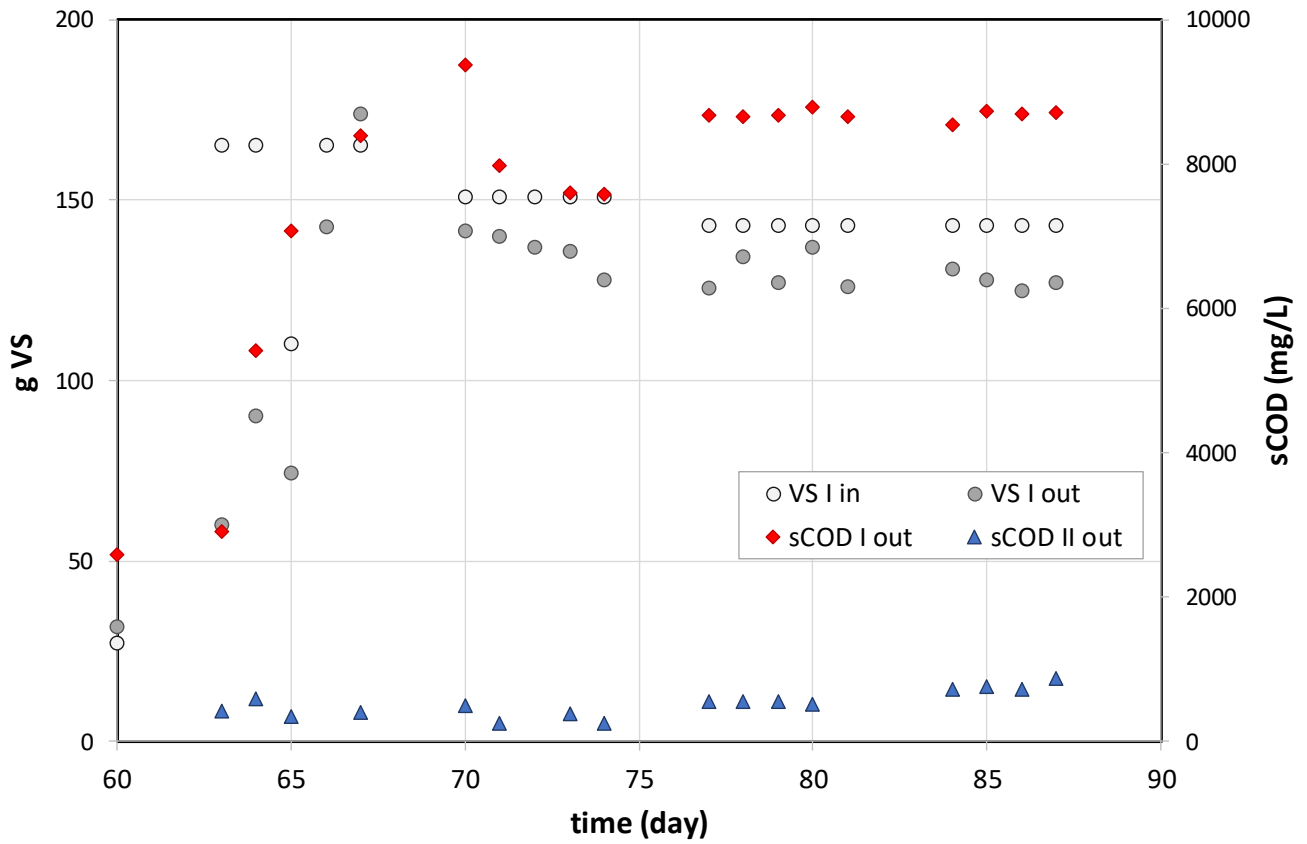


630

631 Figure 2. Daily VS amount fed and extracted from each of the two digesters (I and II) of the TPAD
 632 system (VS in I and VS out I, left y axis; VS in II and VS out II, right y axis)

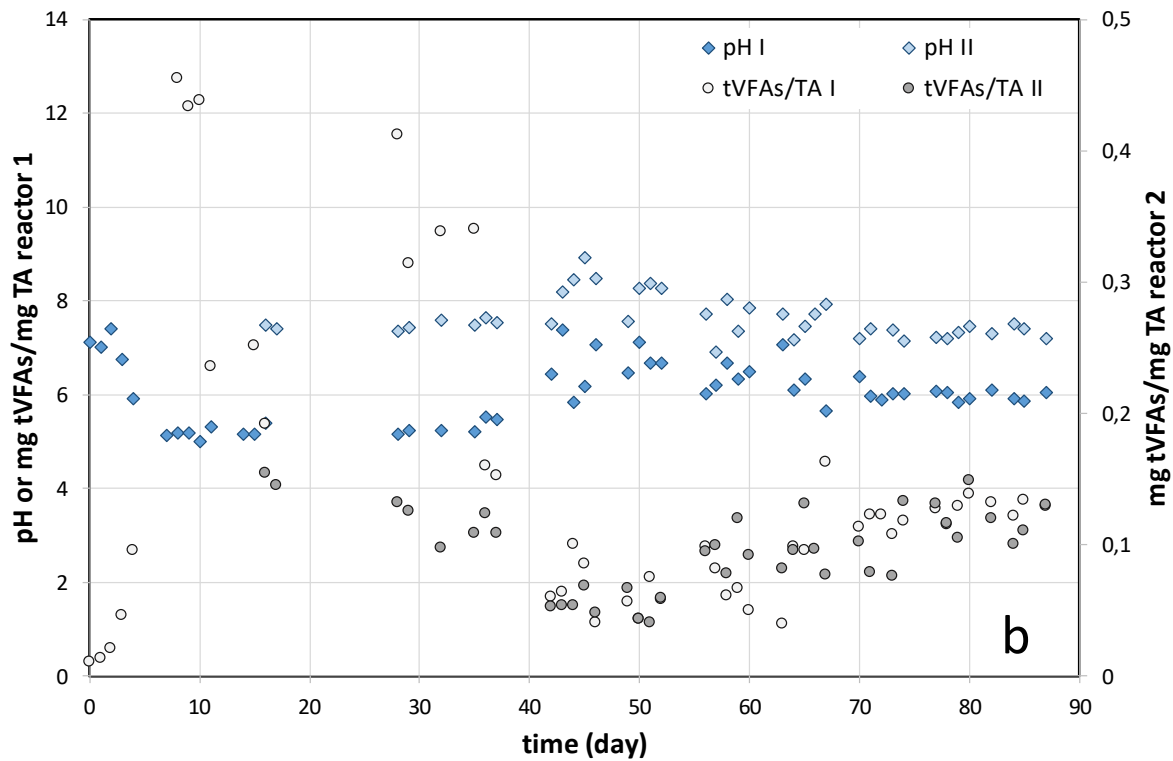
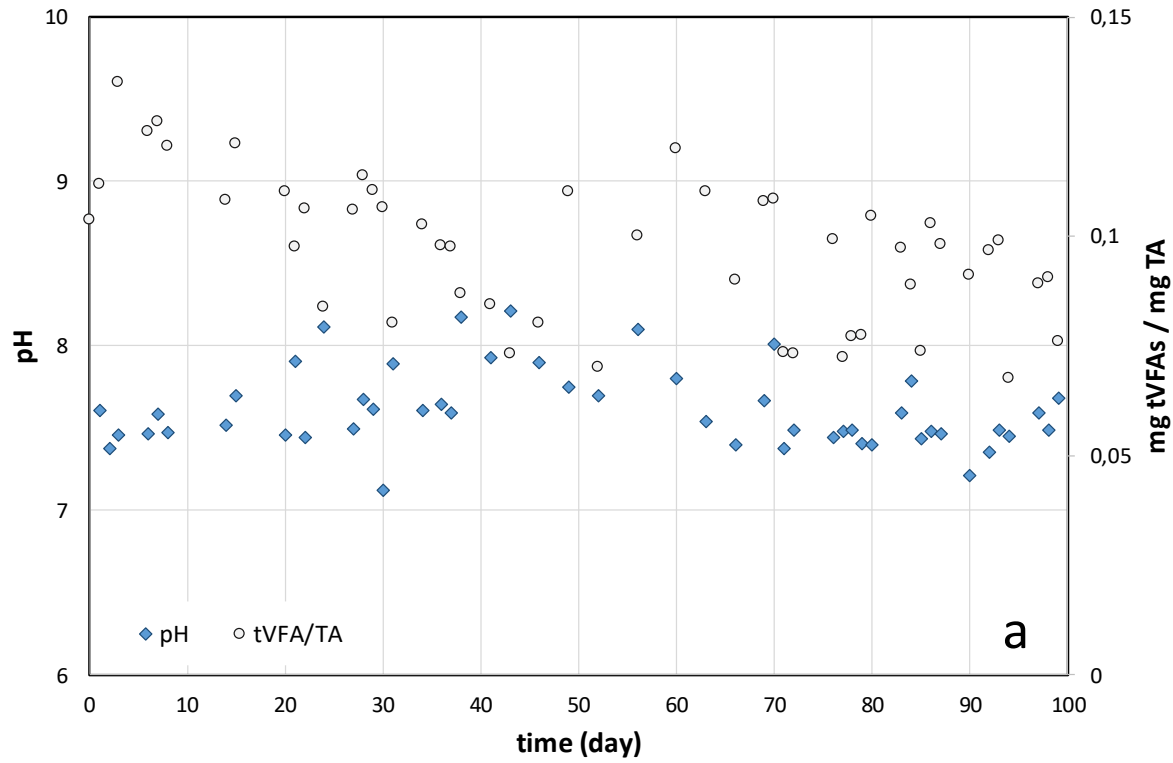
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635

636 Figure 3. Time course of inlet and outlet VS in the AR (left axis). Trend of the sCOD concentration
637 measured at the exit of the AR (I) and MR (II) (right axis)

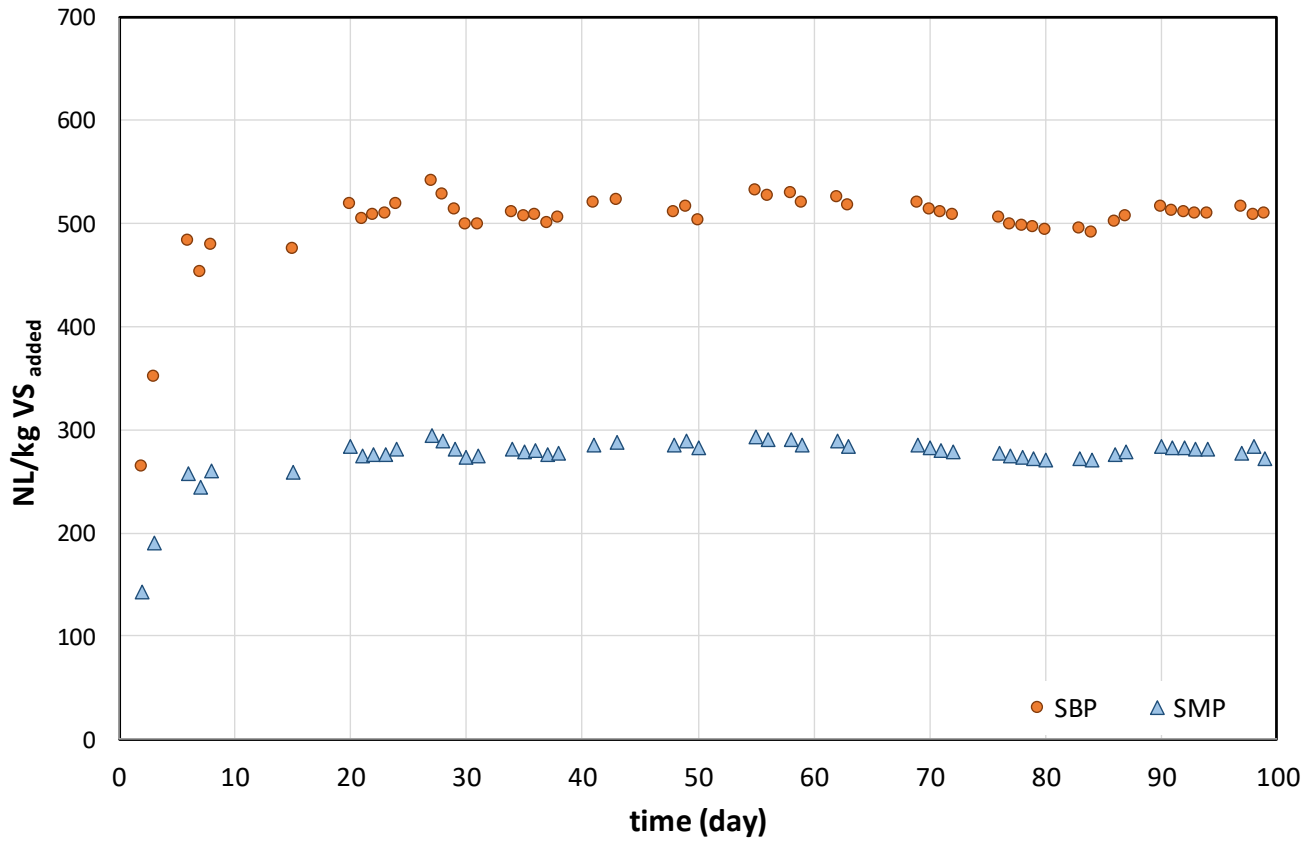


638

639 Figure 4. Time course of the pH and tVFAs/TA in the digestate extracted from the one-stage digester

640 (a) and from the TPAD system (b)

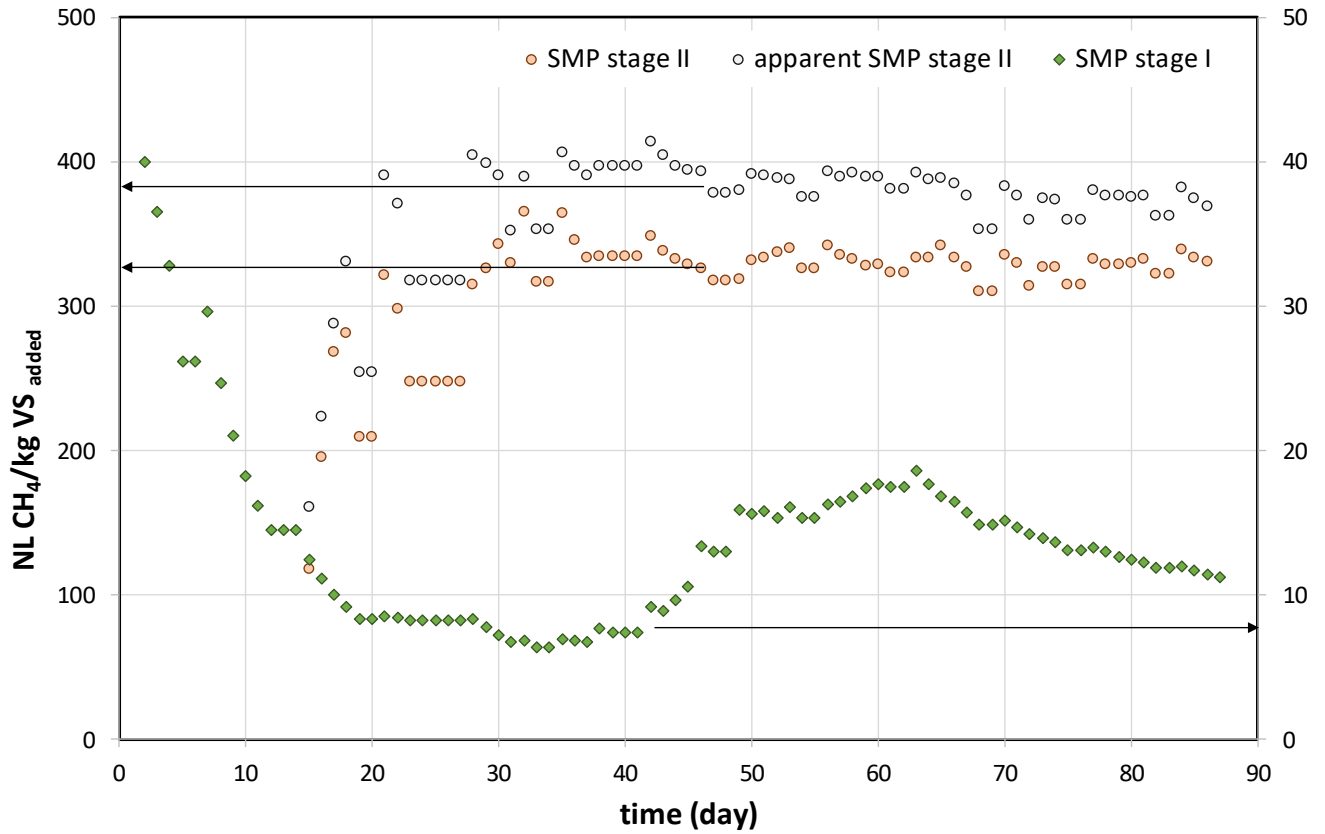
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642

643 Figure 5. Trend of SBP and SMP from the test carried out in the one-stage digester

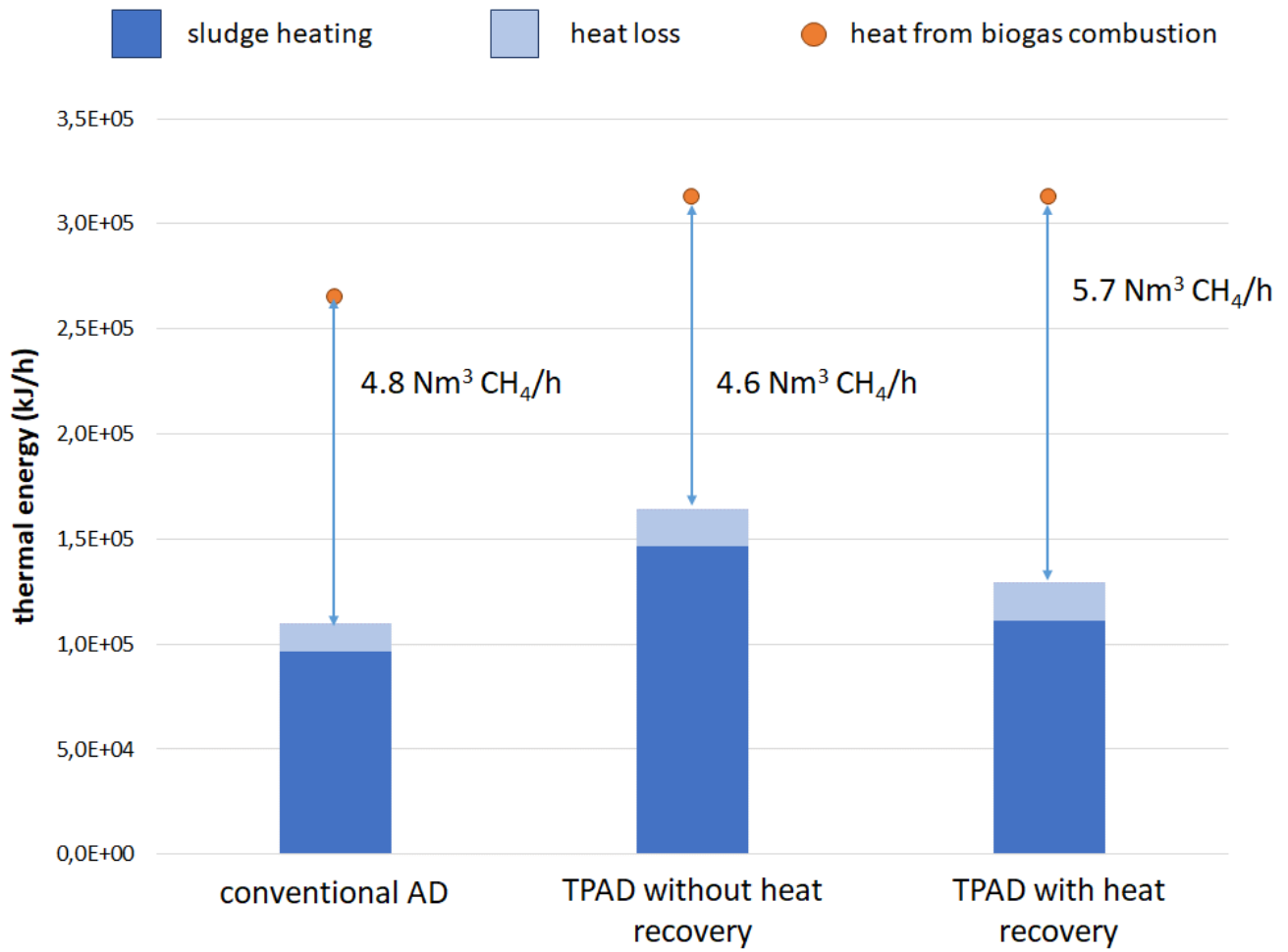
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645

646 Figure 6. Trend of cumulative SMP in the first and in the second digester of the TPAD system

647



648

649 Figure 7. Results of the energy balance