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Full Length Article

Temperature-phased enhanced the single-stage anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure: Perspectives for the circular economy

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A B S T R A C T

The effect of hydraulic retention time (HRT) on single-stage (mesophilic and thermophilic range) and temperature-phase anaerobic co-digestion processes (TPAD) of sewage sludge, wine vinasse, poultry manure was studied. The HRTs studied were 20, 15, 13 and 10 days for the single-stage and TPAD process. For the TPAD with an HRT of 20 days, 65 % of total chemical oxygen demand (TCOD) were removed, which was higher than the single-stage process (26 % and 17 % TCOD removal, respectively for the thermophilic and mesophilic phases). Regarding the volatile solids (VS) removal, TPAD process presented a higher efficiency (90 %) when compared with the thermophilic (41 %) and mesophilic (43 %) single-stage digesters. At an HRT of 13 days 155 $mLCH_4/gVS_{added}$ (thermophilic), 260 $mLCH_4/gVS_{added}$ (mesophilic), and 202 $mLCH_4/gVS_{added}$ (TPAD) were obtained. The maximum methane yield was achieved for the TPAD process operated at an HRT of 20 days (320 $mLCH_4/gVS_{added}$) with the additional benefit of obtaining hydrogen in the first stage (40.41 mLH_2/gVS_{added}). The digestate obtained from the TPAD process were classified as class A biosolids, and could be used for agriculture fertilizer. In conclusion, TPAD waste management process presented better operational performance and methane yield when compared to a single-stage conventional system, direcly contributing for the framework of a circular economy transition of the agri-food industry.

1. Introduction

Single-stage mesophilic anaerobic digestion is one of the most widely used conventional digestion processes for the treatment of organic waste [1]. It has been widely used for the digestion of sewage sludge to obtain energy in the form of methane [2], However, this technology has some limitations. For example, it requires high HRTs, resulting in partial reduction of volatile solids and pathogens, which leads to a negative use of the resulting biosolids. To overcome these problems, thermophilic anaerobic digestion has been proposed, which has some advantages over mesophilic anaerobic digestion. For example, greater reduction in volatile solids is obtained and higher pathogen removal rates are achieved. However, it also has disadvantages compared to mesophilic ranges, as it requires additional energy to heat the digesters [2].

In general, anaerobic digestion has pH control problems when the process occurs at high levels of OLR, producing negative effects on methanogenic activity [3], so it is necessary to find viable alternatives. To overcome these limitations, anaerobic co-digestion is proposed, which consists of a mixture of various substrates establishing a nutritional balance, determining the appropriate proportion of each substrate when carrying out anaerobic digestion, achieving a better performance of the process. This method provides numerous advantages as it achieves balance and positive synergy in the substrate to be digested, with benefits such as: a better functioning of the biodigester, an adequate C/N

Abbreviations: S, Sewage Sludge; V, Wine Vinasse; PM, Poultry manure; T, Single-stage anaerobic codigestion in thermophilic range; M, Single-stage anaerobic codigestion in mesophilic range; TPAD, Temperature-Phase Anaerobic Digestion; TCOD, Total chemical oxygen demand, in milligrams per liter; SCOD, Soluble chemical oxygen demand, in milligrams per liter; TVFA, Total volatile fatty acids, in milligrams acetic equivalent per liter; TS, Total solids, in grams per liter; VS, Volatile solids, in grams per liter; TAN, Total Ammonial Nitrogen, in grams per liter; C, Carbon; N, Nitrogen; C/N, Carbon/nitrogen ratio; HRT, Hydraulic Retention Time; OLR, Organic Load Rate; VFA/Alk, Volatile fatty acids/alkalinity ratio.

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ratio reducing the concentration of nitrogen and producing better methane yields, it also improves the buffering capacity of the system, increases biodegradability and balances the metabolic activities [4–11]. Therefore, the combination of compatible wastes was considered appropriate, with the possibility of achieving all the reported benefits.

Sewage sludge is a semi-solid waste whose composition depends on the type of treatment system and the origin of the wastewater. In general, it contains large amounts of organic matter, nitrogen and phosphorus, which is interesting from an agronomic point of view [12]. However, they also contain heavy metals and a high load of pathogens, so technologies must be applied that reduce the hazardousness of sewage sludge and allow for its agronomic applicability after treatment [13,14]. Wine vinasse is a waste generated during the production of Brandy de Jerez, more specifically from distillation. This waste has a high organic load measured as chemical oxygen demand (COD) of around 43 g/L and an acid pH of around 3 [15,16]. Poultry manure is a magnificent fertiliser due to its high content of organic matter and nutrients [17]. However, it can not be applied directly to the soil as it requires a prior process of the organic matter degradation and the dose to be applied must be taken into account so as not to damage the crops. The combination of these three wastes, in a suitable proportion, provides a stable equilibrium suitable for anaerobic co-digestion, which allows numerous final benefits to be obtained [18].

On the other hand, temperature-phase anaerobic processes (TPAD) combines the advantages of operating in different temperature ranges getting better efficiencies of organic matter removal and higher methane productivities than single-stage anaerobic digestion [19,20]. This technology can be combined with two-phase anaerobic digestion processes, which proposed the physical separation of acid-formers and methane-formers in two separate reactors, where optimum environmental conditions for each group of organisms would be provided to enhance the overall processstability and control [21].

The resulting process consists of two bioreactors connected in series: the first one operates at thermophilic temperatures (55 °C) and acidic pH (5.5) and the second one is set at mesophilic temperatures (35 °C) and pH around 7.5. Acid-forming microorganisms are favoured in the first reactor and methanogenic bacteria in the second reactor, independently of each other [1,22,23]. The thermophilic digester can improve hydrolysis and acidogenesis, reducing feedstock recalcitrance and increasing metabolism. The mesophilic digester provides stable acetogenesis and methanogenesis at lower temperatures thus reducing toxicity [3,24–28].

Numerous benefits of TPAD have been reported in the literature, the most relevant being: ability to operate stably at higher organic loading rates (OLR), acceleration of the hydrolysis process in the thermophilic stage, greater pathogen inactivation by initially integrating thermophilic temperatures, greater ability to withstand shock loads, and the ability to withstand the effects of the thermophilic stage [1,2], higher vS and volatile fatty acid (VFA) removal rates, increased methane yield and production, reduced foaming, reduced propionic acid and total ammonia nitrogen (TAN) accumulation, and the majority of the process is under mesophilic conditions with consequent energy savings [4,22,24,29,30].

Conventional anaerobic digestion has been used for sludge stabilisation efficiently. However it does not achieve the necessary reduction in pathogens to classify the effluent as class A biosolids when operating at mesophilic temperatures. By choosing a TPAD system it combines both temperature ranges and, by opting for anaerobic co-digestion, sufficiently reduce the parasites and pathogens in the final biosolids. The pathogen requirement to be classified as class A biosolids is defined according to the US EPA and the European Regulation (EU) 2019/1009 of the European Parliament and the Council [31,32]. Therefore, thermophilic anaerobic digestion has advantages in pathogen reduction over mesophilic anaerobic digestion, although it has disadvantages due to VFA accumulation and destabilisation of the process. Two-stage and temperature-phase anaerobic digestion combines the individual advantages provided by each process, in the thermophilic range (pathogen destruction and improved hydrolysis, with increased vS removal) and in the mesophilic range (avoiding VFA accumulation) while avoiding the disadvantages of each, increasing buffer capacity and providing process stability [33].

The objective of this test was to compare the results obtained in two-stage TPAD systems with conventional, single-stage reactors, in mesophilic and thermophilic range separately. For this purpose, the same raw feed conditions were used, with anaerobic co-digestion of sewage sludge, vinasse and poultry manure in a ratio (49.5:49.5:1). The HRTs studied were 20, 15, 13 and 10 days, which in the TPAD systems correspond to HRTs of the first/second stage as follows ((5/15), (5/10), (5/8) and (5/5)), coinciding with the same HRTs of the single-stage reactors. Ultimately, the aim was to combine the advantages and benefits of anaerobic co-digestion and two-phase TPAD and to determine the optimal HRT which maximize vS removal and methane yield. The importance of this work lies in comparing the anaerobic co-digestion of three substrates, in different temperature ranges, with the combination of both in a TPAD system, analysing the most important parameters to assess the most optimal biomethane and biofertiliser production. This study gathers valid information for the design of these systems on a larger scale in the concept of circular economy.

2. Materials and methods

2.1. Substrates and inoculum

Initially the digesters were loaded with a mixture of substrates and inoculum in a ratio of 80:20, which was considered optimal for the start of the process [2].

The inocula used came from two different digesters (thermophilic and mesophilic) of 5 L capacity, operating at a steady state HRT of 20 days and fed with sewage sludge.

The substrates used in the anaerobic co-digestion were sewage sludge, wine vinasse and poultry manure in ratio (S:V:PM) (49.5:49.5:1). This ratio was studied in previous work, where it was determined as the optimal mixture of these three substrates with the help of biochemical hydrogen and methane potential tests [34,35]. The sewage sludge was supplied by the Guadalete wastewater treatment plant and the wine vinasse was supplied by the González Byass winery, both located in Jerez de la Frontera, Cádiz. The poultry manure was

Table 1
Characterisation of the inocula, the substrate mixture for anaerobic co-digestion (feedstock SVPM) and the effluents SVPM with its corresponding inoculum.

Parameters	Thermophilic Inoculum (TI)	Mesophilic Inoculum (MI)	Feedstock (SVPM)	SVPM + TI	SVPM + MI
pH	8.15 ± 0.06	7.96 ± 0.08	4.53 ± 0.10	6.64 ± 0.09	6.48 ± 0.15
TCOD (g/L)	39.02 ± 1.02	44.18 ± 0.93	47.17 ± 1.59	45.49 ± 1.67	45.85 ± 1.83
SCOD (g/L)	25.76 ± 0.88	26.40 ± 0.98	27.83 ± 1.20	26.04 ± 1.44	26.82 ± 1.05
TS (g/L)	26.60 ± 1.41	28.47 ± 1.54	36.74 ± 1.39	33.61 ± 2.01	35.13 ± 1.25
vS (g/L)	18.97 ± 0.86	20.00 ± 1.22	30.18 ± 1.28	26.59 ± 1.33	27.27 ± 1.27
C/N	15.80 ± 1.36	13.69 ± 0.63	43.13 ± 1.74	31.12 ± 1.17	28.35 ± 0.84
TVFA (g/L)	2780 ± 66	519 ± 12	1150 ± 81	2165 ± 94	803 ± 19
Acetic Ac. (g/L)	734 ± 13	346 ± 12	708 ± 20	836 ± 24	341 ± 16
Butyric Ac. (g/L)	345 ± 11	79 ± 9	120 ± 7	309 ± 11	112 ± 6
Propionic Ac. (g/L)	1133 ± 32	34 ± 5	312 ± 14	805 ± 22	278 ± 14

collected from the poultry farm Marta Aragón S.L., located in Chiclana de la Frontera, Cádiz. Table 1 shows the analysed parameters of the inocula, the mixture of substrates, and the mixture of substrates with the thermophilic or mesophilic inoculum, with which the trials were started.

As can be seen in Table 1, both inocula have a high TCOD, being higher in the mesophilic inoculum. The rest of the parameters were very similar. However, the greater quantity of VFA that the thermophilic inoculum contributes to the mixture in relation to the mesophilic inoculum is noteworthy. The consequences of the difference in VFA concentration contributed by the inoculums were detailed in the following section. Finally, the mixture of raw material and inoculum provides an initial C/N ratio of around 30, a recommended value for a balanced anaerobic digestion process [9,15,43,34,36–42].

2.2. Operating conditions

Continuous stirred tank reactors (CSTR) equipped with a heating plate were used for the single-stage anaerobic co-digestion tests. They were programmed at different temperatures, in mesophilic range (35 °C) (M) and in thermophilic range (55 °C) (T). For the TPAD system, two digesters were connected in series, the first one in thermophilic range and the second one in mesophilic range. All digesters had a volume of 3 L, with a working volume of 2 L. The agitation of each digester was set at 40 rpm. All digesters were equipped with a temperature probe and a 5-litre Tedlar bag was connected to hold the biogas produced daily. Samples were taken from the contents of the Tedlar bag with a gas syringe to determine its composition. They also have different ports for the feed inlet and effluent outlet, as well as chemical agents for pH control. Each experiment was carried out in triplicate.

For this study, the influence of increasing OLR in each system was tested by decreasing the HRT. The HRTs studied were 20, 15, 13 and 10 days, which in the TPAD systems correspond to HRTs of the first/second stage as follows ((5/15), (5/10), (5/8) and (5/5)), coinciding with the same HRTs of the single-stage. The operating parameters were shown in Table 2.

All the operating conditions shown in Table 2 were maintained until steady state were reached. The achievement of steady state was verified after an initial period (three times the HRT) by checking whether the characteristic values of the effluent were still in the average of the previous measurements [2].

2.3. Analytical methods

An initial characterization of the substrates and the feed was carried out, in terms of pH, total solids (TS), volatile solids (VS), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), volatile fatty acids (VFA), total ammonia nitrogen (TAN), ammonia, alkalinity and carbon/nitrogen ratio (C/N ratio).

For TS, vS TCOD and SCOD parameters, the Standard Methods APHA-AWWA-WPFC [44] were followed. For the characterization of the pH, a HACH sensION + pH meter was used. The individual VFAs were determined by gas chromatography, using a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector (FID) system and a capillary column packed with Nukol [44,45]. Acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic and heptanoic acids were quantified in mg/L and the total content of TVFA acids expressed as mgAcHequivalent/L was calculated. For the characterization of both total organic carbon and total nitrogen, a total organic carbon analyzer (Shimadzu TOC-L CSH/CSN) was used, according to the standard APHA-AWWA-WPFC methods [44]. Alkalinity, total ammonia nitrogen (TAN) and ammonia were measured using the HANNA multiparameter photometer (HI83399), following the standard APHA-AWWA-WPFC methods [44]. The volume and composition of the biogas produced was measured daily using a Ritter TG1 gas flow meter and KNF Laboport gas suction pump. The composition of the biogas was analyzed with a gas chromatograph (Shimadzu GC-2010) equipped with a thermal conductivity detector (TCD). The colum used to determine the composition of H_2, CO_2 and CH_4 was a Supelco Carboxen 1010 plot column [8].

Table 2
Operating conditions tested in the different systems.

HRT (days)	20	15	13	10
OLR ($gVSL^{-1}d$)	3.02	4.02	4.65	6.04
Flow rate (mL/d)	100	133	154	200

2.4. Class A biosolids

The analysis of *Salmonella* and *Faecal Coliform* density was carried out in the effluent of the digesters at the end of the stable periods of each HRT tested in each system, with the objective of knowing their possible classification as class A biosolids. *Total Coliforms*, *Escherichia Coli (E-coli)* and *Salmonella* were quantified, as well as the degree of pathogens removal in the effluents. A quantity of <1000 most probable number (MPN) *Faecal Coliforms*/gTS, 3(MNP) *Salmonella*/gTS and that the density of *E-coli* in the effluent does not exceed 1000 colony forming units (CFU)/gTS and that *Salmonella* must be absent in 25 mL of sample in an organic fertilizer [8,12,31–33,46–48]. The determination of *total coliforms, E-Coli* [Method 9222H] and *Salmonella* [Method 9260B] was carried out according to standard methods [44]. *Total coliforms* were calculated through *E-Coli*, since it represents approximately 90 % of them [8].

2.5. Statistical analysis

To analize the correlation between the different HRTs tested and the performance of each system in terms of methane production, methane yield and vS removal, Pearson's correlation coefficients and their p-values were studied. A significant correlation was declared at $p < 0.05$. Correlations were determined from the data of each TPAD System and the data of the thermophilic and mesophilic digesters separately. In addition, the means of methane yields, vS removal and HRT decline were compared in each case. Statistical significance was declared at $p < 0.05$.

3. Results and discussion

3.1. Effluent characterisation and process stability

The stability of the three anaerobic co-digestion systems studied (thermophilic monostage, mesophilic monostage and the TPAD system) was measured by monitoring pH evolution, VFA evolution, alkalinity, VFA/Alk ratio and TAN concentration. Fig. 1 shows the different parameters analysed throughout the process as HRT decreased in each of them.

The pH values in the thermophilic and mesophilic single-stage effluents tended to decrease as the HRT decreased. On several occasions it was necessary to correct the low pH with NaOH (10 M). The digester operating under thermophilic conditions presented the most problems in terms of pH stability, constantly decreasing its value for the HRT of 15 and 13 days, requiring adjustment, as can be seen in Fig. 1a. Regarding the TPAD system, it presented a stable pH throughout the whole process, in an average range of 7.5 and 7.9. There was no need to adjust the pH at any time.

Single-stage thermophilic anaerobic co-digestion presented problems of VFA accumulation during the whole process, with values around 5000 mg/L at 15-day HRT (Fig. 1b). The VFA production was 4 times higher than that of the supplied feed. The high temperatures were conducive to this, causing pH fluctuations and affecting the instability of the process. The thermophilic inoculum also negatively affected VFA accumulation, as it had a higher contribution than the mesophilic inoculum (2780 mg/L and 519 mg/L, respectively). The excessive VFA

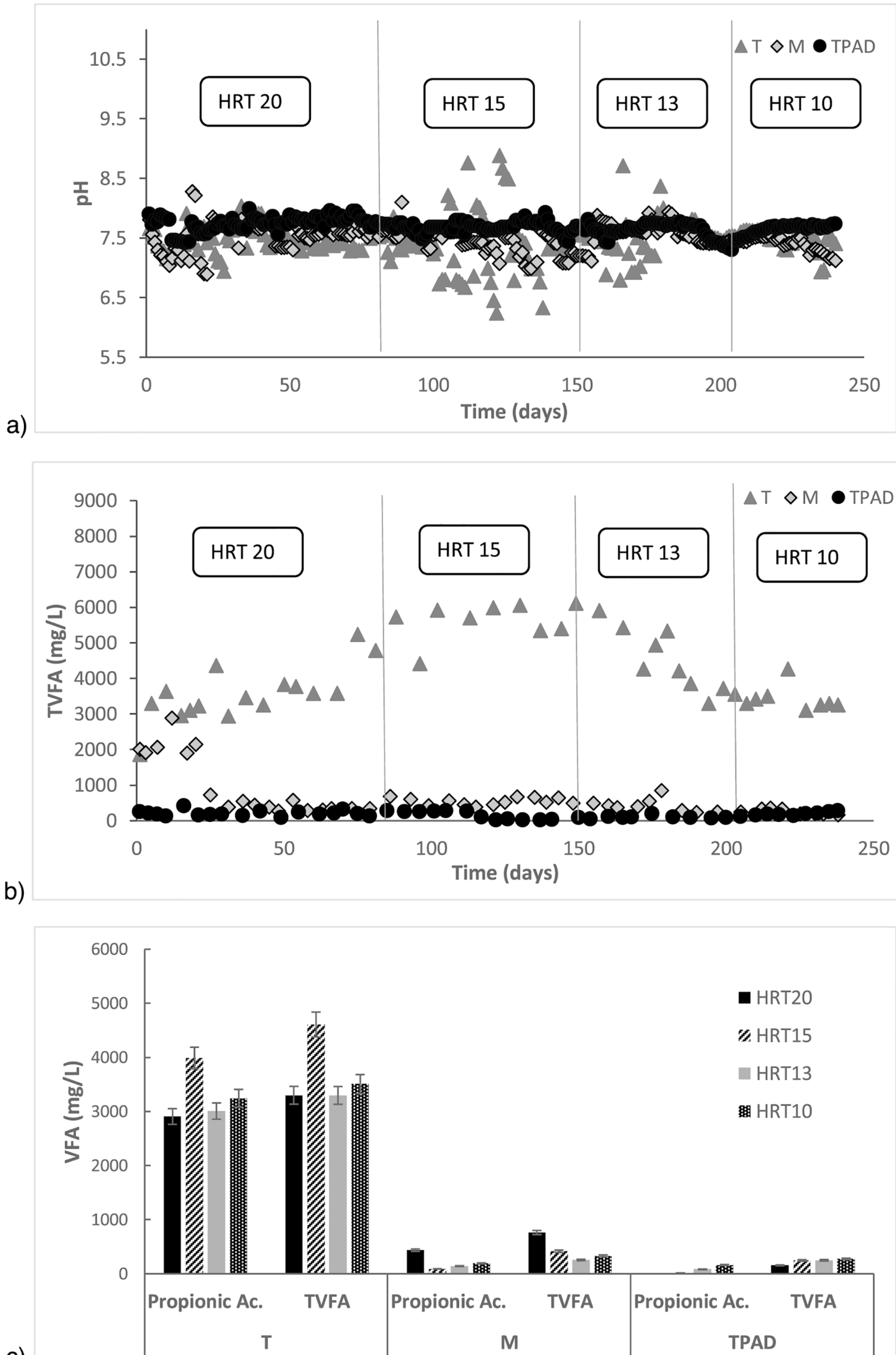

Fig. 1. Evolution parameters measured throughout the whole process of the different experiments (thermophilic single-stage digesters (T), mesophilic single-stage digesters (M), and the TPAD system), in the different HRT tested (20, 15, 13 and 10 days). a) pH evolution, b) VFA concentration evolution, c) propionic acid concentration in reference to the total VFA registered, d) alkalinity evolution, e) VFA/Alk ratio and f) TAN concentration evolution.

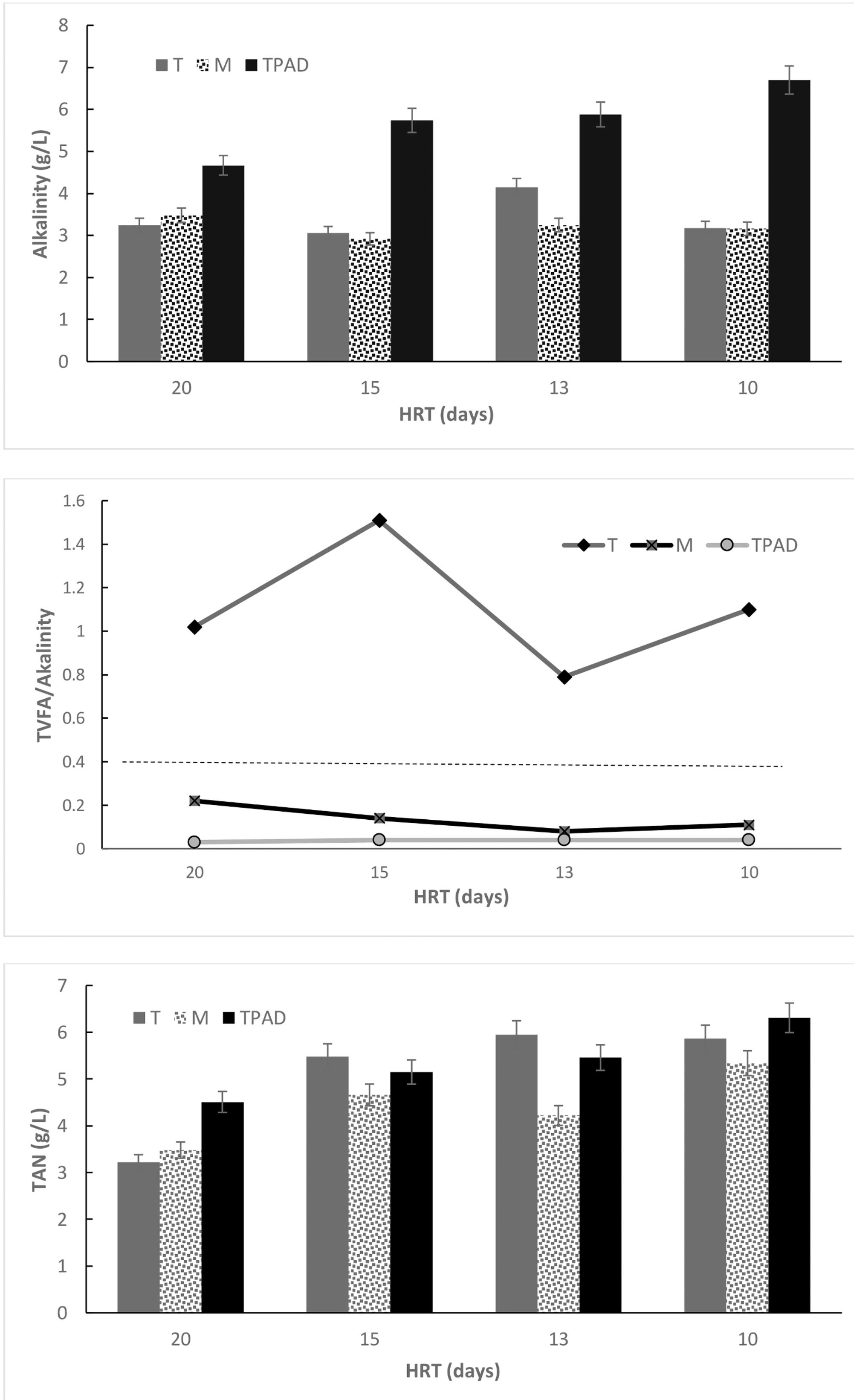

Fig. 1. (*continued*).

accumulation caused a decrease in biogas generation and a loss of reactor stability [24], as will be discussed in the next section. The high total VFA concentration fluctuations under thermophilic conditions in a single stage reflects a kinetic imbalance between acid producers and consumers which is typical for systems under stress conditions [29]. Propionic acid accumulation when operating in the thermophilic range

(Fig. 1c) caused reactor failure in single-stage processes [15]. However, mesophilic conditions favoured the elimination of VFA for all the HRT tested, showing values below 1000 mg/L throughout the process. On the other hand, anaerobic co-digestion in TPAD showed the highest VFA removal values. Considering that the TVFA values at the end of the first stage ranged between 6000 and 7000 mg/L, removal percentages higher than 76 % were achieved for the shortest HRT, reaching up to 87 % removal in the effluent for the 20-day HRT, showing a very low concentration of VFA in the effluent throughout the process. It was worth noting the absence of propionic acid at 20-day HRT and as the HRT decreases, propionic acid starts to appear in small quantities in the effluent of the methanogenic digesters. This again demonstrates the stability of the TPAD process against both mesophilic and thermophilic single-stage anaerobic co-digestion. Although the VFA values for the mesophilic and TPAD digesters were always below the described inhibition values [4,49].

Total alkalinity was an indicator of the buffering capacity of the system and was monitored throughout the procedure for the one and two-stage digesters. The results were shown in Fig. 1d. For the thermophilic digesters, the alkalinity presented stable values between 3.06 and 4.15 g/L throughout the process, being the highest at 13 days of HRT and decreasing for a 10-day HRT. For the mesophilic digesters, alkalinity was also very stable in all the tested HRTs with a maximum of 3.48 in the 20-day HRT and a minimum of 2.92 g/L for the 15-day HRT. However, the TPAD system presented a different trend, increasing the alkalinity concentration in the shorter HRTs, from 4.67 g/L to 6.70 g/L in the 10-day HRT. The higher alkalinity values recorded in the TPAD system compared to the single-stage digesters reflect higher degradation activity towards nitrogenous compounds [2]. Stability in the digester was described with an alkalinity between 2 and 5gL [1,50], however, higher values were obtained in the effluents of the TPAD systems. In the first stage, where hydrolysis and acidogenesis occurs, there was a concumption of alkalinity, registering an average of 1.08 g/L in the acidogenic digester effluents, while in the second stage, during methanogenesis, alkalinity was produced and acidity was compensated. The high alkalinity in the TPAD system counteracts the increase in VFA by keeping the pH constant throughout the process and in all the HRTs tested, demonstrating the buffering capacity of the system and, thus, favouring the development of methanogenic archaea [49]. This allows stable operation at lower HRT, resulting in higher biomethane production without pH fluctuations [27].

The ratio of VFA to alkalinity of the three tested systems for the different HRTs were monitored to compare the buffering capacities of the pH changes that occurred (Fig. 1e). It was known that the buffering capacity of the system was sufficient when the VFA/Alk ratio was below 0.4. and furthermore, it was recommended that this value was around 0.1 [2,28,51,52]. As can be seen in Fig. 1e, thermophilic anaerobic co-digestion presented a VFA/Alkalinity ratio well above the recommended values, with a high accumulation of VFA and revealing a high instability of the system. For mesophilic anaerobic co-digestion, all values were below 0.4, presenting a maximum value at 20-day HRT (0.22) and dropping to 0.08 for 13-day HRT. This parameter reflected the stability of the system under mesophilic conditions. Finally, the TPAD process was the most stable, with values between 0.03 and 0.04 during the whole process. Therefore, it can be stated that the TPAD system was much more resistant to increases in OLR (decreases in HRT) demonstrating the robustness and robustness of the process throughout the experiment.

TAN control during the process was a very important factor, due to the poultry manure cosubstrate, which contributes significant amounts to the feed. The TAN concentration was an indicator of the buffering capacity of the system. At adequate concentrations >500 mg/L, they ensure successful operation, providing a source of nitrogen to methanogenic microorganisms using acetate [27]. However, the concentration of TAN that produces inhibition should be studied, as it depends on the degree of acclimatisation of the microorganisms. Wide ranges of inhibition have been described (1.7–14 g/L) [19,53,54]. In this study, the values recorded in all the systems tested were within this wide range but their concentration caused different reactions in each system. In the single-stage thermophilic digesters, the high TAN concentration affected the propionate-utilising microorganisms, causing the propionic acid accumulation in the system. High temperatures were related to TAN accumulation and fluctuations in pH, leading to imbalances in the anaerobic co-digestion process [55]. This also had consequences on the methanogenic activity, causing inhibition [27,56,57]. With the increase in OLR came an increase in propionic acid production exceeding its consumption rate, which was a slow process requiring high HRT [56,58]. This affected the thermophilic digesters, which was the least stable system with the highest VFA/Alk ratio, above the values established as limits for a stable system with high buffering capacity. In the mesophilic digesters, no inhibition of methanogenic activity by TAN accumulation was observed, reaching values close to 6 g/L for a HRT of 13 days, where the maximum methane yield was recorded for this system, as will be seen in the next section. The inhibition of methane yield in the thermophilic reactors at the same TAN concentration as in the mesophilic reactors may be due to the effect of higher temperatures which would lead to higher concentrations of free ammonia nitrogen, causing higher toxicity for the thermophilic anaerobic co-digestion system [55]. TPAD systems are an alternative to alleviate the toxicity caused by TAN accumulation, being more stable systems, with higher buffering capacity that did not suffer inhibition in methanogenic activity at TAN concentrations around 4.5 g/L.

In summary, for all the parameters analysed, the TPAD system was more stable and showed higher buffering capacity than the single-stage systems. And among the single-stage systems, the one that operates in mesophilic ranges showed better results and higher stability than the thermophilic anaerobic co-digestion system.

3.2. Removal efficiencies

The performance of the digesters in terms of solids and COD removal was presented in Fig. 2. For the single-stage digesters, at long HRTs of 20 and 15 days, those operating in the mesophilic range were able to remove a higher percentage of COD than the thermophilic digesters, however for 13-day HRT, the thermophilic digesters removed a higher percentage of COD. When compared to the TPAD system, the TPAD system was superior in all tested HRTs, except for the 13-day HRT, where the thermophilic digesters were superior. In general, for the three systems, for HRT below 10 days, the COD elimination percentages decrease. Thus, an increase in the OLR caused a decrease in the elimination of COD, which indicates that the degradation capacity of the microorganisms decreases.

Fig. 2b shows the vS removal data average for each HRT tested and for each assay. For the single-stage digesters, similar vS removals were observed for all tested HRTs, being higher in the 13-day HRT with values around 57 %. The TPAD system was far superior in terms of vS removal in all tested HRTs, reaching values of 90 % for HRTs of 20 and 15 days, and >80 % in shorter HRTs of 13 and 10 days. This can be explained by the first stage of the TPAD which aids the solubilisation of the particulate fraction of the sludge [46]. The improved performance in terms of vS removal in the TPAD system was mainly attributed to the enhanced hydrolytic activity that occurred in the thermophilic digesters that make up the first stage of the process. The TPAD system was able to dissolve the particulate organic material and therefore, the vS reductions in the TPAD system were high and stable throughout the process [29], confirming the advantages of TPAD systems over single-stage digesters.

In general, for all three systems, the vS removal rates were relatively constant throughout the process. This shows that the increase in OLR does not affect the degradation microorganism activity. The increase in vS reduction enhancement was directly correlated with methane production [1]. This coincides with the results obtained, where the highest methane yield coincides with the maximum vS removal value in each

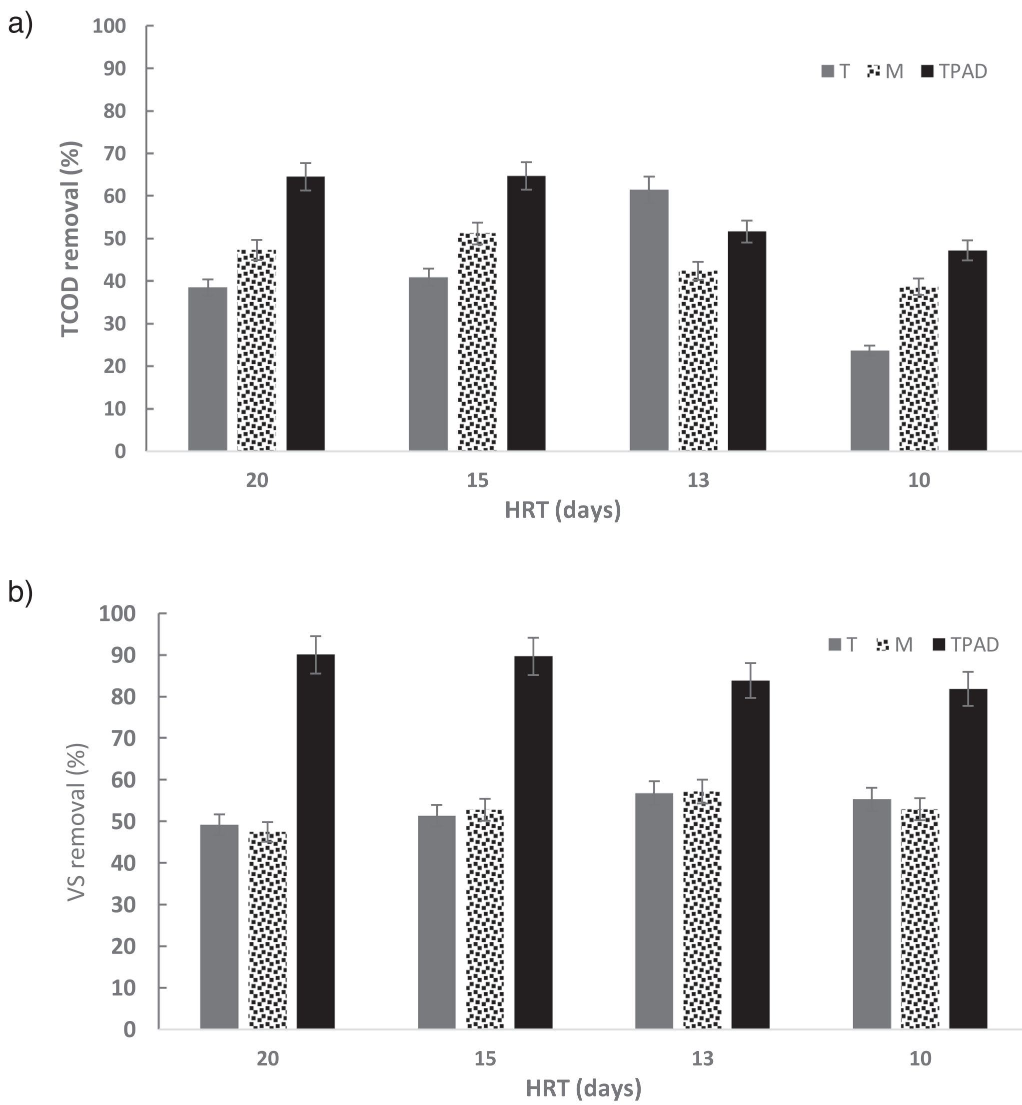

Fig. 2. Average removal efficiencies recorded in each trial, a) evolution of % TCOD removal, b) evolution of % vS removal.

system, for the single-stage digesters for a 13-day HRT and for TPAD a 20-day HRT.

In summary, for 20-day HRT, the TPAD system reached 26 % and 17 % more TCOD removal than the single-stage thermophilic and mesophilic systems, respectively. Regarding the percentage of vS removal, this was 41 % and 43 % higher for the TPAD system than for the thermophilic and mesophilic single-stage systems, respectively. As with the anaerobic co-digestion of the mixture of sewage sludge, vinasse and poultry manure, where the COD and vS removal rates were higher for the TPAD system, compared to the mesophilic and thermophilic single-stage systems, vS removal rates Close to 72.6 % were found for 10/10 TPAD systems of anaerobic co-digestion of sludge and beet pulp compared to 46.8 % and 40.5 % of SV removal in the corresponding thermophilic and mesophilic monodigestion processes, respectively [2]. Simmilarly, for the anaerobic digestion of the organic fraction of municipal solid waste, a TPAD system showed 15 % higher COD removal than a mesophilic monostage system, and 34 % higher vS removal than a thermophilic monostage system [59].

3.3. Yields and biogas production

The average methane yield in each HRT produced for the single-stage reactors (thermophilic and mesophilic) and that produced in the second stage of the TPAD were shown in Fig. 3.

The methane yields varied greatly depending on the type of reactor and the HRT tested. For the single-stage thermophilic digesters, the maximum yield was obtained at 13-day HRT with a value of 155mLCH_4/gVS_{added}, this system being the one that reported the lowest methane yield data. The system showed a trend of increasing performance as HRT decreased to 13-day values, after which it began to decrease for shorter HRT conditions. The mesophilic single-stage digesters recorded the best average methane yield data at 13-day HRT with values of 261mLCH_4/gVS_{added}. This trend was similar in single-stage thermophilic digesters, showing an increase in yield as HRT decreased up to 13 days and then yield decreased at shorter HRT. Thus, it was observed that as the HRT decreased below 10 days, there was a drastic inhibition of methane production that caused the failure of the digesters (data not shown). Comparing these results with those of TPAD, it can be seen that this system recorded the highest yield values (320mLCH_4/gVS_{added}), at HRT of 20 days (HRT first/second stage of 5/15 days). Unlike the mesophilic digesters, the tendency was to decrease the yield as the HRT decreased, obtaining 185mLCH_4/gVS_{added} in the shortest HRT, and a higher value than all the records of the single-stage thermophilic digesters.

Another notable difference was the high percentage of methane contained in the biogas for the case of the second stage in TPAD compared to the single-stage systems. For the second stage, TPAD at 20-

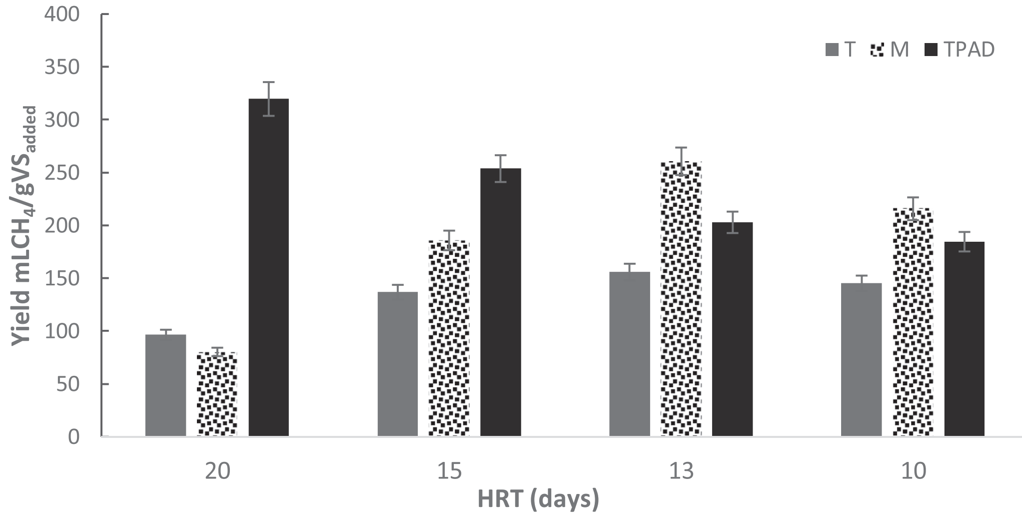

Fig. 3. Methane yields for the different experiments in each HRT tested.

day HRT the average value was 67.2 % compared to 60.8 % in the single-stage mesophilic digesters and 56.3 % in the single-stage thermophilic digesters for a 13-day HRT.

Determining the production and yield of methane in each experiment was necessary to identify the optimal HRT in each system and to be able to compare its performance for the same substrate as feed. Under these conditions, it was determined that methane yield is related to the degree of organic matter solubilization and that higher yield values correspond to higher SV removal rates in each study. In contrast to the results obtained by Aslanzadeh et al. (2014), [29], the best results in methane yields for TPAD were observed at longer HTRs (20 days). This trend was also observed by Ariunbaatar et al (2014) [27] for TPAD systems, which decreased methane yield as OLR increased. Montañes et al. (2016), [2] found similar behaviour between single-stage mesophilic and TPAD digesters.

The TPAD system has another very important advantage over single-stage anaerobic digestion processes due to the phase separation of microorganisms: the production of biohydrogen in the first stage of the TPAD process. Thus, hydrogen was obtained by the action of the acidogenic bacteria in the first stage and methane in the second stage by the action of the methanogenic *archaea*. By separating the phases, a more stable system was achieved, allowing for optimal conditions for each microbial population separately [24]. For a HRT of 5 days in the acidogenic stage, an average hydrogen yield of 40.41 mLH_2/gVS was recorded. This hydrogen production can be exploited individually as it has a high energy density of 141 MJ/kg [60]. Likewise, it could also be converted into methane (methanation) by means of the Sabatier reaction [61,62] or combined with methane, receiving the name of biohythane, composed of 10 % hydrogen, 60 % methane and 30 % carbon dioxide. The production of biohythane in a two-stage process has an energy recovery of between 10 and 43 %. The combination of these gases is a form of potential energy, generating more energy with less pollutants [60,63].

In summary, the TPAD systems applied to the anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure show greater stability compared to single-stage systems, with the thermophilic system being the one with the greatest fluctuations in operating parameters and a lower methane production yield. It was confirmed that TPAD systems with mixed temperature ranges were a viable alternative to solve the operational problems of single-stage anaerobic co-digestion processes, in addition to presenting higher energy yields.

3.4. Class A biosolids

An analysis of the effluent was carried out under stable conditions to determine the removal of pathogens during the process and to check if it could be classified as Class A biosolids according to US EPA and European legislation (Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019). In the first place, regarding the analyzes for the detection of *Salmonella*, the presence of *Salmonella* was found in the feed of the reactors. However, all the reactors showed absence in the effluents. This suggests that the anaerobic co-digestion of the selected mixture was effective in the elimination of *Salmonella* in all the HRTs studied. Regarding the results obtained for *E-coli* and *Total Coliforms*, the feed of the acidogenic reactors and the effluents for all the HRTs tested in the methanogenic reactors were also analyzed. *Total Coliforms* determined include *Faecal Coliforms* and *E-Coli*. To check the possible classification of the effluent as class A biosolids, the initial concentration of *E-coli* and *Total Coliforms* in the feed was determined. The results obtained were 19325 CFU/gTS and 29460 CFU/gTS for E-Coli and Total Coliforms, respectively.

The results of the *E-coli* and *Total Coliform* concentrations found for the effluents from the mesophilic and TPAD digesters for each HRT tested were shown in Fig. 4.

No pathogens were detected in the thermophilic digesters. The high temperatures contribute to the complete sanitisation of the effluent. Other authors also witnessed this fact when working in thermophilic temperature ranges [33]. However, this did not occur for the mesophilic digesters and also not for the TPAD process.

The same trend was observed in both processes, with the concentration of *E-coli* in the effluent increasing as the HRT decreases. However, values of 1000 CFU/gTS were not reached in any digester for all tested HRT.

Regarding Total Coliforms, it was observed that the concentration increases as HRT decreases for M and TPAD, reaching $>$1000 CFU/gTS in the mesophilic digesters. Therefore, for HRT $<$13 days it would not be possible to use the mesophilic effluent for agronomic purposes as a class A biosolid, although it could be used as a class B biosolid.

These results indicate that both the anaerobic co-digestion of sewage sludge, vinasse and chicken manure in a single stage, either mesophilic or thermophilic, and in two stages, can be considered adequate methods for the production of class A biosolids, reducing and even eliminating health risks in its subsequent use in agriculture.

On the other hand, for mesophilic anaerobic codigestion, it is confirmed that mixing different substrates adequately allows the HRT to be reduced below 20 days [54] and reach up to 13 days, producing class

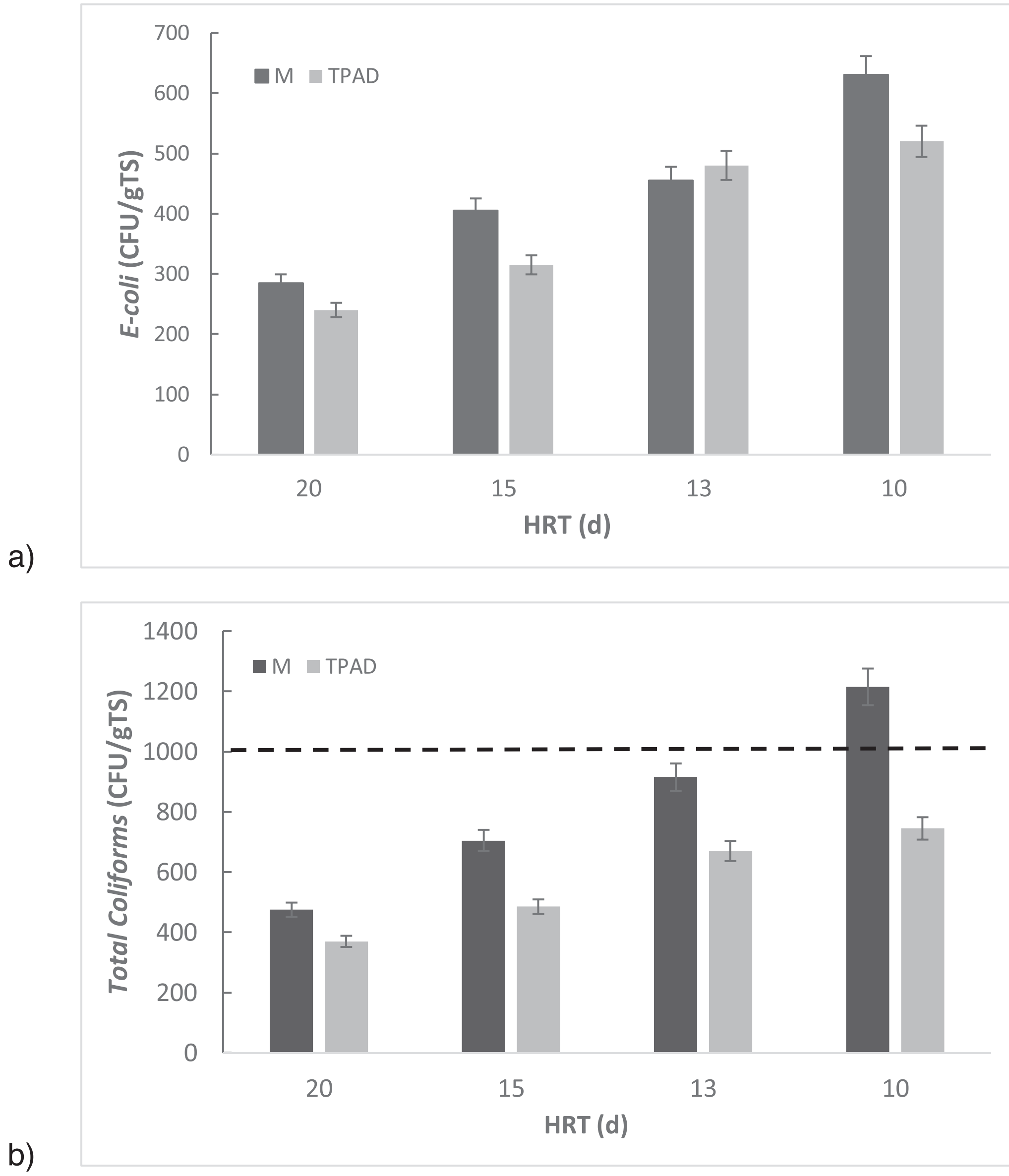

Fig. 4. Pathogen concentration in the effluent for M and TPAD a) *E-coli* concentration, b) *Total Coliform* concentration.

A biosolids. Below this HRT, class B biosolids would be produced.

3.5. Statistical analysis

The results obtained from the statistical analysis showed large differences between the contrasting technologies. Pearson's correlation showed a strong positive and significant correlation in the case of TPAD, between HRT and methane yield (Pearson's coefficient = 0.983, $p < 0.05$) and vS removal (Pearson's coefficient = 0.883, $p < 0.05$). A strong positive correlation was also observed between methane yield and vS removal (Pearson's coefficient = 0.9099, $p < 0.05$). However, for the single-stage anaerobic digestion system, both thermophilic and mesophilic, the correlations of methane yield with decreased HRT were negative (Pearson's coefficient = -0.853, Pearson's coefficient = -0.889, $p < 0.05$, respectively). Comparison of the differences between the means of each trial was significant ($p < 0.05$). This means that TPAD systems respond more predictively to external changes, showing a strong resistance when HRT decreases and OLR increases, showing a strong positive correlation, and that by determining the optimal operating conditions, the stability of the process was ensured by avoiding abrupt changes in system behaviour. In contrast to the single-stage anaerobic digestion, which was more sensitive to changes in OLR or HRT, showing a different response in process performances with a negative correlation in this respect.

The results obtained in this study for the TPAD technology were of great importance to contribute to the circular economy model. Firstly, the waste was collected in areas very close to each other, saving on transport and sharing waste management facilities. In addition to solving an environmental problem of waste management, a sustainable valorisation would be achieved that allows obtaining high added value products such as biohydrogen, biomethane and biofertiliser, suitable for self-supplying the treatment plant and the recovery of natural nutrients in the surrounding crops. Thus closing the circle in the concept of the circular economy.

4. Conclusions

Experimental results have shown significant advantages in the performance of the TPAD system compared to single-stage processes for the anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure (in the thermophilic and mesophilic range).

Regarding the stability of the process, the TPAD process showed constant pH values with low VFA concentration in the effluent, maintaining a constant VFA/Alk ratio throughout the operation and a high buffer capacity against the increase in OLR.

The purification efficiency in terms of removal of TCOD and vS was higher for the TPAD compared to that of the systems that operate in a single stage. For TPAD in HRT of 20 days, elimination values of 65 % TCOD and 90 % vS were reached. This result was 26 % and 17 % higher than the %TCOD and 41 % and 43 % higher than the %vS of the single-stage thermophilic and mesophilic digesters, respectively.

Methane yield showed the highest TPAD values in 20-day HRT, reaching 320mLCH_4/gVS_{added} and 67.2 % CH_4 in biogas. This value was higher than the yields achieved by the thermophilic and mesophilic digesters at HRT of 13 days (260 and 155mLCH_4/gVS_{added}). In addition, average hydrogen yield values of 40.41 mLH_2/gVS_{added} for a fixed HRT of 5 days were achieved in the first TPAD stage.

The classification of the effluents as class A biosolids was possible in the most cases (TPAD and single-stage thermophilic and mesophilic) for all HRTs tested, except for 10-day HRTs in single-stage mesophilic reactors, where the effluent can be classified as class B effluent.

Therefore, the TPAD system allows obtaining higher removal efficiencies, higher methane yields as well as the possibility of obtaining biohythane. The effluent obtained can be classified as class A biosolids, without stability problems, being able to verify the high buffering capacity and robustness of the system compared to systems that work in a single stage. In conclusion, TPAD waste management process presented better operational performance and methane yield when compared to a single-stage conventional system, direcly contributing for the framework of a circular economy transition of the agri-food industry.

CRediT authorship contribution statement

Leonor Sillero: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Montserrat Perez:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Rosario Solera:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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