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

In the last decade several studies have investigated the advantages offered by the application of a TPAD system. Hameed et al. (2019) used two systems made of two semi-continuous reactors each to study the effect of the temperature of the first digestion phase. They found that the main AD reactor, that had received the pretreated sludge, generated approximately the same specific methane potential (SMP, ca. 0.89 m^3 CH_4/kg VS removed), irrespective of the temperature at which the pre-treatment was performed (45 or 55 °C). Zamanzadeh and Parker (2018) carried out several tests in single and dual batch reactors to study the kinetic of the hydrolysis process in traditional and TPAD systems where mesophilic (M) and thermophilic (T) phases had been combined in all the possible ways (M/M, T/T, M/T, T/M). Martín-Pascual et al. (2017), by using a pilot-scale test, compared the efficiency of a conventional mesophilic (33-34 °C) AD reactor with a two-stage system, where the first reactor was kept at the ambient temperature (18-22 °C). No significant differences were found concerning VS and COD reduction, 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 $FeCl_3$-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. Grübel 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 oxidation, with a sludge retention time in the order of approx. 25 days, secondary settling and final filtration on a gravel and anthracite bed.

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

Table 1 shows the average elemental composition of the PS used in the tests carried out in this study (see Section 2.2), to which corresponded the raw formula: $C_{10.7}H_{18.4}O_{9.0}N$. From this information the specific tCOD value (as g O_2/g TS) of the PS was evaluated as in Equation 1 (van Lier et al., 2008).

$$tCOD = \frac{8(4n+a-2b-3d)}{(12n+a+16b+14d)} \; as \; \left(\frac{g\ COD}{g\ C_nH_aO_bN_d}\right) \quad (1)$$

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

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

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

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

2.2 Reactor set up and operations

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

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

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

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

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

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

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

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

NA, not available

2.3 Analytical methods

TS, VS and pH were determined according to the Standard Methods (APHA, AWWA, WEF, 2012). The tVFAs/TA parameter is the ratio between the tVFAs, which stands for volatile fatty acids, expressed in equivalent milligrams of acetic acid per liter, and TA, which stands for Total Alkalinity, expressed in mg equivalent of calcium carbonate per liter. It was obtained by a potentiometric titration, according to the Nordmann method (Nordmann, 1977), by using a SI Analytics automatic titrator. Specifically, a sample of 20 mL of fermentation substrate is titrated by 0.1 N of sulfuric acid solution (H_2SO_4) up to pH 5.0 to calculate the TA value, expressed in mg/L of calcium carbonate ($CaCO_3$). Then the VFA value is obtained after a second titration step between pH 5.0 and pH 4.4. It is expressed in mg/L of acetic acid (CH_3COOH).

Soluble COD, sCOD, is the fraction of COD separated after an initial centrifugation at 15,000 rpm for 10 min and a subsequent filtration of the supernatant on a 0.45 mm nylon membrane filter, as recommended by Roeleveld and van Loosdrecht (2002). The elemental composition analysis was carried out on samples of PS dried at 105 °C and on the residual ashes after combustion at 600 °C. A Flash 2000 ThermoFisher Scientific CHNS analyzer was used for the elemental analysis.

2.4 Calculations

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

$$COD\ solubilization = \frac{(sCOD_f - sCOD_i)}{pCOD_i} \qquad (2)$$

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

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

$$Extent\ of\ solubilization = \frac{COD_{CH4} + sCOD_f - sCOD_i}{tCOD_i - sCOD_i} \qquad (3)$$

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

3. Results and Discussion

3.1 Effect of the TPAD on VS reduction and COD solubilization

This section analyzes the effects of the biological pre-treatment, carried out through the TPAD system, on VS reduction and COD solubilization of the substrate, compared with a conventional, one-stage, mesophilic (38 °C) AD process. Similarly to previous studies, the VS content was used as an indicator of the amount of organic matter contained into the sludge (Arnaiz et al., 2006). Figure 1 compares the daily amount of VS fed to the conventional one-stage digester with the residual amount of VS daily extracted with the digestate. The irregular trend of the VS fed to the reactor was smoothed by the digestion process, that was able to generate a digestate with a nearly constant VS concentration. The steady concentration of VS into the digestate demonstrated that the process had been correctly operated and the digester was well mixed. As it can be seen from Figure 1, in the whole digestion period, lasted approx. 100 days, the overall amounts of VS fed and extracted from the one-stage digester were of 534 g and 309 g respectively, with a consequent VS removal of 42.0%. This value was in general 10-20% lower than those reported in other studies that used PS as substrate for digestion processes, carried out in semi-continuous (or continuous) modality and mesophilic conditions (35-38 °C). For example, Riau et al. (2010b) obtained 42% of VS reduction on a mixed sludge in a continuous digestion process, with a SRT = 15 days. On a similar substrate, Martín-Pascual et al. (2017) found a value 25% higher than that of this study, by carrying out analogous tests (HRT = 22 d, T = 35 °C). In a quite dated study, Ghyoot and Verstraete (1997) found a VS consumption of 57% on pure PS, at an OLR of 1.36 kg VS/m^3·d. Finally, Ersahin (2018) measured a VS reduction of approx. 50% in a single, full-scale, anaerobic digester that treated PS at a HRT of 22 days. The VS reduction is highly correlated with the methane production, being the intrinsic sludge degradability and the SRT of the digestion process the two most relevant 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 12th to day 60th, a VS reduction of 14.0% was found, while in the second period, that is from day 71st 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_2 (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_2 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 occurred in the whole system (first + second stage), it could be concluded that the overall VS reduction was of 55.7% in the system with HRT = 3 days and of 62.2% in the system with HRT = 2 days.

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

The application of Eq.1, to the results of the elemental analysis carried out on the PS used for this study (see Section 2.1), returned a specific tCOD value of 1.05 g O_2/g TS or 1.65 g O_2/g VS. Consequently, the ratio between soluble (sCOD) and total COD (tCOD) of the PS was in the order of 5%. Detailed data of the sCOD/tCOD ratio were shown for some PS sampling dates in Figure 1. As expected, the main fraction of the PS was in the particulate form, and, in line with other studies, it contributed for more than 90% to the tCOD (Zamanzadeh and Parker, 2018). Thus, this indicated the significance of the hydrolysis step for improved biogas production.

The calculation of the two parameters, namely the COD solubilization and the extent of solubilization, was carried out for the second phase of the experimentation (HRT = 2 days). The amount of tCOD daily 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 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 71st – day 90th, 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 ± 0.24) for all the duration of the test. The ratio tVFAs/TA had been at an average value of 0.10, with small variations (± 0.02). The observed tVFAs/TA value was in the expected range for digestion processes of sewage sludge (Ruffino et al., 2019).

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

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

Figure 5 shows the cumulative specific biogas production (SBP) and SMP observed from the test carried out in the one-stage digester. The SBP of the PS in mesophilic conditions (38 °C) was of 511.6 ± 10.7 NL/kg VS added and the SMP was of 280.4 ± 6.2 NL/kg VS added, with an average methane percentage into the biogas of 55.0 ± 3.1 % by volume. Figure 5 highlights a steady cumulative specific production of biogas and methane from day 20th to the end of the test. Values of SBP and SMP returned by this test were in the middle of a range of values found in other experimentations, thus demonstrating that the gas productivity is highly dependent of the characteristics of the substrate and the operating conditions of the test. For example, the batch tests carried out by Yuan and coauthors (2019) returned values of SMP in the order of 180-190 NL/kg VS fed for a PS with a VS/TS ratio in the order of 75%. Conversely, Pinto 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^3/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 ± 3.7 NL/kg VS fed and 12.8 ± 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 30th to day 60th) and of 372 NL/kg VS added in the second phase (from day 70th 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 matter was converted into methane already in the AR; secondly, the method used for the determination of TS and VS did not allow to preserve all the residual organic matter but the most volatile substances were lost during the analytical determinations.

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

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

where B' is the overall methane yield ($NLCH_4$/kg VS added), B_0 is the methane yield of the sludge after the pre-fermentation ($NLCH_4$/kg VS added), and ρ is the VS consumption from the first to the second reactor (g VS final / g VS initial), as in Peces et al (2016).

As detailed in Section 3.1, the VS reduction observed in the first reactor was of 14.0% and 11.0% in the first (HRT = 3 days) and second (HRT = 2 days) phase of the experimentation, respectively. Consequently, the SMPs referred to the initial organic matter content of the substrate were of 333.7 and 331.0 NL/kg VS added in the phases with HRT = 3 and 2 days respectively. These SMP values were very close to those observed in the study of Zamanzadeh and Parker (2018). They carried out tests in a batch mode to compare several single and dual reactor systems and observed the highest methane yield (approx. 0.320 NL/g VS added) for the combination of a thermophilic (HRT = 3.5 days) and a subsequent mesophilic (HRT = 14 days) reactor.

The lower curve of Figure 6 (primary y axis) represented the trend of the cumulated SMP referred to the initial amount of VS. From this curve it was not possible to distinguish the effect of the change of the HRT in the first digester, in fact the SMPs over the two periods were approximately of the same extent. The digestion carried out in the TPAD system allowed to produce 18.6% more methane than the conventional system. In the case it was possible to recover also the methane produced in the first stage, the overall methane yield would be in the order of 345 NL/kg VS added. In a similar test, carried out on 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^3/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^3)	480	48	480
Net heat from biogas combustion (kJ/h)	264,960	312,274	
Heat for sludge heating (kJ/h)	96,278	146,510	
Heat for heat loss compensation (kJ/h)	13,522	4,281	13,522

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

For the calculation of the thermal energy generated from the biogas combustion, it was assumed that only the biogas generated in the second reactor of the TPAD system could be collected and used for thermal valorization. The lower heating value of methane was of 35,880 kJ/m^3 and the boiler efficiency was fixed to 0.9. The thermal energy necessary to heat the sludge was calculated by considering a specific heat capacity (C, 4.18 kJ/kg/°C) and a density (ρ, $1\cdot10^3$ kg/m^3) of the sludge similar to those of water; the ambient temperature was assumed equal to 15 °C. Finally, for the calculation of the heat loss through the digester walls, the heat transfer coefficient (k) was assumed of 0.8 W/m^2/°C and the surface area of the AD reactor walls was calculated from the digester working volume incremented by 20%, considering a radius to height ratio of 1:1 (Passos and Ferrer, 2015).

Figure 7 reports the main results of the energy balance. It could be seen that the both systems, that is the conventional AD process and the TPAD, were completely self-sufficient from an energy point of view. However, the increase in the methane generation observed in the TPAD system was not sufficient to compensate the higher thermal requirements due to sludge heating (up to the temperature of 50 °C) and heat loss, because of the higher temperature difference between the core of the reactor and the external air. In Figure 8 the positive amount of heat that resulted from the heat balance has been accounted in terms of the methane that could be transferred to the local or national gas distribution network. In the case of the conventional AD system the available amount of methane was of 4.8 Nm^3/h, while in the case of the TPAD system it was of only 4.6 Nm^3/h.

Because of the high temperature value of substrate into the AR, for the TPAD system, it was introduced an option of thermal recovery. The extra heat of the sludge at the exit of the AR, due to the difference of temperature between the first (50 °C) and the second (38 °C) reactor, was recovered with an efficiency estimated at 70% to heat the sludge incoming into the AR. In this way, the heat requirement for sludge heating decreased from 146.5 MJ/h to 111.3 MJ/h, with a saving of 24%. With this recovery option, the TPAD offered a larger benefit in terms of the amount of methane to be transferred to the distributing network, that increased from 4.6 to 5.7 Nm^3/h.

4. Conclusions

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

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

- the TPAD showed a superiority in VS reduction, in fact the overall removal of VS increased from 42.0%, in the one-stage reactor, to 55.7% and 62.2% for the TPAD system with a HRT of 3 and 2 days, respectively;
- the COD solubilization, that is the capacity of the hydrolytic / fermentative process, that takes place in the AR, to release soluble substances in the form of saccharides, amino acids, and short and long chain fatty acids, was of approx. 14%;
- the process developed in the two phases of the TPAD was stable for the whole period of the study, as testified by the values of pH and tVFAs/TA ratio;
- the SMP observed in the AR was kept at very low values, in the order of 10-12 NL CH_4/kg VS added, that is approximately 3% of the overall methane production of the TPAD; this was an indication that the status of phase separation between the two acidogenic and methanogenic 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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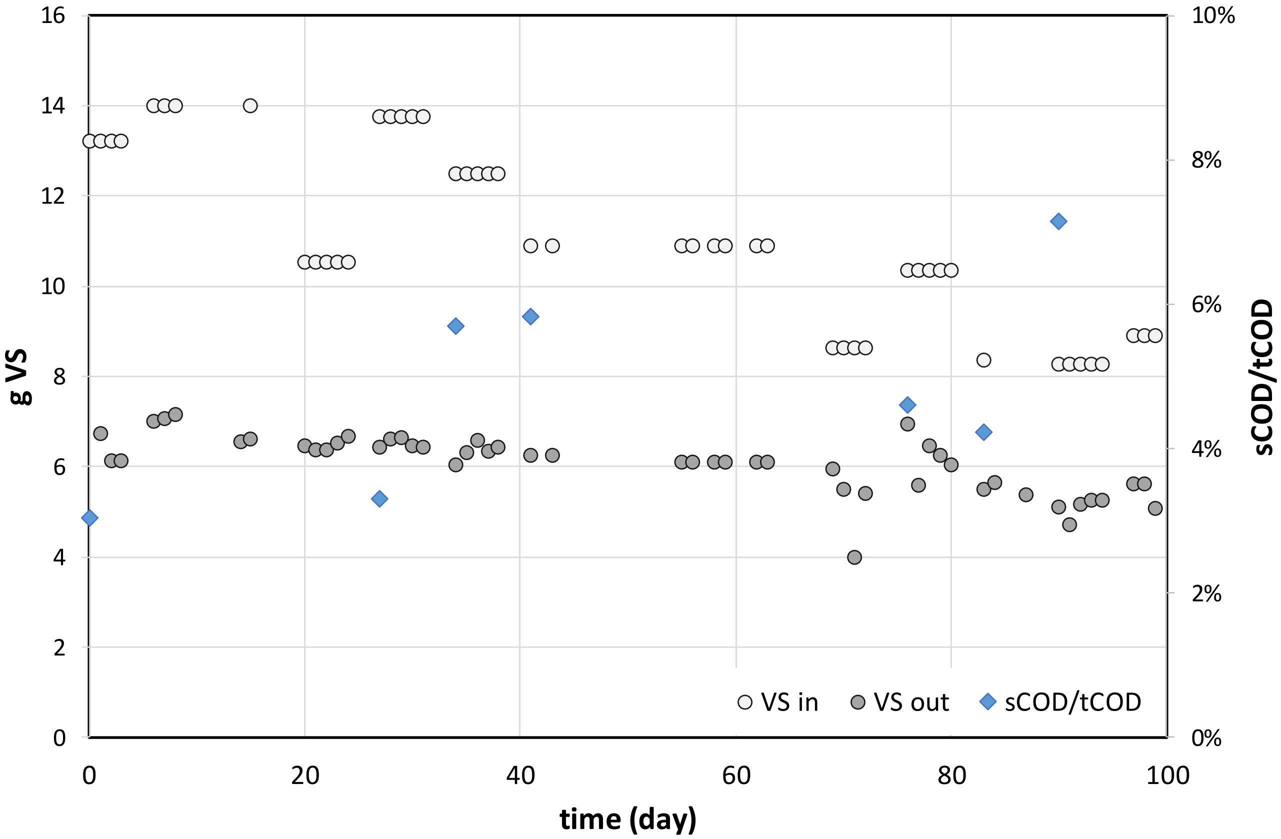


Figure 1. Left axis: daily amount of VS fed to the conventional one-stage digester and residual amount of VS extracted with the digestate. Right axis: sCOD/tCOD ratio of the PS fed to the digester

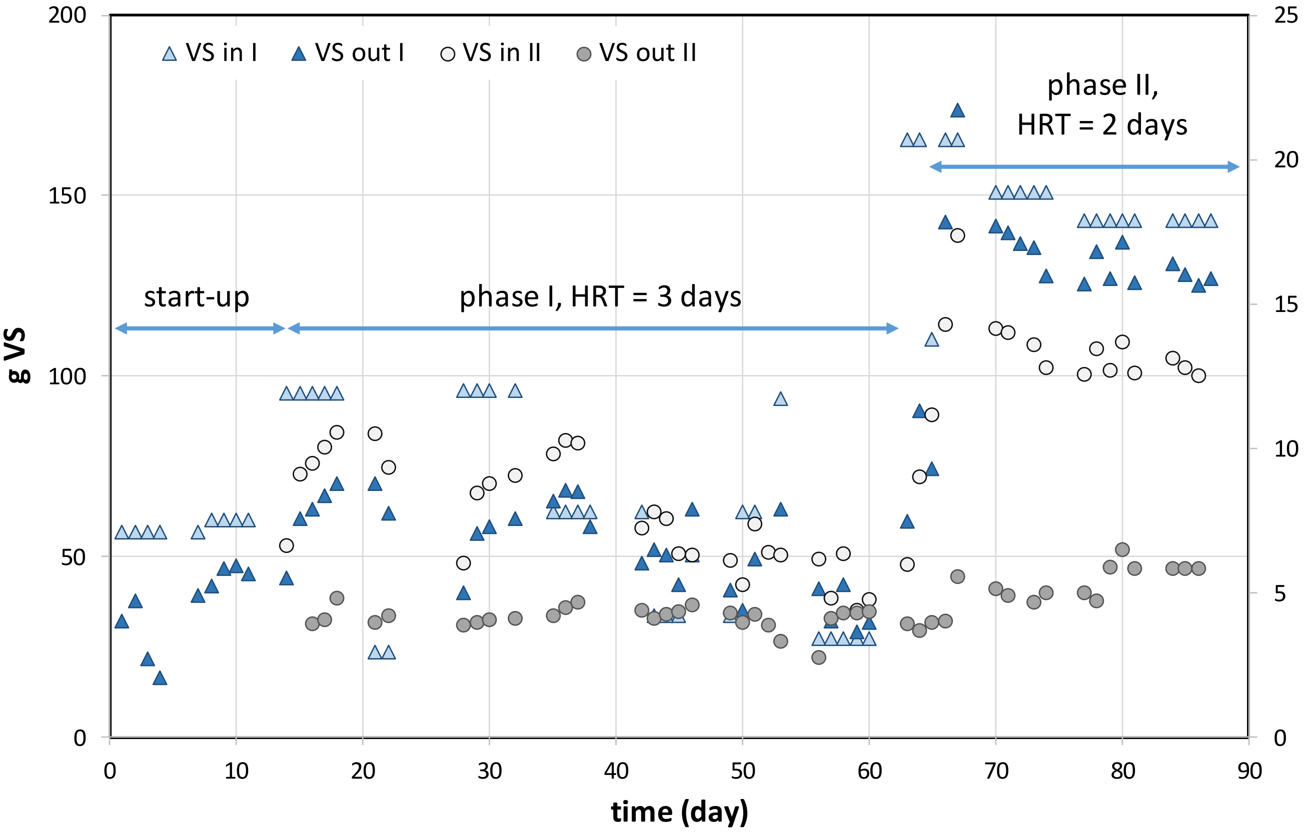


Figure 2. Daily VS amount fed and extracted from each of the two digesters (I and II) of the TPAD system (VS in I and VS out I, left y axis; VS in II and VS out II, right y axis)

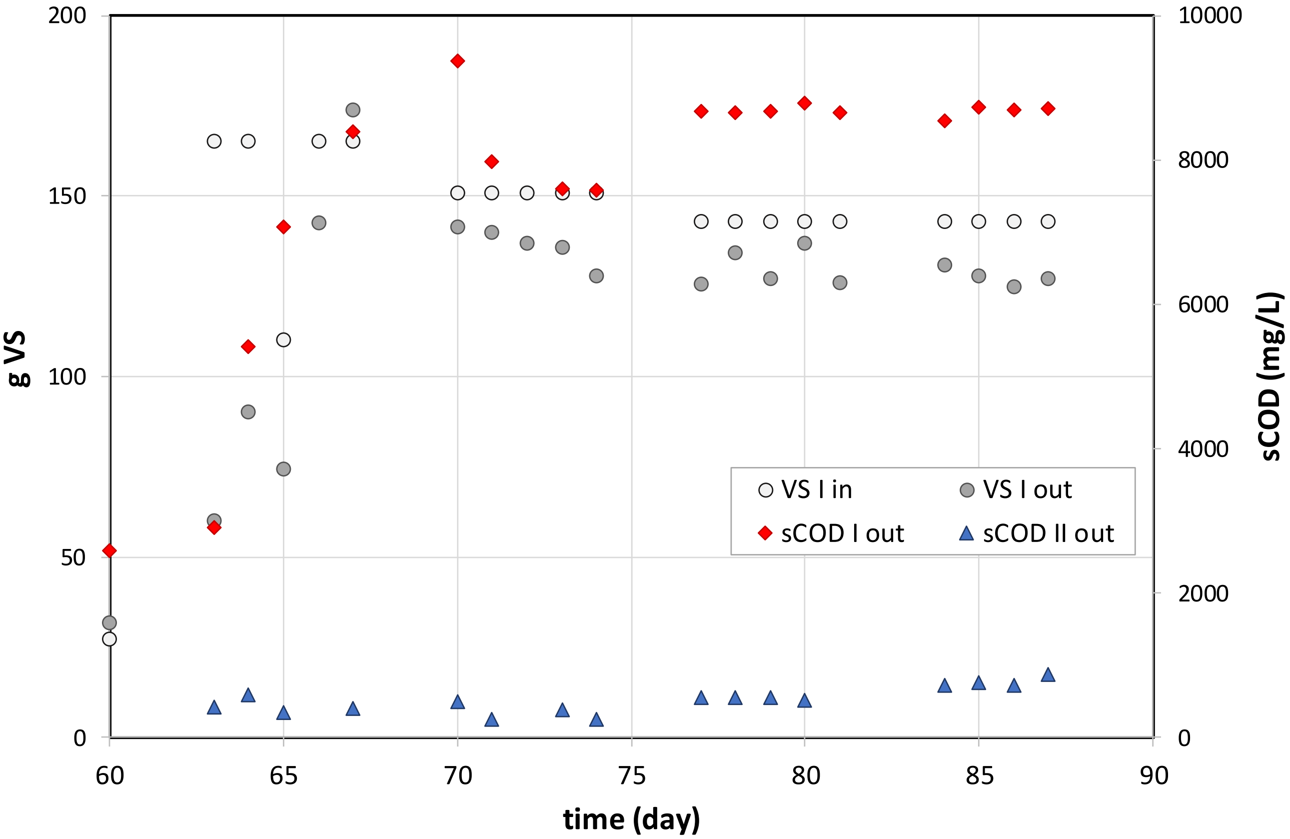


Figure 3. Time course of inlet and outlet VS in the AR (left axis). Trend of the sCOD concentration measured at the exit of the AR (I) and MR (II) (right axis)

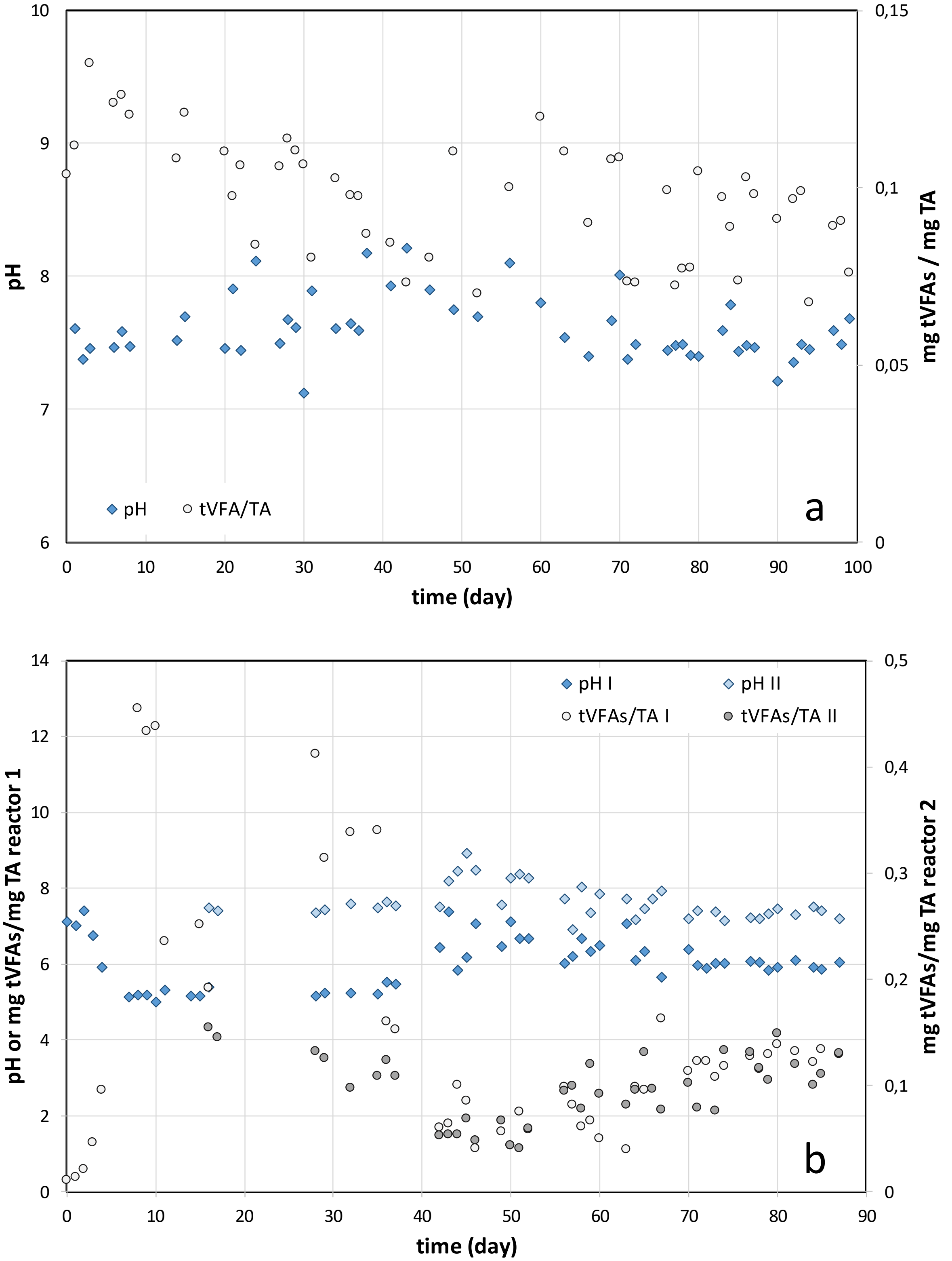


Figure 4. Time course of the pH and tVFAs/TA in the digestate extracted from the one-stage digester (a) and from the TPAD system (b)

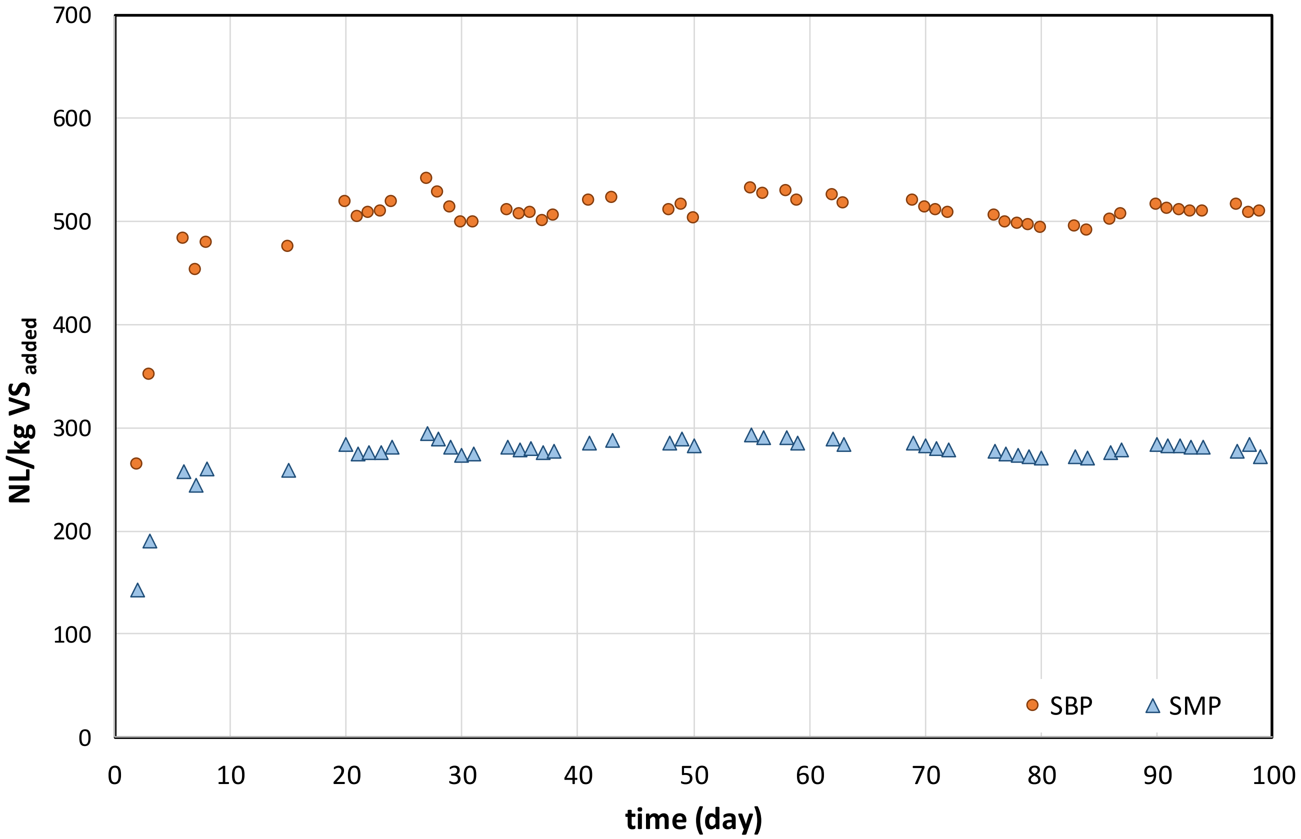


Figure 5. Trend of SBP and SMP from the test carried out in the one-stage digester

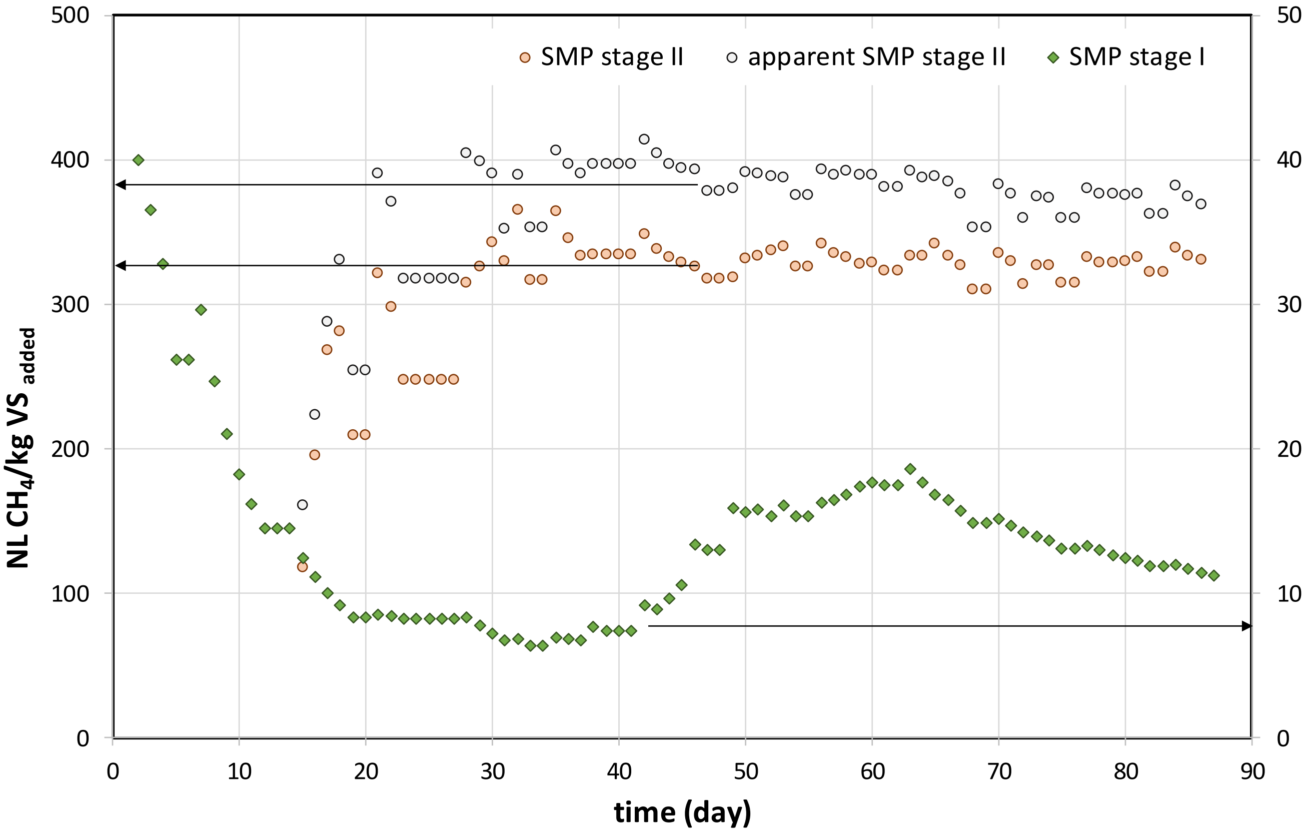
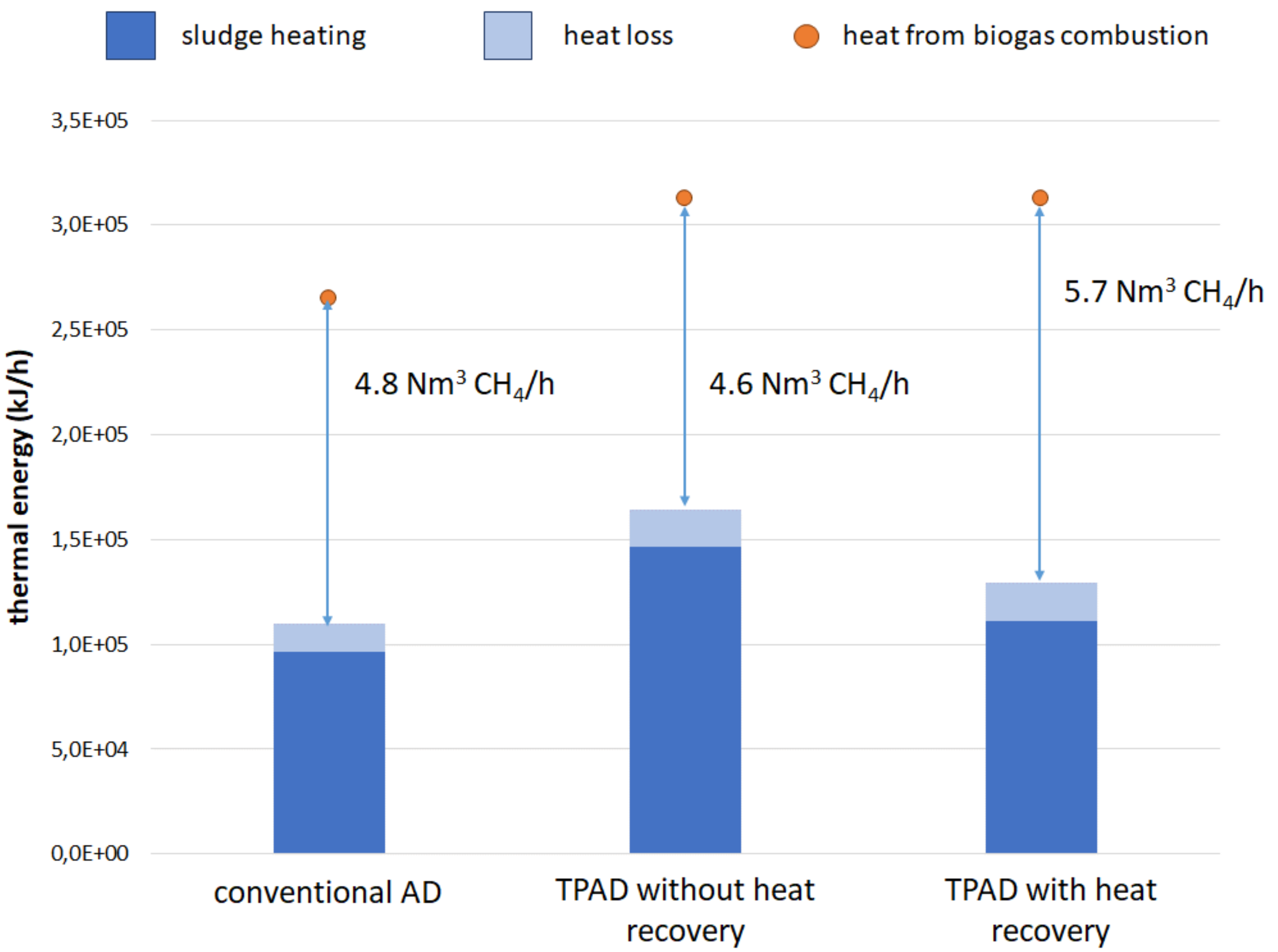


Figure 6. Trend of cumulative SMP in the first and in the second digester of the TPAD system



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649 Figure 7. Results of the energy balance