

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 FeCl<sub>3</sub>-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<sub>10.7</sub>H<sub>18.4</sub>O<sub>9.0</sub>N. From this information the specific  
 154 tCOD value (as g O<sub>2</sub>/g TS) of the PS was evaluated as in Equation 1 (van Lier et al., 2008).

155

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

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 <sup>3</sup> ·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 12<sup>th</sup>, start-up; from day 13<sup>th</sup> to day 60<sup>th</sup>,  
 185 first phase; from day 61<sup>st</sup> to 70<sup>th</sup>, transitional phase in the correspondence of HRT decrease; from day  
 186 71<sup>st</sup> 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 13<sup>th</sup> 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 <sup>3</sup> ·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 (H<sub>2</sub>SO<sub>4</sub>) up to pH 5.0 to  
 203 calculate the TA value, expressed in mg/L of calcium carbonate (CaCO<sub>3</sub>). 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 (CH<sub>3</sub>COOH).

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<sub>f</sub> and sCOD<sub>i</sub> were the outlet and inlet concentrations of soluble COD from and to the AR  
220 respectively, and pCOD<sub>i</sub> 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<sub>CH<sub>4</sub></sub> was the methane production as mg COD from the AR; sCOD<sub>f</sub> and sCOD<sub>i</sub> were the outlet  
226 and inlet concentrations of soluble COD respectively; and tCOD<sub>i</sub> 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/m<sup>3</sup>·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 12<sup>th</sup> to day 60<sup>th</sup>, a VS reduction of 14.0% was found, while in the second period, that is from  
262 day 71<sup>st</sup> 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 CO<sub>2</sub> (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 CO<sub>2</sub> 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 O<sub>2</sub>/g TS or 1.65 g O<sub>2</sub>/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 71<sup>st</sup> – day 90<sup>th</sup>, 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 ( $7.59 \pm 0.24$ ) for all the duration of the test. The ratio tVFAs/TA

322 had been at an average value of 0.10, with small variations ( $\pm 0.02$ ). 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 ( $\pm 0.67$ ). 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 40<sup>th</sup> 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 40<sup>th</sup>. From that moment it showed a more  
330 regular trend, stabilizing on an average value of 2.52 ( $\pm 0.94$ ).

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  $511.6 \pm 10.7$   
338 NL/kg VS added and the SMP was of  $280.4 \pm 6.2$  NL/kg VS added, with an average methane percentage  
339 into the biogas of  $55.0 \pm 3.1$  % by volume. Figure 5 highlights a steady cumulative specific production  
340 of biogas and methane from day 20<sup>th</sup> 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 Nm<sup>3</sup>/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  $10.7 \pm 3.7$  NL/kg VS fed and  $12.8 \pm 1.1$  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 30<sup>th</sup> to day 60<sup>th</sup>) and of 372 NL/kg VS added in the second phase  
364 (from day 70<sup>th</sup> 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 (NLCH<sub>4</sub>/kg VS added), B<sub>0</sub> is the methane yield of the sludge after  
377 the pre-fermentation (NLCH<sub>4</sub>/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 m<sup>3</sup>/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 <sup>3</sup> )	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/m<sup>3</sup> 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 ( $\rho$ , 1·10<sup>3</sup> kg/m<sup>3</sup>) 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/m<sup>2</sup>/°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 Nm<sup>3</sup>/h, while in the case  
432 of the TPAD system it was of only 4.6 Nm<sup>3</sup>/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 Nm<sup>3</sup>/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 CH<sub>4</sub>/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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476 **References**

- 477 Akgul D., Cella M.A., Eskicioglu C., 2017. Influences of low-energy input microwave and ultrasonic  
478 pretreatments on single-stage and temperature-phased anaerobic digestion (TPAD) of municipal  
479 wastewater sludge. *Energy* 123, 271-282. <http://dx.doi.org/10.1016/j.energy.2017.01.152>
- 480 APHA, AWWA, WEF, 2012. *Standard Methods for Examination of Water and Wastewater*, twenty-  
481 second ed. American Public Health Association, Washington. 1360 pp. ISBN 978-087553-013-0
- 482 Arnaiz C., Gutierrez J. C., Lebrato J., 2006. Biomass stabilization in the anaerobic digestion of  
483 wastewater sludges. *Bioresource Technol.*, 97(10), 1179–1184.
- 484 Athanasoulia E., Melidis P., Aivasidis A., 2012. Optimization of biogas production from waste activated  
485 sludge through serial digestion. *Renewable Energy*, 47, 147-151. doi:10.1016/j.renene.2012.04.038
- 486 Batstone D.J., Keller J., Angelidaki I., Kalyuzhnyi S.V., Pavlostathis S.G., Rozzi A., Sanders W.T.,  
487 Siegrist H., Vavilin V.A., 2002. The IWA anaerobic digestion model no 1 (ADM1), *Water Science &*  
488 *Technology*, 45, 65–73.
- 489 Campo G., Cerutti A., Zanetti M.C., Scibilia G., Lorenzi E., Ruffino B., 2017. Pre- and intermediate  
490 hybrid treatments for the improvement of anaerobic digestion of sewage sludge: Preliminary results.  
491 *Journal of Environmental Engineering*, 143(9), 04017052. DOI: 10.1061/(ASCE)EE.1943-  
492 7870.0001249
- 493 Carrère H., Dumas C., Battimelli A., Batstone D.J., Delgenès J.P., Steyer J.P., Ferrer I., 2010.  
494 Pretreatment methods to improve sludge anaerobic degradability: a review. *J. Hazard. Mater.* 183, 1–15.
- 495 Carrère H., Antonopoulou G., Affes R., Passos F., Battimelli A., Lyberatos G., Ferrer I., 2016. Review  
496 of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-  
497 scale application. *Bioresource Technol.* 199, 386-397
- 498 Castro L., H. Escalante H., J. Jaimes-Estévez J., L.J. Díaz L.J., K. Vecino K., G. Rojas G., L. Mantilla  
499 L., 2017. Low cost digester monitoring under realistic conditions: Rural use of biogas and digestate  
500 quality. *Bioresource Technology* 239, 311–317. <http://dx.doi.org/10.1016/j.biortech.2017.05.035>
- 501 Collivignarelli M.C., Abbà A., Carnevale Miino M., Torretta V., 2019. What Advanced Treatments Can  
502 Be Used to Minimize the Production of Sewage Sludge in WWTPs? *Appl. Sci.* 9, 2650;  
503 doi:10.3390/app9132650
- 504 Crutchik D., Frison N., Eusebi A.L., Fatone F., 2018. Biorefinery of cellulosic primary sludge towards  
505 targeted Short Chain Fatty Acids, phosphorus and methane recovery. *Water Research* 136, 112-119.  
506 <https://doi.org/10.1016/j.watres.2018.02.047>
- 507 Ding H.H., Chang S., Liu Y., 2017. Biological hydrolysis pretreatment on secondary sludge:  
508 Enhancement of anaerobic digestion and mechanism study. *Bioresource Technology* 244, 989–995.  
509 <http://dx.doi.org/10.1016/j.biortech.2017.08.064>
- 510 Elalami D., Carrere H., Monlau F., Abdelouahdi K., Oukarroum A., Barakat A., 2019. Pretreatment and  
511 co-digestion of wastewater sludge for biogas production: Recent research advances and trends.  
512 *Renewable and Sustainable Energy Reviews* 114, 109287. <https://doi.org/10.1016/j.rser.2019.109287>

513 Ersahin M.E., 2018. Modeling the dynamic performance of full-scale anaerobic primary sludge digester  
514 using Anaerobic Digestion Model No. 1 (ADM1). *Bioprocess and Biosystems Engineering*, 41, 1539–  
515 1545. <https://doi.org/10.1007/s00449-018-1981-5>

516 Fu B., Wang Y., Liu H., Chen Y., Jiang Q., Liu H., 2014. Balancing energy production and pathogen  
517 removal during temperature phased anaerobic digestion of sewage sludge. *Fresenius Environmental*  
518 *Bulletin*, 23 (7a), 1643 – 1649.

519 Ge H., Jensen P.D., Batstone D.J., 2010. Pre-treatment mechanisms during thermophilic–mesophilic  
520 temperature phased anaerobic digestion of primary sludge. *Water Res* 44, 123-130.  
521 doi:10.1016/j.watres.2009.09.005

522 Ge H., Jensen P.D., Batstone D.J., 2011a. Increased temperature in the thermophilic stage in temperature  
523 phased anaerobic digestion (TPAD) improves degradability of waste activated sludge. *J. Hazard. Mater.*  
524 187, 355–361. doi:10.1016/j.jhazmat.2011.01.032

525 Ge H., Jensen P.D., Batstone D.J., 2011b. Temperature phased anaerobic digestion increases apparent  
526 hydrolysis rate for waste activated sludge. *Water Res* 45, 1597-1606. doi:10.1016/j.watres.2010.11.042

527 Ghyoot W., Verstraete W., 1997. Anaerobic digestion of primary sludge from chemical pre-precipitation  
528 *Water Science and Technology*, 36 (6–7), 357-365. [https://doi.org/10.1016/S0273-1223\(97\)00543-X](https://doi.org/10.1016/S0273-1223(97)00543-X)

529 Grübel K., Suschka J., 2015. Hybrid alkali-hydrodynamic disintegration of waste-activated sludge before  
530 two-stage anaerobic digestion process. *Environ Sci Pollut Res*, 22, 7258–7270. DOI 10.1007/s11356-  
531 014-3705-y

532 Hameed S.A., Riffat R., Li B., Naz I., Badshah M., Ahmeda S., Ali N., 2019. Microbial population  
533 dynamics in temperature-phased anaerobic digestion of municipal wastewater sludge. *J Chem Technol*  
534 *Biotechnol* 94, 1816–1831. DOI 10.1002/jctb.5955

535 Kiselev A., Magaril E., Magaril R., Panepinto D., Ravina M., Zanetti M.C., 2019. Towards Circular  
536 Economy: Evaluation of Sewage Sludge Biogas Solutions. *Resources*, 8(2), 91.  
537 <https://doi.org/10.3390/resources8020091>

538 Kobayashi T., Xu K.Q., Li Y.Y., Inamori Y., 2012. Effect of sludge recirculation on characteristics of  
539 hydrogen production in a two-stage hydrogen–methane fermentation process treating food wastes. *Int. J.*  
540 *Hydrogen Energy* 37 (7), 5602–5611.

541 Kor-Bicakci G., Eskicioglu C., 2019. Recent developments on thermal municipal sludge pretreatment  
542 technologies for enhanced anaerobic digestion. *Renewable and Sustainable Energy Reviews*, 110, 423–  
543 443. <https://doi.org/10.1016/j.rser.2019.05.002>

544 Lin L., Li X.Y., 2018. Acidogenic fermentation of iron-enhanced primary sedimentation sludge under  
545 different pH conditions for production of volatile fatty acids. *Chemosphere* 194, 692-700.  
546 <https://doi.org/10.1016/j.chemosphere.2017.12.024>

547 Madsen, M., Holm-Nielsen, J.B., Esbensen, K.H., 2011. Monitoring of anaerobic digestion processes: a  
548 review perspective. *Renew. Sustain. Energy. Rev.* 15, 3141–3155.

549 Martín-Pascual J., Rueda-Pérez J.J., Jóver M., Hontoria E., Poyatos J.M., 2017. Optimization of the Acid  
550 Stage of a Two-Stage Anaerobic Digestion Process to Treat Urban Wastewater Sludge. *J. Environ. Eng.*,  
551 143(9): 04017038

552 Metcalf E., Tchobanoglous G., Stensel H.D., Tsuchihashi R., Burton F., 2013. *Wastewater Engineering:  
553 Treatment and Resource Recovery*. McGraw-Hill, New York, NY, USA.

554 Nordmann W. Die Überwachung der Schlammfäulung. KA-Informationen für das Betriebspersonal,  
555 Beilage zur Korrespondenz Abwasser, 3/77; 1977 (in German)

556 Passos F., Ferrer I., 2015. Influence of hydrothermal pretreatment on microalgal biomass anaerobic  
557 digestion and bioenergy production. *Water Res.*, 68, 364–373.  
558 <https://doi.org/10.1016/j.watres.2014.10.015>

559 Peces M., Astals S., Clarke W.P., Jensen P.D., 2016. Semi-aerobic fermentation as a novel pre-treatment  
560 to obtain VFA and increase methane yield from primary sludge. *Bioresource Technology* 200, 631–638.  
561 <http://dx.doi.org/10.1016/j.biortech.2015.10.085>

562 Pinto N., Carvalho A., Pacheco J., Duarte E., 2016. Study of different ratios of primary and waste  
563 activated sludges to enhance the methane yield. *Water and Environment Journal* 30, 203–210.  
564 [doi:10.1111/wej.12188](https://doi.org/10.1111/wej.12188)

565 Przydatek G., Wota A.K., 2020. Analysis of the comprehensive management of sewage sludge in Poland.  
566 *Journal of Material Cycles and Waste Management*, 22, 80–88. <https://doi.org/10.1007/s10163-019-00937-y>  
567

568 Qin Y, Higashimori A., Wu L.J., Hojo T., Kubota K., Li Y.Y., 2017. Phase separation and microbial  
569 distribution in the hyperthermophilic-mesophilic-type temperature-phased anaerobic digestion (TPAD)  
570 of waste activated sludge (WAS). *Bioresource Technology*, 245, 401–410.  
571 <http://dx.doi.org/10.1016/j.biortech.2017.08.124>

572 Riau V., de la Rubia M.A., Pérez M., 2010a. Temperature-phased anaerobic digestion (TPAD) to obtain  
573 Class A biosolids. A discontinuous study. *Bioresource Technology* 101, 65–70.  
574 [doi:10.1016/j.biortech.2009.07.072](https://doi.org/10.1016/j.biortech.2009.07.072)

575 Riau V., de la Rubia M.A., Pérez M., 2010b. Temperature-phased anaerobic digestion (TPAD) to obtain  
576 class A biosolids: A semi-continuous study. *Bioresource Technology* 101, 2706–2712.  
577 [doi:10.1016/j.biortech.2009.11.101](https://doi.org/10.1016/j.biortech.2009.11.101)

578 Riau V., de la Rubia M.A., Pérez M., 2015. Upgrading the temperature-phased anaerobic digestion of  
579 waste activated sludge by ultrasonic pretreatment. *Chemical Engineering Journal*, 259, 672–681.  
580 <http://dx.doi.org/10.1016/j.cej.2014.08.032>

581 Roeleveld P.J., van Loosdrecht M.C., 2002. Experience with guidelines for wastewater characterisation  
582 in The Netherlands. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* 45 (6), 77-87.

583 Ruffino B., Campo G., Zanetti M.C., Genon G., 2014. Improvement of activated sludge anaerobic  
584 digestion: thermal and economical perspectives. *WIT Trans. Ecol. Environ.* 190, 979–991. DOI:  
585 10.2495/EQ140922

586 Ruffino B., Campo G., Cerutti A., Zanetti M.C., Lorenzi E., Scibilia G., Genon G., 2016. Preliminary  
587 Technical and Economic Analysis of Alkali and Low Temperature Thermo-alkali Pretreatments for the  
588 Anaerobic Digestion of Waste Activated Sludge. *Waste Biomass Valor.* 7, 667-675. DOI  
589 10.1007/s12649-016-9537-x

590 Ruffino B., Cerutti A., Campo G., Scibilia G., Lorenzi E., Zanetti M.C., 2019. Improvement of energy  
591 recovery from the digestion of waste activated sludge (WAS) through intermediate treatments: The effect  
592 of the hydraulic retention time (HRT) of the first-stage digestion. *Appl Energ* 240, 191-204. DOI:  
593 10.1016/j.apenergy.2019.02.061

594 Sarwar R., Elbeshbishy E., Parker W.J., 2018. Codigestion of High Pressure Thermal Hydrolysis-Treated  
595 Thickened Waste Activated Sludge with Primary Sludge in Two-Stage Anaerobic Digestion.  
596 *Environmental Progress & Sustainable Energy*, 37(1), 425-433. DOI 10.1002/ep

597 Shaddel S., Bakhtiary-Davijany H., Kabbe C., Dadgar F., Østerhus S.W., 2019. Sustainable Sewage  
598 Sludge Management: From Current Practices to Emerging Nutrient Recovery Technologies.  
599 *Sustainability*, 11, 3435; doi:10.3390/su11123435

600 Teo C.W., 2016. Unified Theory and Model for Anaerobic Hydrolysis in Municipal Wastewater  
601 Treatment: Review of Enzymological Aspects and Hydrolysis Assessments, *J. Environ. Eng.*, 04016041.  
602 DOI: 10.1061/(ASCE)EE.1943-7870.0001110

603 Wahidunnabi A.K., Eskicioglu C., 2014. High pressure homogenization and two-phased anaerobic  
604 digestion for enhanced biogas conversion from municipal waste sludge. *Water Research*, 66, 430-446.  
605 <http://dx.doi.org/10.1016/j.watres.2014.08.045>

606 van Lier J.B., Mahmoud N., Zeeman G., 2008. Anaerobic wastewater treatment. In: *Biological*  
607 *Wastewater Treatment. Principles modeling and Design*. Chapter 16. Henze, M., van Loosdrecht,  
608 M.C.M., Ekama, G.A., Brdjanovic, D. eds. ISBN: 9781843391883. IWA Published London UK.

609 Wu L.J., Qin Y., Hojo T., Li Y.Y., 2015. Upgrading of anaerobic digestion of waste activated sludge by  
610 temperature-phased process with recycle. *Energy* 87, 381-389.  
611 <http://dx.doi.org/10.1016/j.energy.2015.04.110>

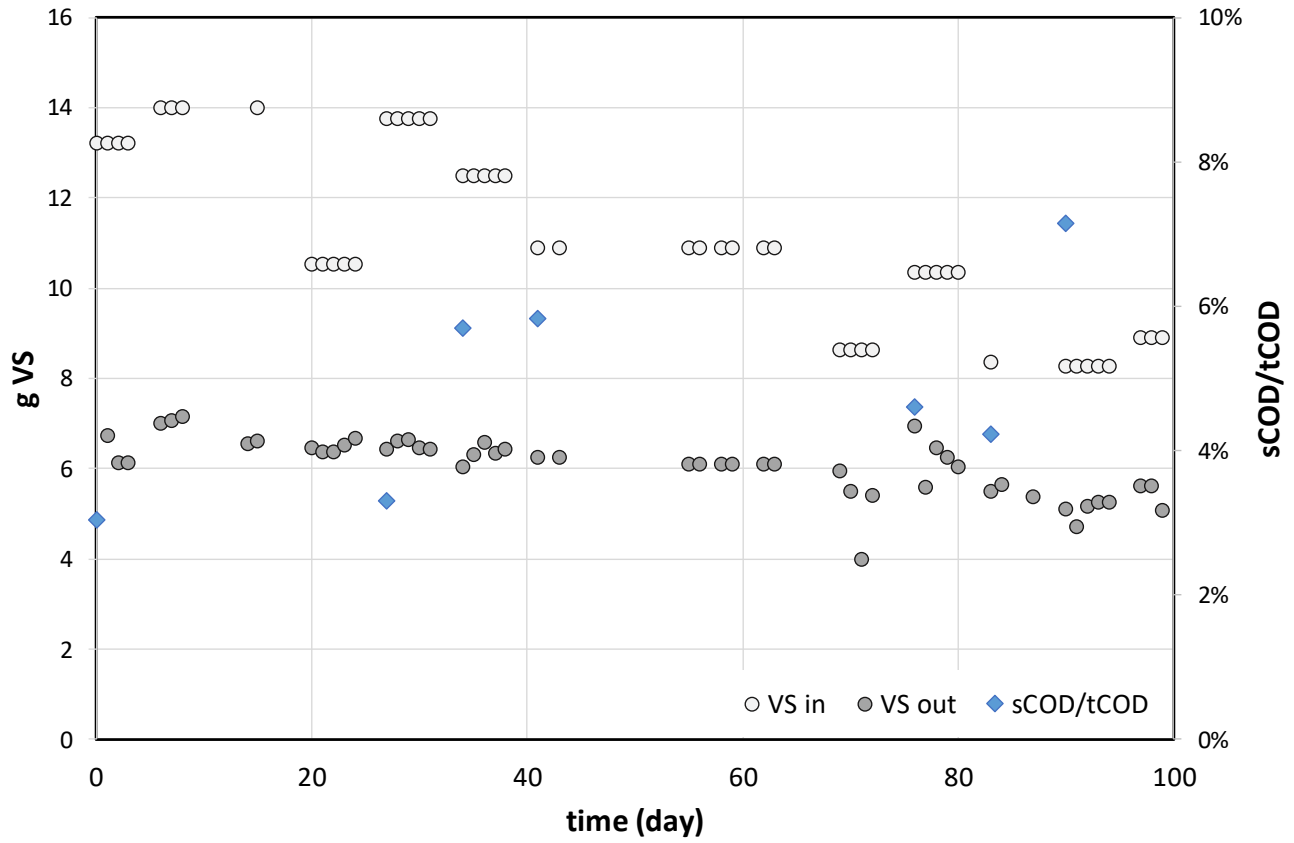
612 Xiao B., Qin Y., Zhang W., Wu J., Qiang H., Liu J., Li Y.Y., 2018. Temperature-phased anaerobic  
613 digestion of food waste: A comparison with single-stage digestions based on performance and energy  
614 balance. *Bioresource Technology*, 249, 826–834. <http://dx.doi.org/10.1016/j.biortech.2017.10.084>

615 Yuan T., Cheng Y., Zhang Z., Lei Z., Shimizu K., 2019. Comparative study on hydrothermal treatment  
616 as pre- and post-treatment of anaerobic digestion of primary sludge: Focus on energy balance, resources  
617 transformation and sludge dewaterability. *Applied Energy*, 239, 171–180.  
618 <https://doi.org/10.1016/j.apenergy.2019.01.206>

619 Zamanzadeh M., Parker W.J., 2018. Characterization of Hydrolysis Kinetics in Staged Anaerobic  
620 Digestion of Wastewater Treatment Sludge. *Water Environ. Res.*, 90, 5.  
621 doi:10.2175/106143017X15054988926497

622 Zhen G., Lu X., Kato H., Zhao Y., Li Y.Y., 2017. Overview of pretreatment strategies for enhancing  
623 sewage sludge disintegration and subsequent anaerobic digestion: Current advances, full-scale  
624 application and future perspectives. *Renew. Sust. Energ. Rev.*, 69, 559-577

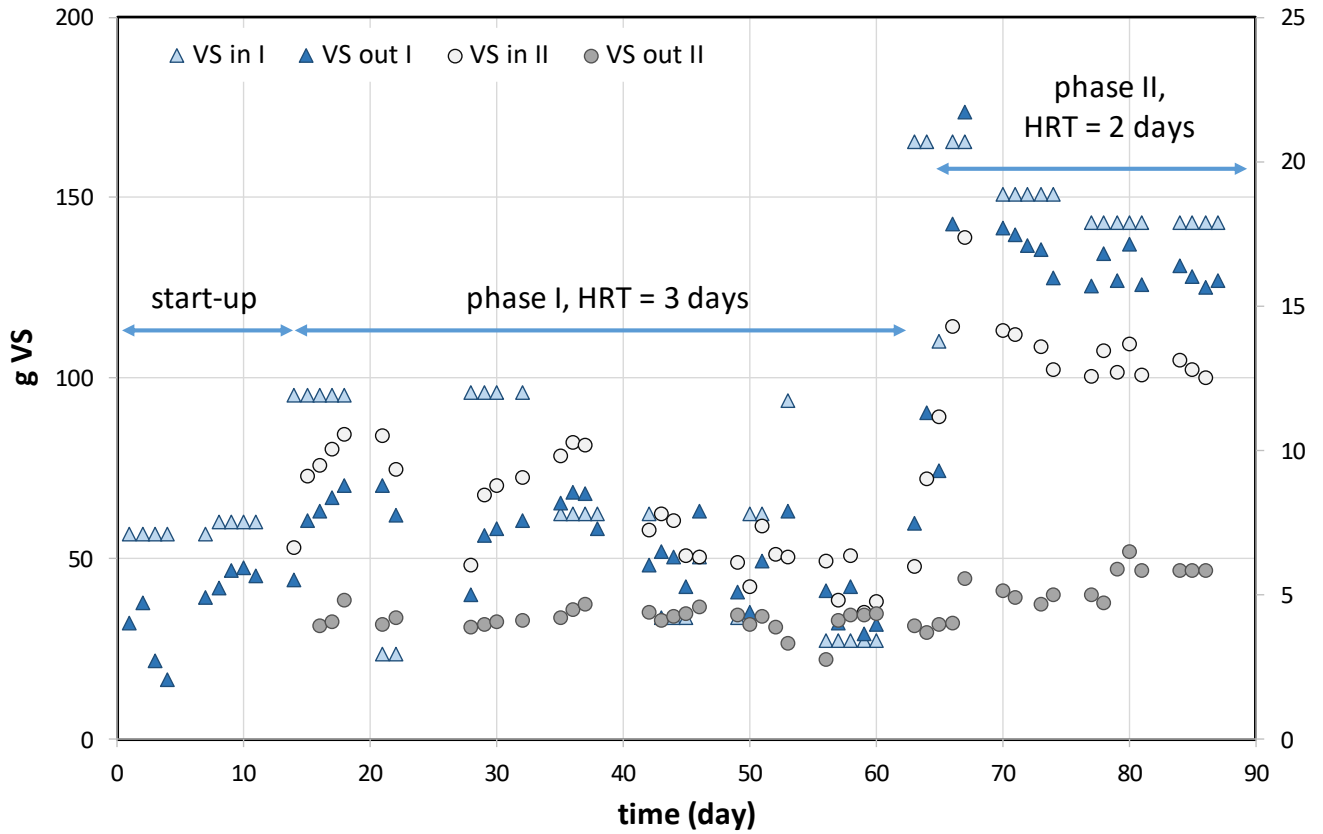
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627 Figure 1. Left axis: daily amount of VS fed to the conventional one-stage digester and residual amount  
 628 of VS extracted with the digestate. Right axis: sCOD/tCOD ratio of the PS fed to the digester

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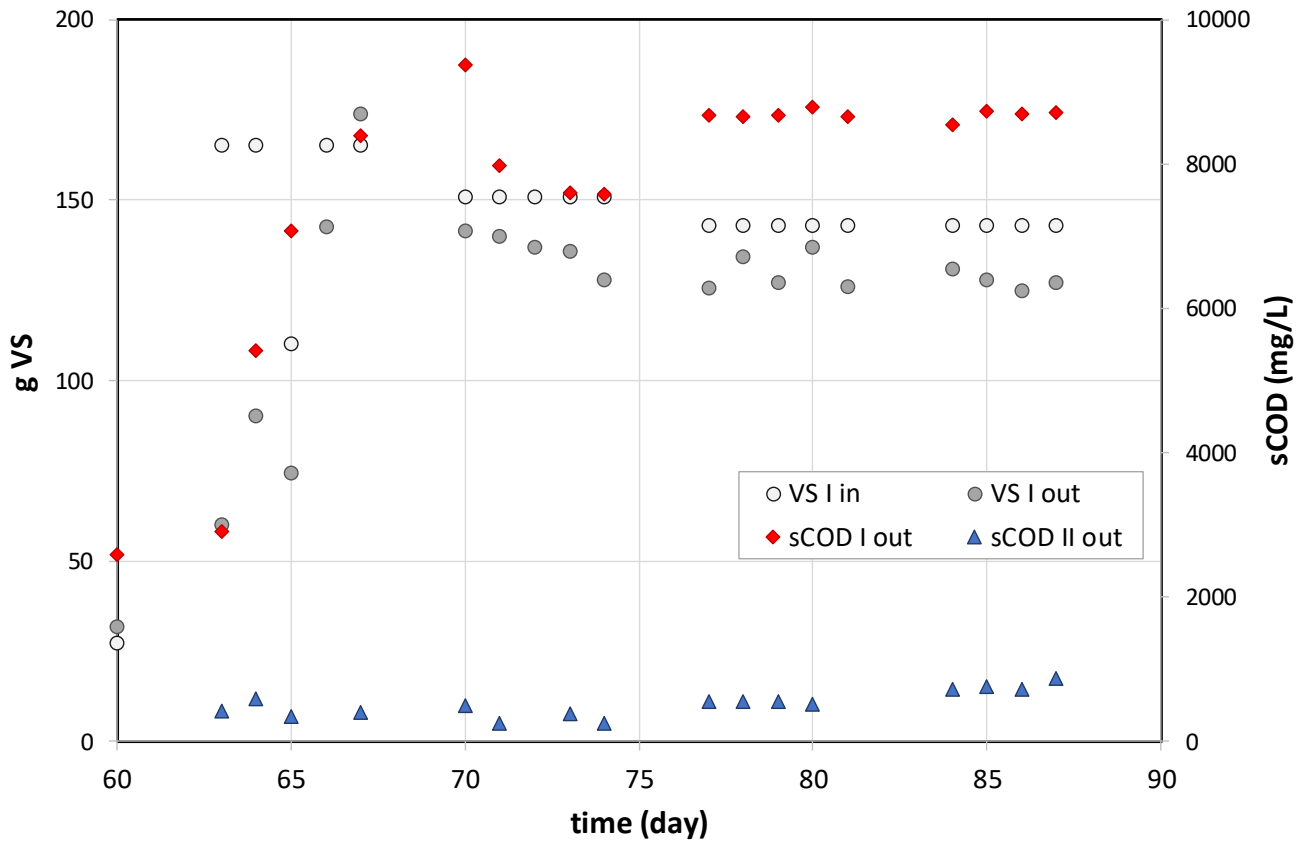


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631 Figure 2. Daily VS amount fed and extracted from each of the two digesters (I and II) of the TPAD  
 632 system (VS in I and VS out I, left y axis; VS in II and VS out II, right y axis)

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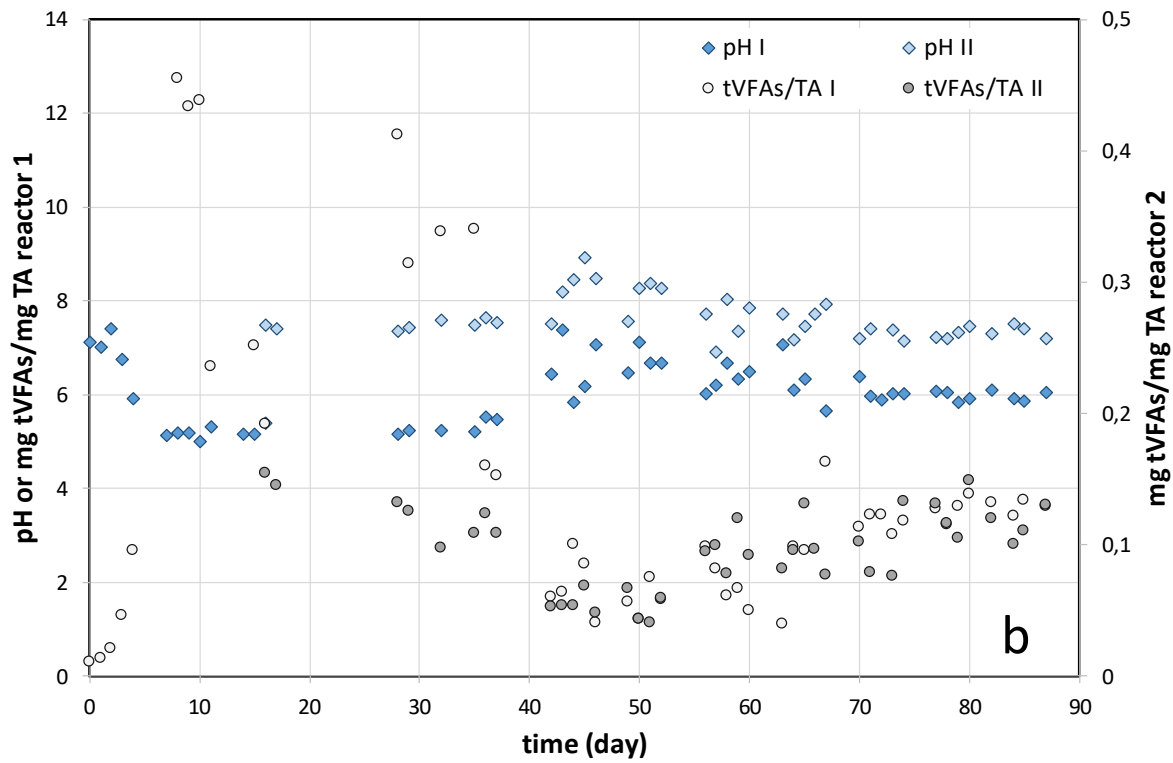
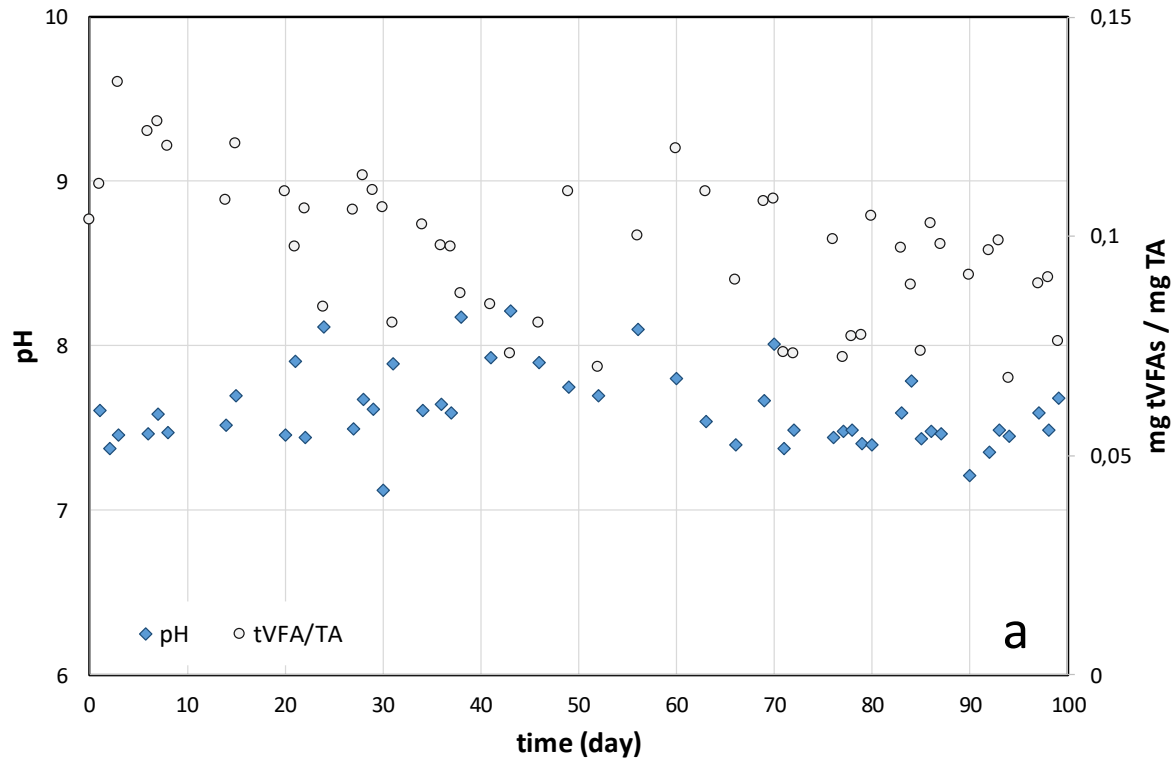
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636 Figure 3. Time course of inlet and outlet VS in the AR (left axis). Trend of the sCOD concentration  
637 measured at the exit of the AR (I) and MR (II) (right axis)



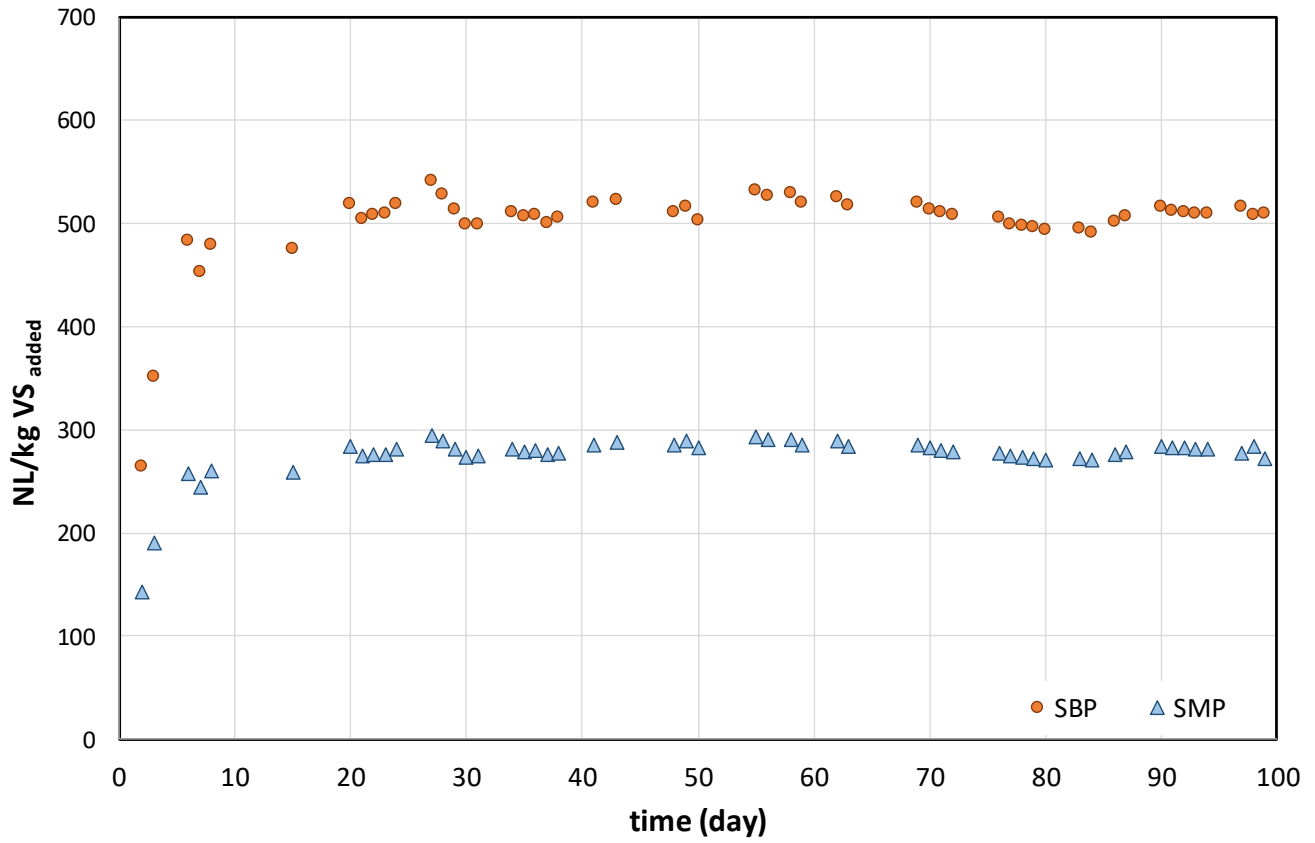


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639 Figure 4. Time course of the pH and tVFAs/TA in the digestate extracted from the one-stage digester

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

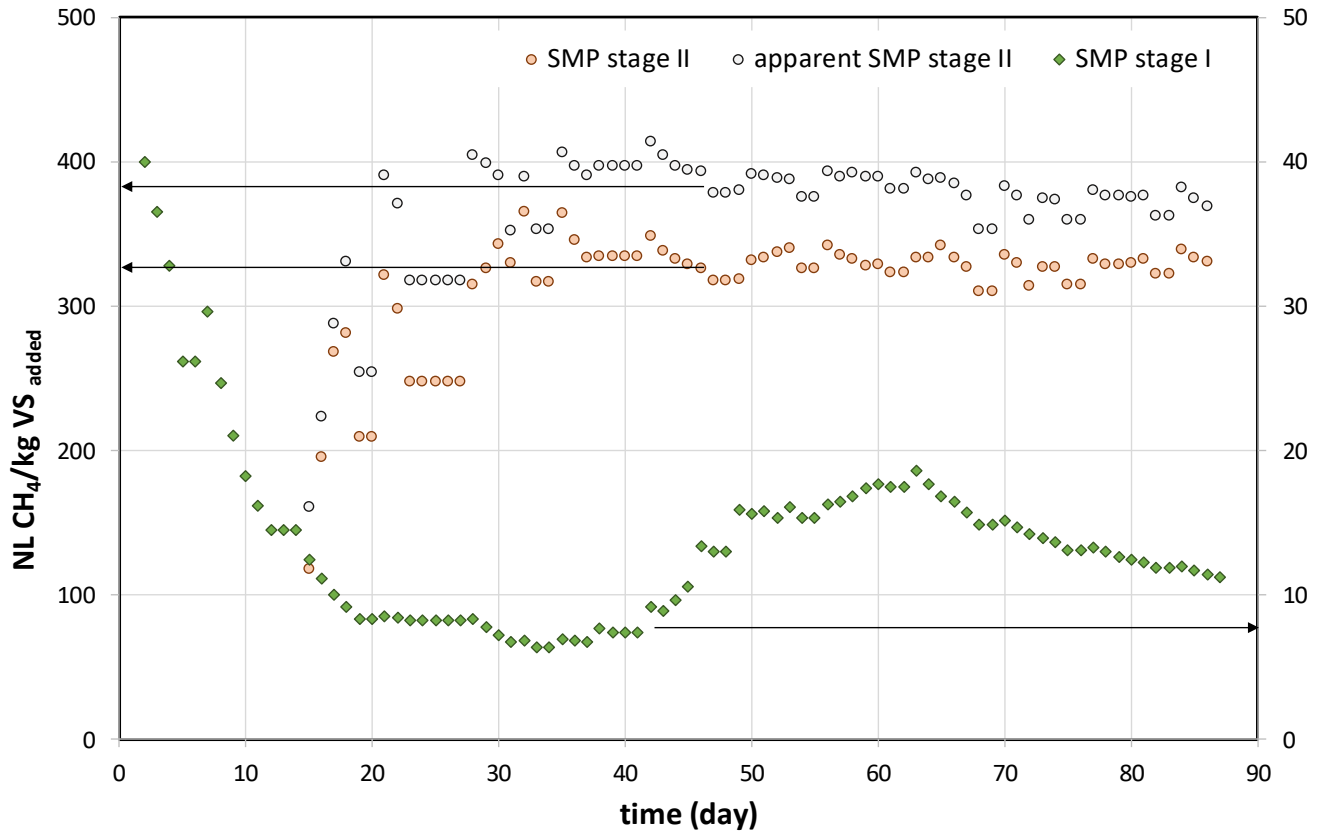
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643 Figure 5. Trend of SBP and SMP from the test carried out in the one-stage digester

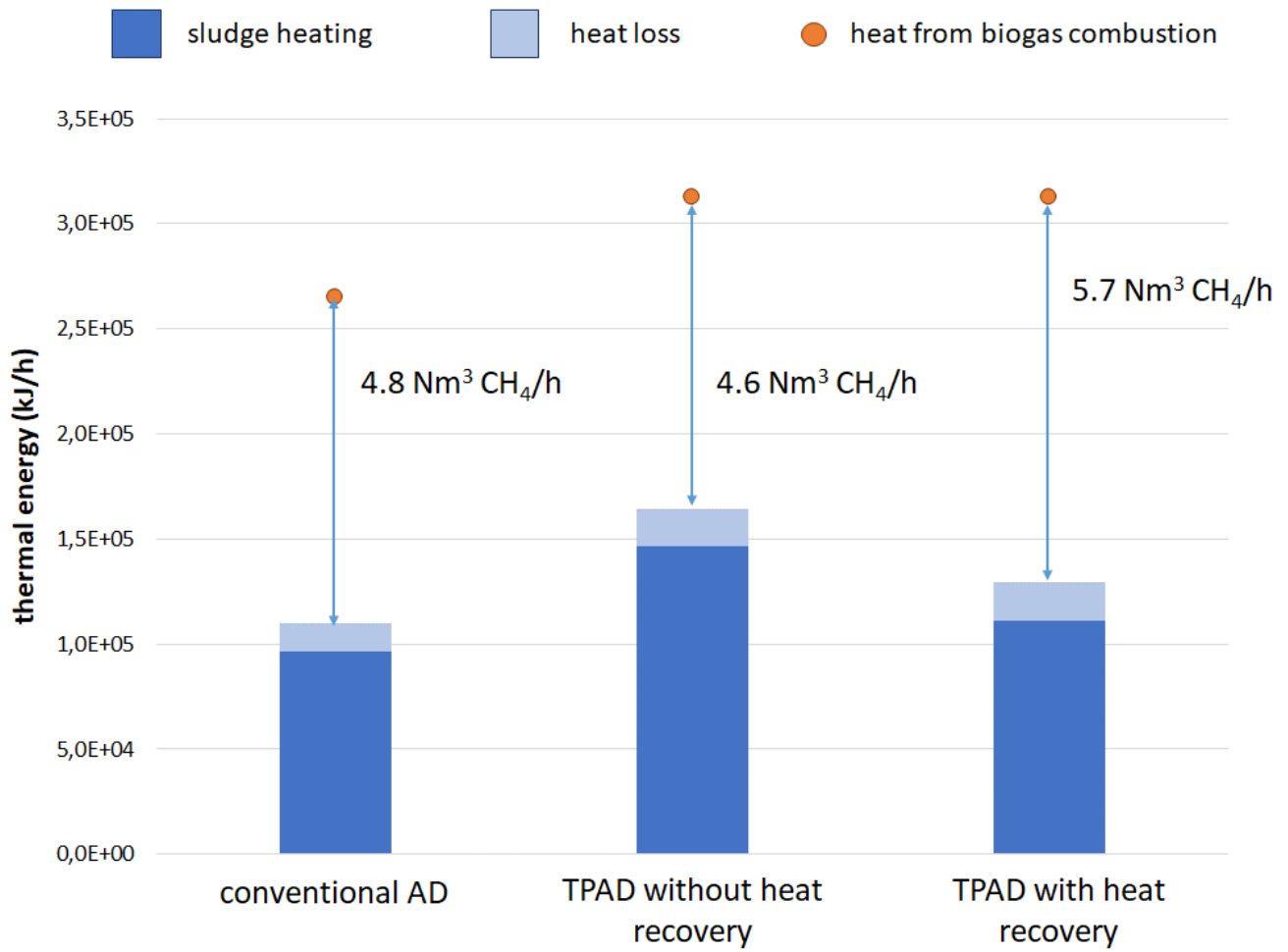
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646 Figure 6. Trend of cumulative SMP in the first and in the second digester of the TPAD system

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649 Figure 7. Results of the energy balance