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Monitoring of the process, resources transformation and energy balance

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**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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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

## 1. Introduction

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

the conventional reactor. The increase of the temperature in the first reactor, from the first to the last cycle of test, in combination with the reduction of HRT positively affected the production of methane. Other studies were aimed to find the best pre-treatment conditions to optimize both the extraction of VFAs and the production of methane and, eventually, to promote the inactivation of pathogens. They used batch tests to reproduce the pre-fermentation phase and BMP tests to quantify the substrate biodegradability after the pre-treatment (Ding et al., 2017; Peces et al., 2016; Riau et al, 2010a). Lin and Li (2018) applied the acidogenic fermentation process in a TPAD system to treat a primary sludge obtained from the  $\text{FeCl}_3$ -based chemical enhanced primary sludge process to convert organic substances of the sludge to VFAs.

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

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

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

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

## **2. Materials and Methods**

### **2.1 Substrate**

The substrate used in this study was the PS obtained from the SMAT WWTP located in Castiglione Torinese (NW Italy). A detailed description of the WWTP water and sludge lines was provided in a previous paper (Ruffino et al., 2014). Shortly, the WWTP has a standard configuration that includes 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 
$$tCOD = \frac{8(4n+a-2b-3d)}{(12n+a+16b+14d)} \text{ as } \left( \frac{g \text{ COD}}{g \text{ C}_n\text{H}_a\text{O}_b\text{N}_d} \right) \quad (1)$$

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

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

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

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

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).

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

Figure 2 compares the amount of the VS daily fed to the first digester of the TPAD system (AR) with the amount of VS daily extracted with the pre-treated sludge. The trend of the inlet VS well highlighted the two phases of the experimentation, in which the first-stage digester was run at HRT = 3 days for approx. 50 days, and, subsequently, at HRT = 2 days, for the final 30 days of the test. In the first period, that is 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 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 observed between the inlet and the outlet of the AR was a consequence of the processes that take place in it. In a two-stage digestion system, the AR converts biodegradable COD to VFAs through the processes of hydrolysis and fermentation (Ge et al., 2010; Ge et al., 2011a). The products of hydrolysis are typically sugars, long chain fatty acids and amino acids; the subsequent process of fermentation will transform some of these compounds to VFAs and CO<sub>2</sub> (Batstone et al., 2002). Furthermore, if the status of phase separation between the two reactors is not completely achieved, some VFAs could be converted to acetates and, finally, to methane (see Section 3.2). The generation of CO<sub>2</sub> and, potentially, of methane, determines a reduction of the VS into the AR.

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

and approx. 12 g/day for the two subsequent weeks. The concentration of sCOD measured at the exit of the reactor was reported in Figure 3, together with the inlet and outlet VSs, and was of 8,600 – 8,700 mg/L. Consequently, the daily load of discharged sCOD was of approx. 43 g and the COD solubilization of the biological pre-treatment of 13.8%. The average daily methane production, over the period between 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 contribution due to the methane generation allowed to calculate an extent of solubilization of 14.7%. It could be seen from the values of these two parameters that only a very reduced amount (6.5%) of the substances made readily degradable by the BH pre-treatment was transformed into methane already in the AR. This was a demonstration that the status of phase separation between the two reactors was quite successfully achieved. Ge et al (2011b) observed an extent of solubilization similar to that of this study (15%) after a 2-day biological thermophilic (50 °C) pre-treatment carried out on a waste activated sludge. It has to be noted that, in that study, a relevant amount (ca. 40%) of readily biodegradable organic matter was transformed into methane already in the BH stage. The sCOD released in the BH stage was consumed for almost 90% in the AD process carried out in the second-stage reactor, in fact the concentrations of 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 outlet of the MR.

### **3.2 Process stability and methane production**

The stability of the AD process was monitored through the measurement of pH, tVFAs and TA. The ratio between tVFAs and TA, also known as FOS/TAC ratio in the German technical literature, is an easy-to-do and reliable measure of the risk of acidification of a digester (Madsen et al., 2011; Castro et al., 2017). As it can be seen from Figure 4a, the pH value of the digestate extracted from the one-stage digester had 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

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

As it can be seen from Figure 6, the second reactor showed an apparent SMP of 388 NL/kg VS added in 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 (from day 70<sup>th</sup> to the end), represented by the upper curve. The apparent SMP was calculated by dividing the cumulative production of methane by the amount of VS introduced into the second reactor (and reported in Figure 2). However, the methane yield of the pre-fermented sludge had to be referred to the initial organic matter content of the substrate. For that, it was necessary to take into account the losses of organic substances that originated from both the processes of hydrolysis and fermentation, that take place 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),  $B_0$  is the methane yield of the sludge after  
377 the pre-fermentation (NLCH<sub>4</sub>/kg VS added), and  $\rho$  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

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

### 3.3 Energy balance

The comparison between a traditional AD process carried in mesophilic conditions and a TPAD system, that included a thermophilic (50 °C) BH, was performed in terms of an energy balance. The energy balance did not include consideration of energy consumption for sludge loading/pumping/mixing. The 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 same characteristics of the sludge employed in this study. The TS of the sludge was assumed to be of 4%, a value that can be obtained with an efficient gravity thickening process. As in this study, the VS/TS ratio was of 0.74.

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

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

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;

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

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

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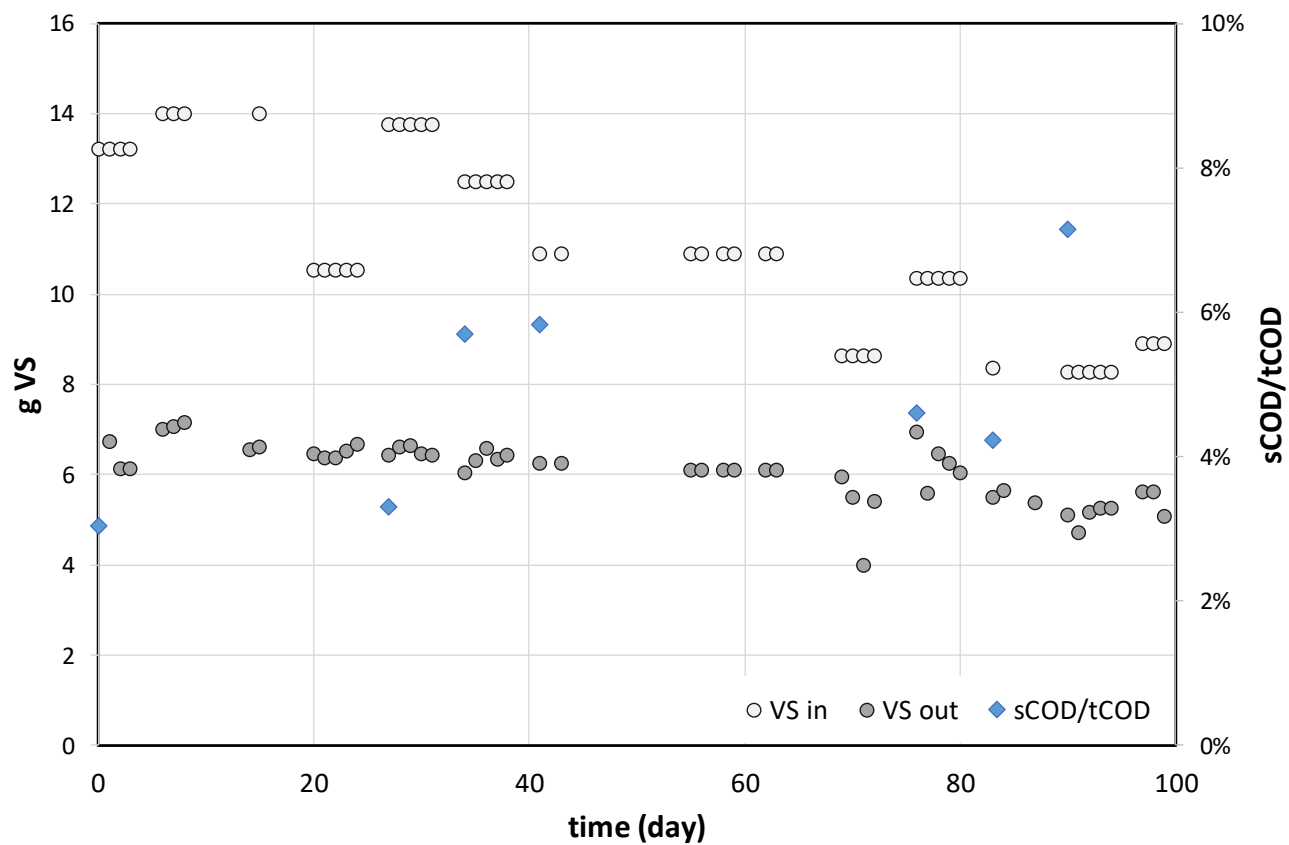
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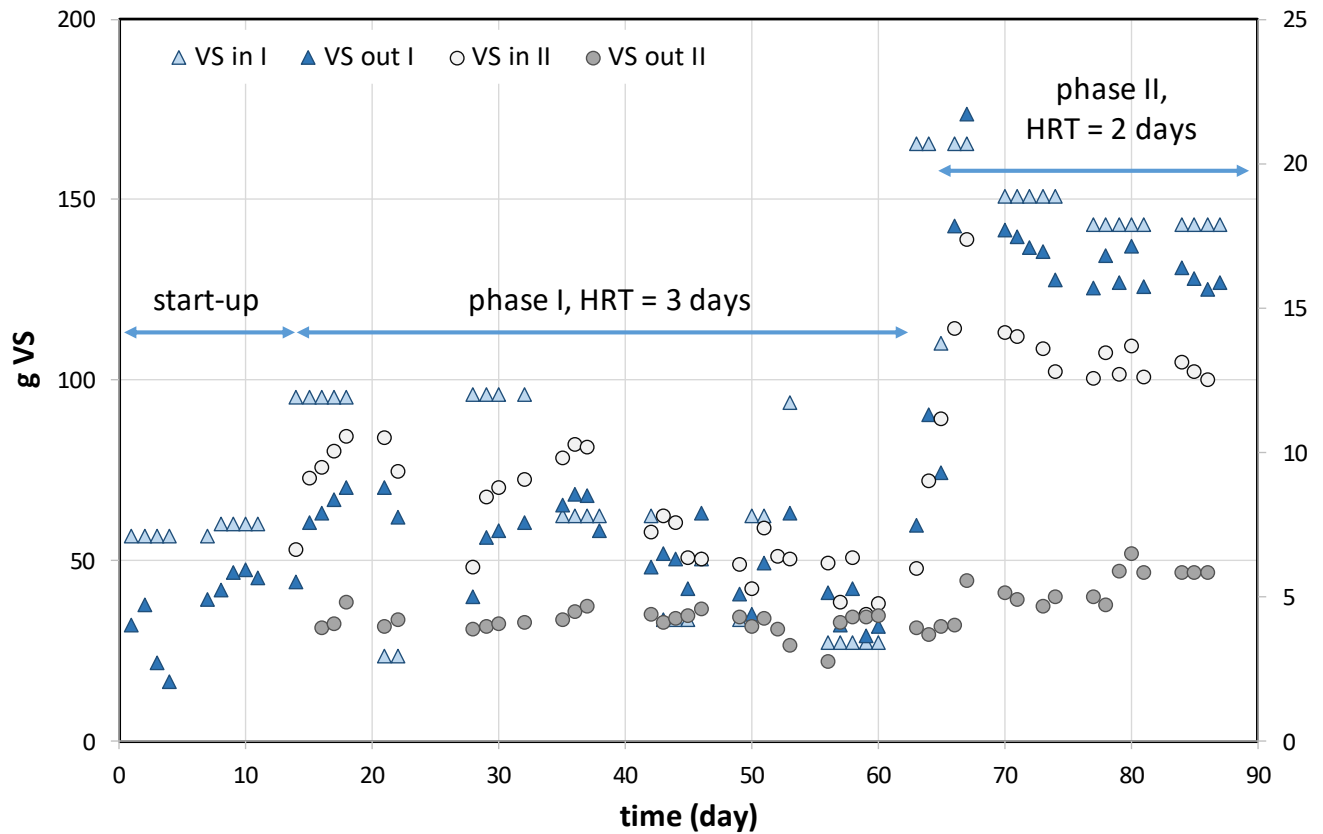
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627 Figure 1. Left axis: daily amount of VS fed to the conventional one-stage digester and residual amount  
 628 of VS extracted with the digestate. Right axis: sCOD/tCOD ratio of the PS fed to the digester

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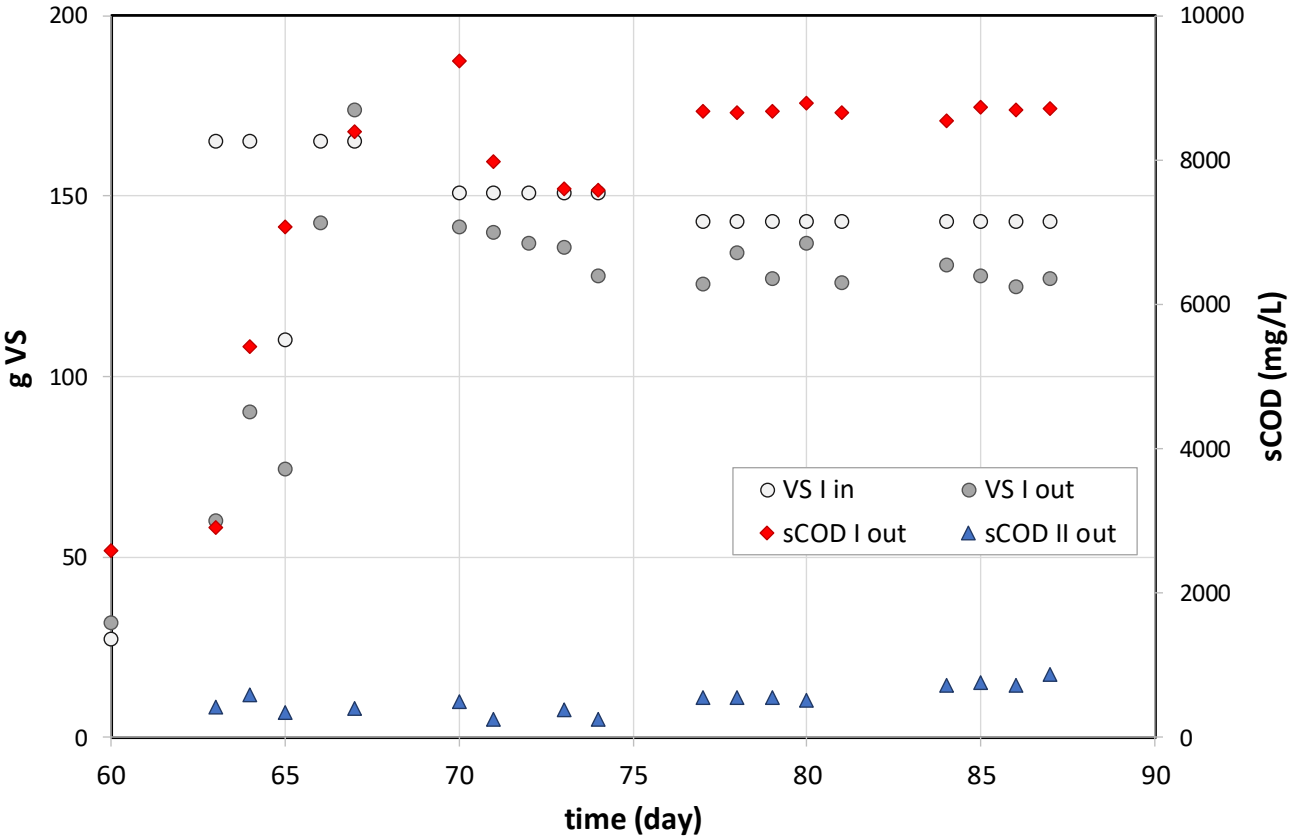


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631 Figure 2. Daily VS amount fed and extracted from each of the two digesters (I and II) of the TPAD  
 632 system (VS in I and VS out I, left y axis; VS in II and VS out II, right y axis)

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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)

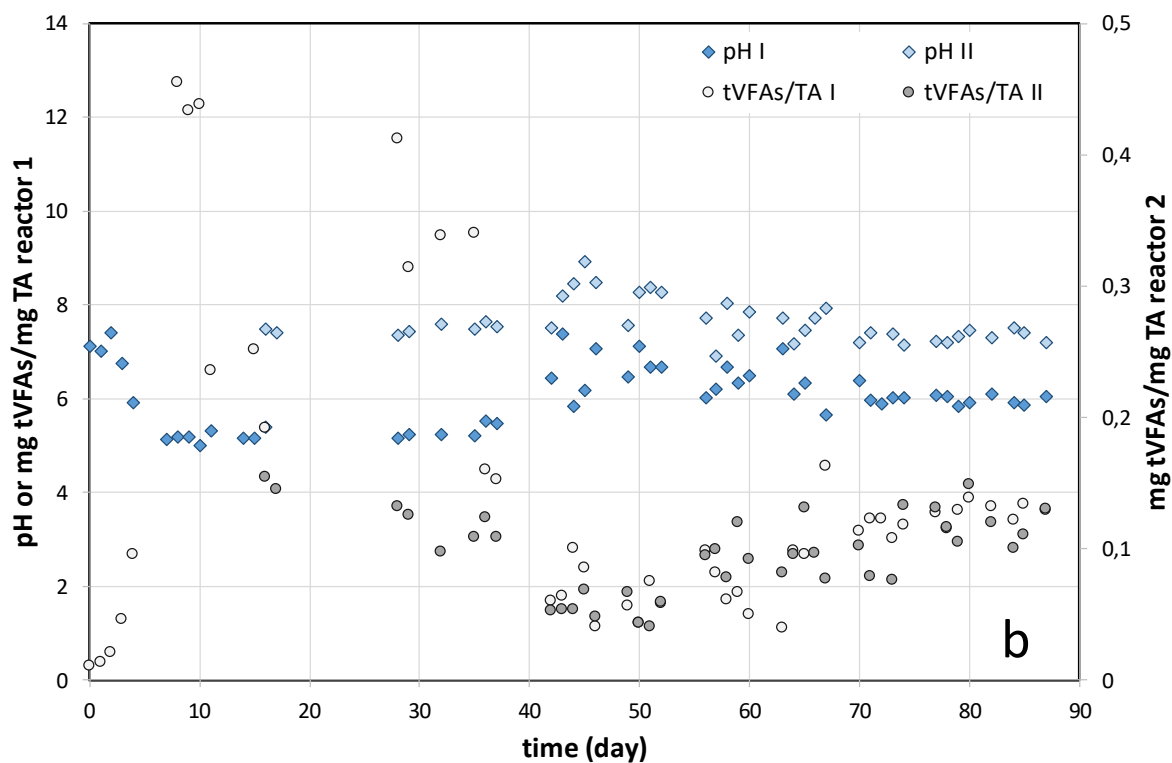
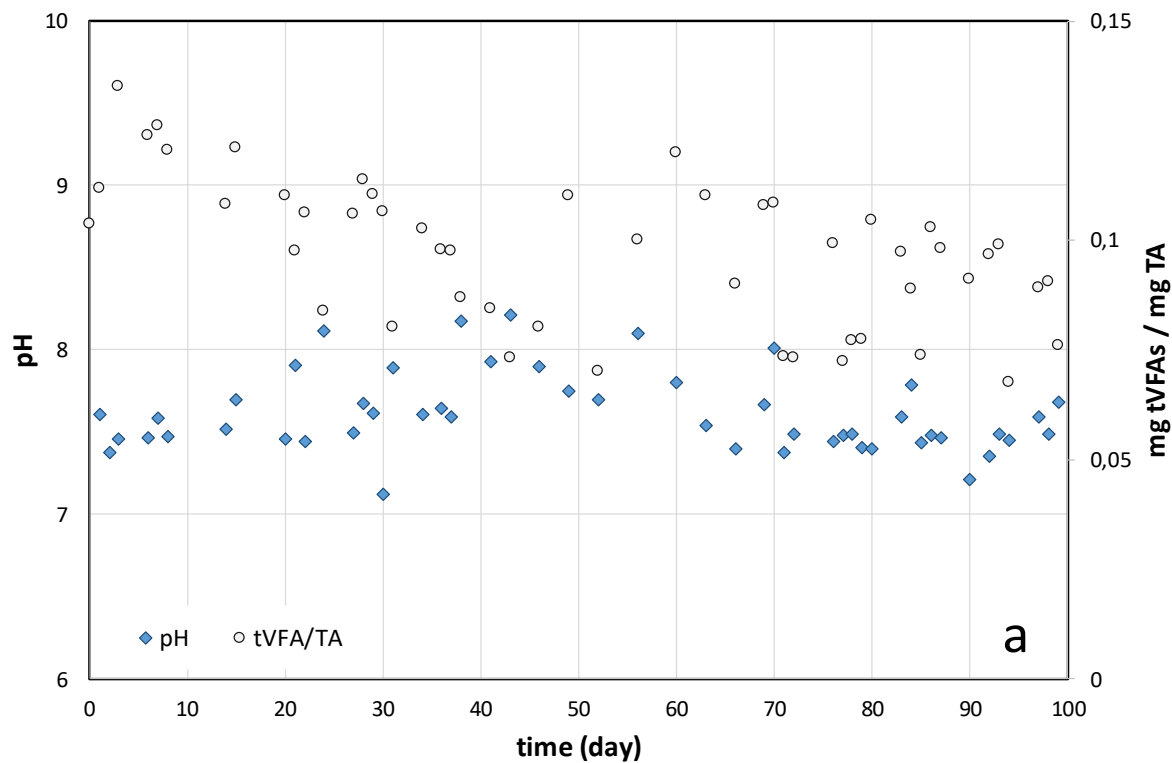
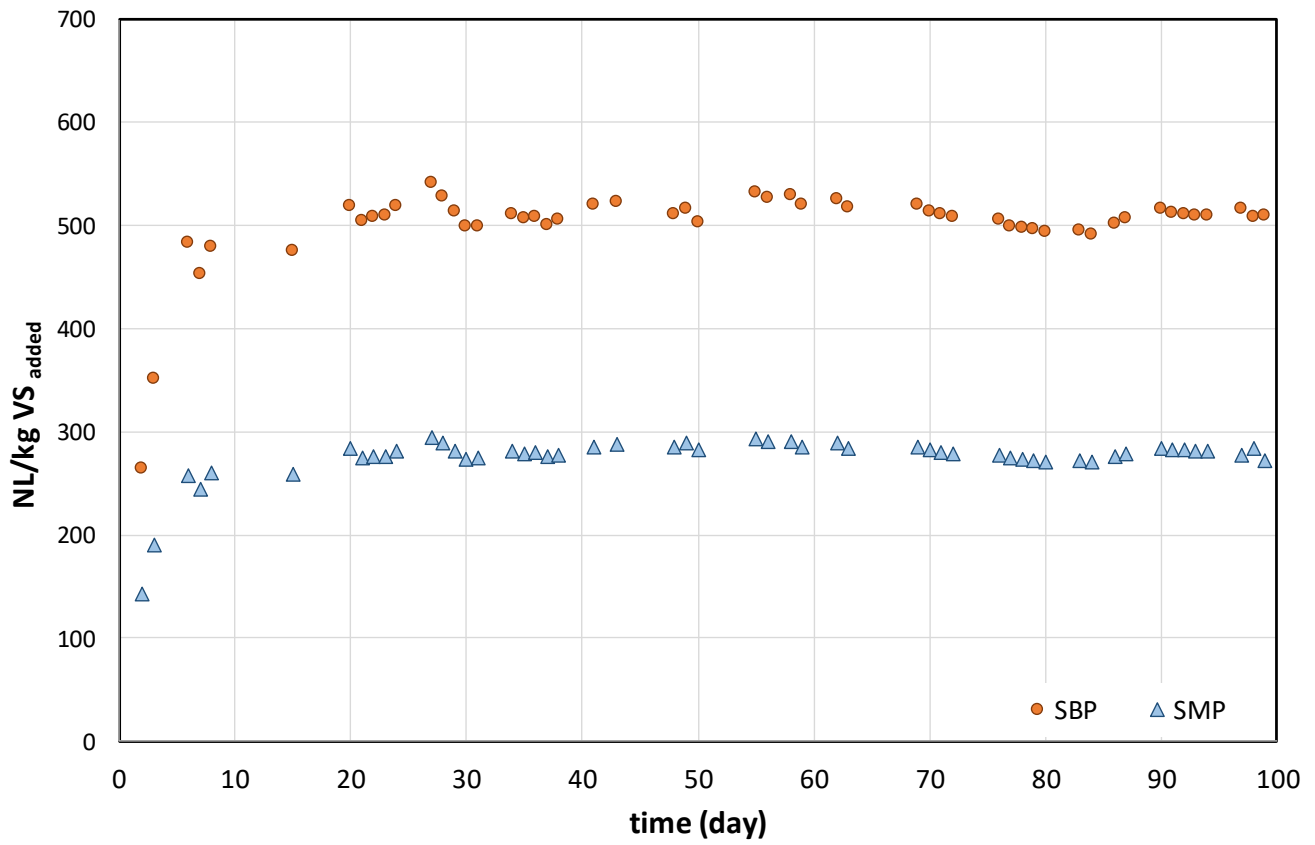


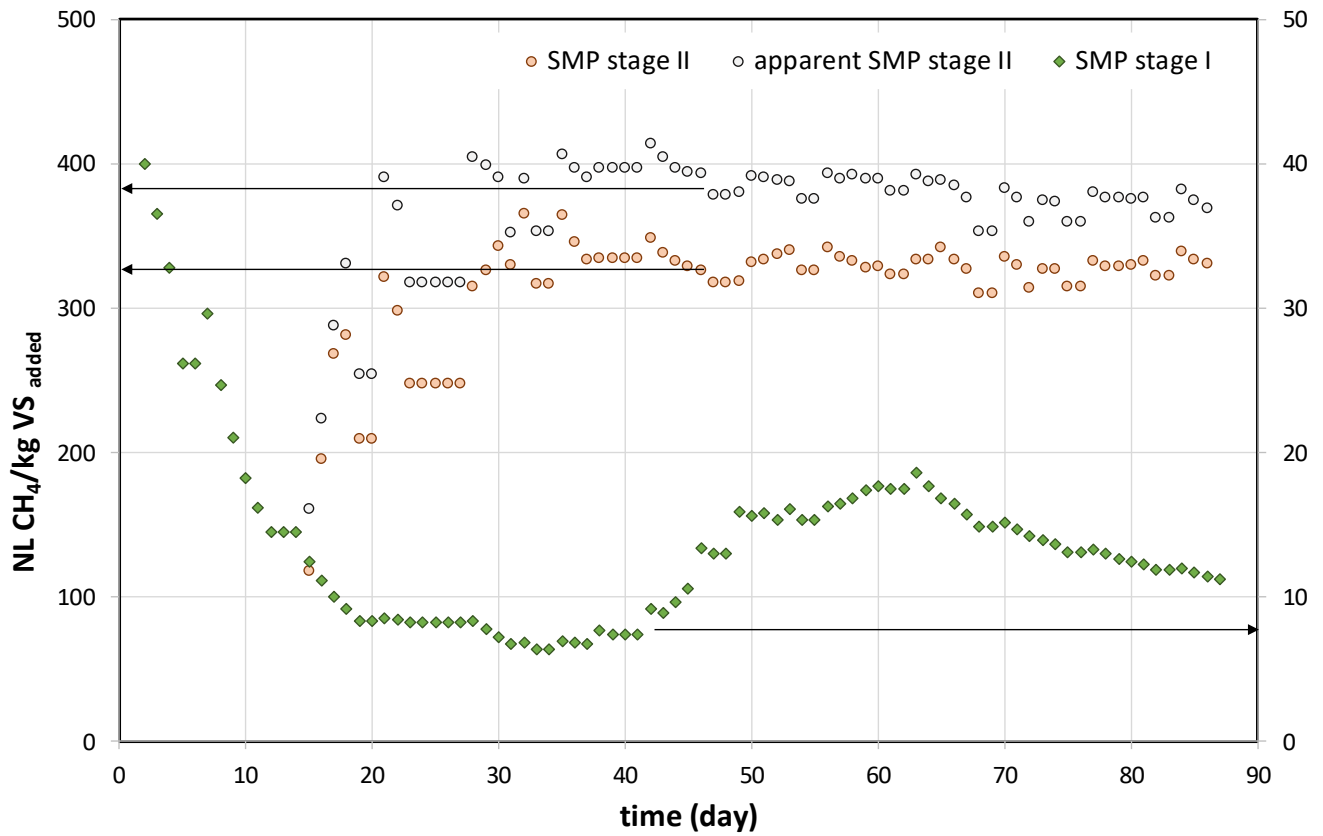
Figure 4. Time course of the pH and tVFAs/TA in the digestate extracted from the one-stage digester (a) and from the TPAD system (b)



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

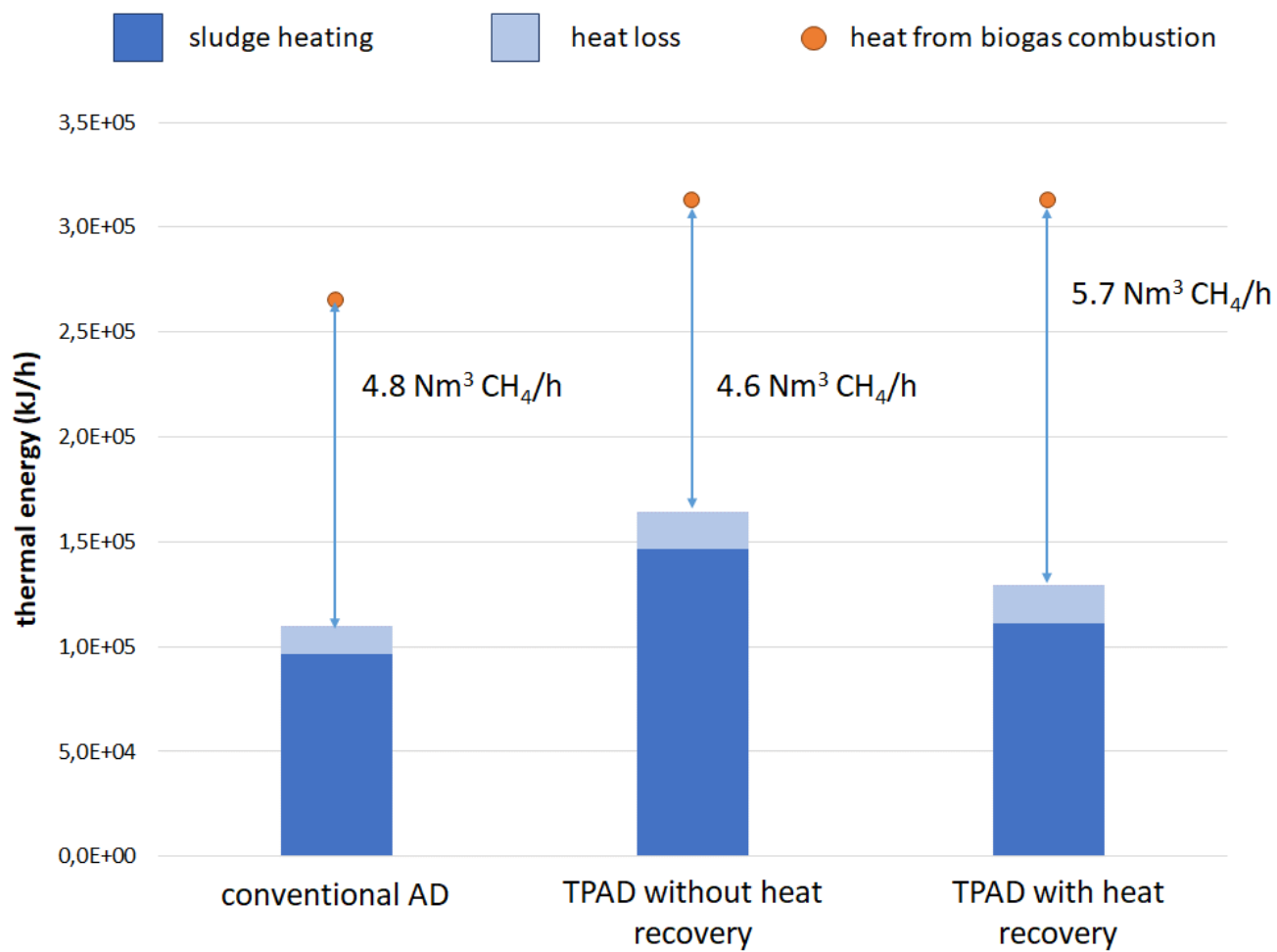
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646 Figure 6. Trend of cumulative SMP in the first and in the second digester of the TPAD system

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649 Figure 7. Results of the energy balance