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Improvement of the anaerobic digestion of sewage sludge by co-digestion with wine vinasse and poultry manure: Effect of different hydraulic retention times

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ABSTRACT

This study arose in response to the management of different organic wastes generated locally, which cause environmental damage. These wastes could be used to obtain biogas and class A biosolids through anaerobic digestion. For this purpose, the mesophilic anaerobic tri-digestion of sewage sludge (S), wine vinasse (V) and poultry manure (PM) (49.5:49.5:1) was studied to obtain biogas in an improved way compared to bi-digestion of SV (50:50) and mono-digestion of S. Tests were carried out in anaerobic digesters, at laboratory scale, to compare the benefits at different hydraulic retention time (HRT) (20, 15, 13, 10, 8 and 6 days). From the results obtained, anaerobic tri-digestion of the waste gave the best results, with a total chemical oxygen demand (TCOD) removal efficiency of 51%, volatile solids (VS) removal efficiency of 57%, and methane yield values of 261 $mLCH_4/gVS_{added}$ at a HRT of 13 days. In relation to the pathogens, both co-digestion studies addressed managed to inactivate enough pathogens in the effluent to be classified as class A biosolids. This would allow considering the anaerobic tri-digestion as a promising technology, which allows valorizing organic waste, obtaining biogas and class A biosolids, moving towards a circular bioeconomy.

1. Introduction

One of the environmental problems in southern Spain is the generation of different organic wastes, which cause local pollution and greenhouse gases emissions, damaging the environment. Among them are sewage sludge, with high organic content and pathogens, mainly. On the other hand, there is agri-food waste, such as wine vinasse and poultry manure, respectively produced in wineries as a result of distillation during wine production and poultry farms dedicated to the export of meat [1–3]. One of the alternative for the management of these wastes can be anaerobic digestion [2,4–7]. The anaerobic digestion consists of a biological process in the absence of oxygen that converts biodegradable organic matter into biogas and a biosolids that can be used for agronomic purposes [1,8–10]. The biogas produced is mainly a mixture of methane and carbon dioxide.

Sewage sludge is one of the most profitable substrates in biogas production through anaerobic digestion [11–13]. However, it has been shown that with the combination with other residues that complement and balance them, better results of methane yield are obtained at the end of the process. This variant is called anaerobic co-digestion, and with it many advantages can be obtained over anaerobic mono-digestion. Some of these advantages would be: improved performance and biogas production, greater reduction of volatile solids (VS), greater biodegradability of organic matter, improved dilution and stabilization of heavy metals and other toxins, nutrient balance and therefore a suitable C/N ratio [1,8,11,14–20]. Anaerobic co-digestion helps to increase the effectiveness of anaerobic digestion and also achieves bioenergy production, technological and economic savings by sharing treatment

Abbreviations: S, Sewage Sludge; V, Wine Vinasse; PM, Poultry manure; RS, Single mesophilic reactor fed with sewage sludge; RSV, Single mesophilic reactor fed with sewage sludge and wine vinasse; RSVPM, Single mesophilic reactor fed with sewage sludge, Wine vinasse and poultry manure; 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 Loading Rate; LR, Loading Rate; VFA/Alk, Volatile Fatty Acids/Alkalinity ratio.

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facilities for different wastes [8,16,17,21–23]. Some examples of improving digestion by anaerobic co-digestion are found in the literature, using sewage sludge with one or more other co-substrates and obtaining good results. Some co-substrates were wine vinasse [24–26], sugar beet pulp [27], organic fraction of urban solid waste [18], food waste [28,29], organic fraction of municipal solid waste and grease trap [17], agricultural straw waste [30], and pig manure [31].

However, it is necessary to select the wastes to be co-digested according to their physicochemical characteristics and their generation, considering both the volume and the frequency and location of the facilities, with the idea of proposing their joint management. For this purpose, in Andalusia community (Spain), wine vinasse and poultry manure are available, which meet the requirements indicated. All three substrates have a high organic load and very significant differences in terms of pH and nutrient concentration. For example, wine vinasse has a very acidic pH (around 3), which is offset by the pH of poultry manure (close to 10). Another example would be the low nitrogen concentration in sewage sludge and wine vinasse. Poultry manure would be the perfect co-substrate to supplement the nutrient deficiencies of sewage sludge and wine vinasse. However, in order to carry out the anaerobic co-digestion of these three wastes, the appropriate ratio must be studied to favour this process and achieve maximum yields in methane production [32].

On the other hand, at the end of the anaerobic co-digestion a biosolid is obtained whose application to the soil provides organic matter and nutrients that help to improve the physical and biological properties of the soils, providing improvements in the ecosystem. The application of biosolids to the soil offers numerous benefits but at the same time, it can contain components that are dangerous for the environment, animals or human health. These components are pathogens and heavy metals that must be analyzed before agronomic use [21,33,34]. Depending on the set operating temperature and hydraulic retention time (HRT), this process can take hours or days [33]. It is intended that this end product of anaerobic co-digestion can be classified as class A biosolids, for this it is necessary to comply with the legal requirements indicated by the United States Environmental Protection Agency (US EPA) and Regulation (EU) 2019/1009 of the European Parliament and the Council of June 5, 2019 [35–37].

The anaerobic co-digestion of sewage sludge with residues from the agri-food sector represents an attractive technology within the framework of the circular economy. It is a low-cost, well-established technology for the treatment of organic waste. Anaerobic co-digestion is a promising technique for producing biofuels due to obtaining biogas as clean energy and stabilized sewage sludge. With biogas, electrical or thermal energy can be obtained that can be used in the agri-food industry itself, and with the application of biosolids to the soil, the recovery of mineral and organic components is achieved, thus improving agricultural crops, being able to reduce or even to the substitution of chemical fertilizers. This energy circle is adapted to the concept of circular economy, thus closing the cycle between energy consumption, food production and subsequent waste disposal [2,8,21,38].

The objective of this study was to verify the benefits of the anaerobic co-digestion of three substrates (sewage sludge, wine vinasse and poultry manure) compared to the anaerobic co-digestion of two substrates (sewage sludge and wine vinasse) and the anaerobic mono-digestion of sewage sludge. The reactors were operated at different HRTs to determine the best results in terms of Chemical Oxygen Demand (COD) and Volatile Solids (VS) removal efficiency, methane production and yield. In addition, the content of pathogens in the effluent from the reactors were checked, to classify them as Class A Biosolids for later use as an agronomic amendment. The aim is to demonstrate that anaerobic tri-digestion is suitable to be carried out in wastewater treatment plants (WWTPs), achieving the valorization of local agri-food waste, to improve the obtaining of biogas and agronomic amendment.

2. Materials and methods

2.1. Reactors and operating conditions

Continuously stirred tank reactors (CSTR) equipped with stainless steel blades driven by motors programmed for 40 rpm stirring were used for this experiment. Each reactor has three liters of capacity with two liters of working volume. The reactors were deposited on a heating plate programmed at 35 °C for those in mesophilic conditions. Each reactor has a port where a 5-liter capacity Tedlar bag was connected to collect the daily biogas produced, a port where a temperature probe was installed, and another port for feeding and effluent outlet (Fig. 1).

The HRT was progressively reduced each time the reactors were stable for a given HRT, in order to increase the methane production and see which time was the most beneficial in terms of VS reduction and methane yield. The HRTs tested were 20, 15, 13, 10, 8 and last 6 days.

A replicate of each of the reactors analyzed in this study was available, which were the following: RS: Mesophilic reactor fed with sewage sludge; RSV: Mesophilic reactor fed with a mixture of sewage sludge and wine vinasse (50:50); and RSVPM: Mesophilic reactor fed with sewage sludge, wine vinasse (50:50) adding 10 g/L of poultry manure. Tests were carried out to determine the optimal proportion of poultry manure that would be used in the feeding of the reactors, with which to achieve better results in terms of methane production and yield, without being inhibited by high concentrations of total ammonia nitrogen (TAN) [3].

2.2. Characterization of the substrates and the feed

The most relevant characteristics of the substrates used in the anaerobic co-digestion studied were shown, as well as the mixtures prepared for each feed depending on the reactor. The feeding of the RSV reactor consisted of a mixture of sewage sludge and wine vinasse in

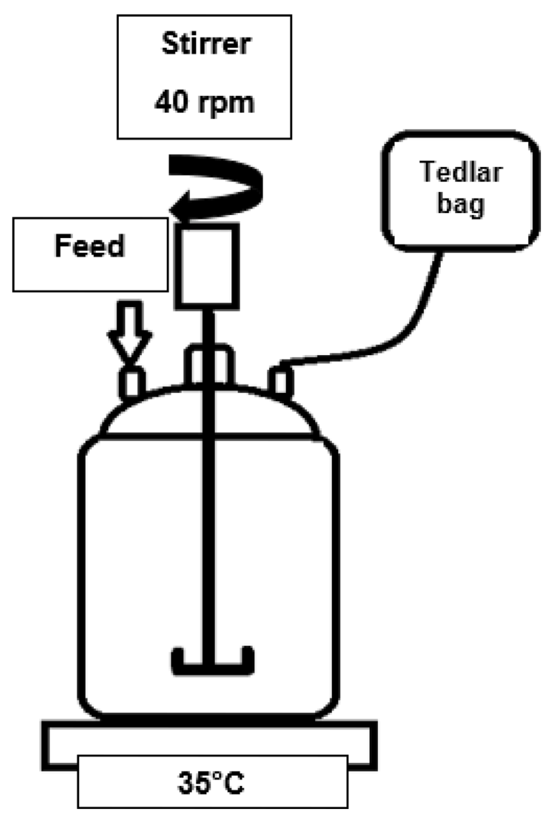

Fig. 1. Scheme of the mesophilic reactor used in the tests and operating conditions. At the top were the different ports for connecting the temperature probe, tedlar bag, feed inlet and effluent outlet.

proportion (50:50), and the feeding of the RSVPM reactor was of sewage sludge and wine vinasse in proportion (50:50) adding 10 g/L of poultry manure.

Table 1 shows the characteristics of each substrate individually and of the mixtures that configured the feed of each tested system. All substrates have a high amount of organic matter, especially poultry manure (PM), which provides a significant amount of organic matter to the SVPM feed. The wine vinasse (V) contributes to lowering the pH and increasing the SCOD in the SV and SVPM feed. This fact was extremely important because it was possible to increase the organic matter accessible to microorganisms, solving one of the main drawbacks of anaerobic mono-digestion of sewage sludge (S) [39]. Also noteworthy is the high concentration of TAN provided by poultry manure, achieving an adequate C/N ratio of around 30 for the SVPM feed [40,41] and a final less acidic pH around 5.5.

2.3. Feed comparison: Organic loading rates

Fig. 2 shows the different organic loading rates of the raw material in each system (Sewage sludge (S), sewage sludge and wine vinasse (SV), sewage sludge, wine vinasse and poultry manure (SVPM)) for the different HRT tests. With respect to a 6-day HRT, only the data referring to SVPM were shown, since this system allowed shorter HRTs to be operated than the others. It is observed in all cases that when HRT decreases, the organic load increases. PM and S provide the highest content of TCOD, VS and TS, while V provides a high content of dissolved organic matter in OLR. For this, the raw material S and SVPM have similar TCOD values, and the mixtures containing V have high values of SCOD, which increases with the addition of PM.

The HRT was decreased and was kept constant during each HRT tested until the steady-state conditions were reached. The attainment of the steady state was verified after an initial period (three times the HRT) by checking whether the constant effluent characteristic values (COD removal, VS removal and methane production) were the mean of the last measurements in each HRT [42].

2.4. 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,

Table 1
Parameters analyzed in an initial characterization of the substrates used and the feed created for the experiments.

Parameters	SEWAGE SLUDGE	WINE VINASSE	POULTRY MANURE	FEED SV	FEED SVPM
pH	6.43 ± 0.23	3.25 ± 0.14	9.76 ± 0.32	4.61 ± 0.26	5.35 ± 0.36
TCOD (g/L)	56.91 ± 0.14	40.22 ± 0.15	272.31 ± 0.33	42.23 ± 2.29	56.16 ± 5.21
SCOD (g/L)	15.61 ± 0.13	39.59 ± 0.09	143.68 ± 0.23	21.99 ± 1.14	35.58 ± 2.18
TS (g/L)	42.17 ± 0.11	22.42 ± 0.09	472.12 ± 0.29	25.86 ± 1.48	35.64 ± 3.07
VS (g/L)	35.74 ± 0.09	19.31 ± 0.08	393.01 ± 0.19	21.62 ± 2.32	27.95 ± 2.11
TVFA (mgAcH/L)	2492 ± 51	1147 ± 37	0.00 ± 0.00	1785 ± 71	1899 ± 54
C/N	14.25 ± 0.77	130.00 ± 3.18	3.24 ± 0.86	44.07 ± 1.22	30.22 ± 1.01
TAN (g/L)	0.22 ± 0.02	0.25 ± 0.04	22.54 ± 1.12	1.02 ± 0.08	1.99 ± 0.03
Alkalinity (g/L)	1.3 ± 0.21	n.d.	35.20 ± 0.18	0.45 ± 0.06	0.56 ± 0.02

The results were expressed as average ± standard deviation. Analyses conducted in triplicates (n = 3).

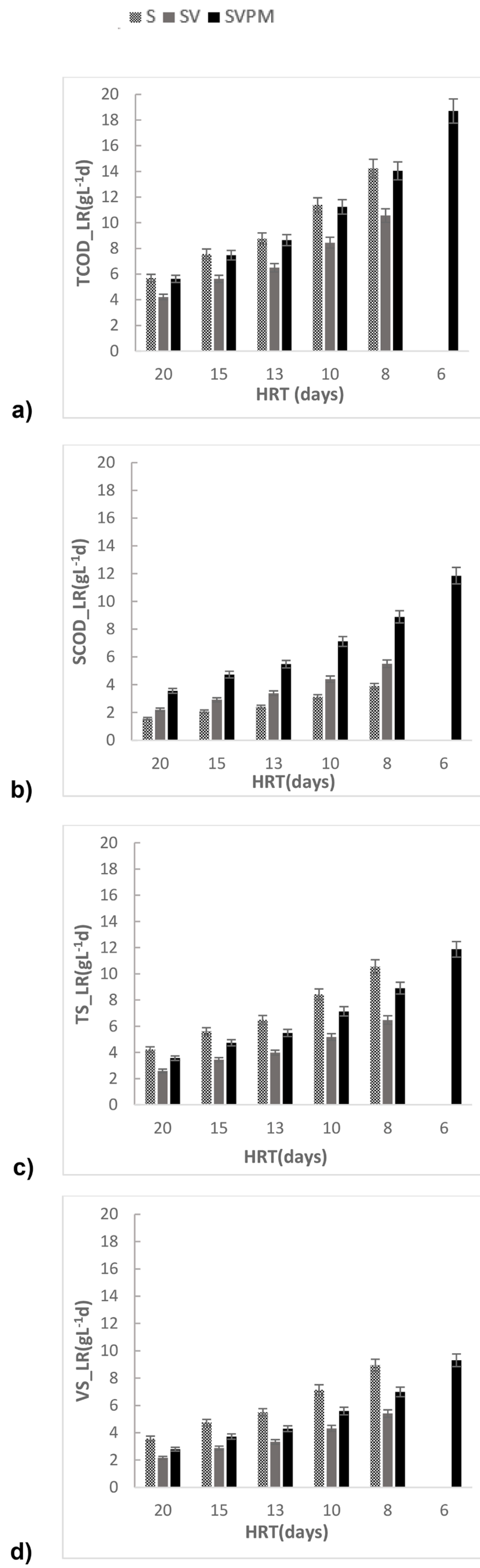

Fig. 2. Effect of HRT on the different organic loading rates (OLR) tested. a) evolution of TCOD_LR, b) evolution of SCOD_LR, c) evolution of TS_LR and d) evolution of VS_LR of the feedstock used in each system (S, SV and SVPM).

alkalinity and carbon/nitrogen ratio (C/N ratio).

For the determination of TS, VS, TCOD and SCOD the Standard Methods APHA-AWWA-WPFC [43] were followed. For the determination 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 [43,44]. 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 determination of 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 [43]. Alkalinity and total ammonia nitrogen (TAN) were measured using the HANNA multiparameter photometer (HI83399), following the standard APHA-AWWA-WPFC methods [43]. The volume and composition of the biogas produced were 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), H_2, CO_2, CH_4 and O_2 were analyzed by means of a thermal conductivity detector (TCD) using a Supelco Carboxen 1010 Plot column. The determination was performed under the following operating conditions: split: 100; constant pressure at the injection port: 70 kPa; 2 min at 40 °C, ramp at 40 °C/min up to 200 °C, 1.5 min at 200 °C; detector temperature: 250 °C; injector temperature: 200 °C. The carrier gas used was helium (266.2 mL/min); synthetic air (120 mL/min) and hydrogen (80 mL/min) as mixtures for the flame; and Helium (8 mL/min) as auxiliary gas for compensation [7,45,46].

2.5. *Effluent classification as biosolid class a*

The pathogenic microorganisms in the feeding of the reactors and in the effluents of these were determined in the most stable HRTs of each reactor in order to quantify *total coliforms, Escherichia coli (E-Coli) and Salmonella*, and check if the values were met required to be classified as class A biosolids, as well as the degree of pathogen reduction after the thermophilic and mesophilic anaerobic codigestion process and the anaerobic monodigestion of sewage sludge. The United States Environmental Protection Agency (US EPA) determines a quantity less than 1000 *Fecal Coliforms*/gTS and 3 most probable number/4gTS for *Salmonella*, in order to classify the effluent as class A biosolids [21,34–36]. On the other hand, according to Regulation (EU) 2019/1009 of the European Parliament and the Council of June 5, 2019, it establishes that the density of *E-Coli* must not exceed the limit of 1000 colony-forming units (CFU)/gTS and that *Salmonella* must be absent in 25 mL of sample in an organic fertilizer [21,33,37,47–49]. The determination of *total coliforms, E-Coli* [Method 9222H] and *Salmonella* [Method 9260B] was carried out according to standard methods [43]. *Total coliforms* were calculated through *E-Coli*, since it represents approximately 90% of them [21].

3. Results and discussion

3.1. *Process stability and effluent quality*

The stability of the process was studied through the evolution of the pH in each system, because this parameter is key in monitoring the anaerobic degradation process [7]. For RS and RSV the pH tended to become more acidic as the HRTs tested were shorter. It was necessary to add NaOH (10 M) on several occasions, which can be seen in Fig. 3. when the pH dropped to values close to 7. RSV was the least stable system, this could be due to the vinasse, which has a very acidic pH and tends to acidify the influent used, thus causing acidification in the system. This problem is very common in single stage reactors as the organic loading rate increases, VFA build up can occur, leading to acidification [50,51]. pH control was important when HRT falls due to the negative effect it has on methanogenic activity [4,52].

On the other hand, RSVPM showed the most stable pH throughout the process, without the need to resort to external agents to correct it, being in a stable mean range of 7.5. The three substrates used have very different pH values (Table 1), although when it was mixed it presented acid values in the feed, during anaerobic tri-digestion it managed to maintain higher values, in the optimum range for methanogenic archaea. Therefore, the anaerobic co-digestion of 3 substrates occurred in a balanced way, helping to keep the pH evolution stable throughout the process.

As to effluent quality, the organic matter content in the effluent was determined by measuring VS, SCOD, TCOD, TS, VFA, TAN, alkalinity and ratio VFA/Alk, in both streams. Fig. 4. And Fig. 5. Shows the evolution of these parameters after reaching the steady state at each tested HRT.

Higher values of organic matter were detected in the effluent of RS

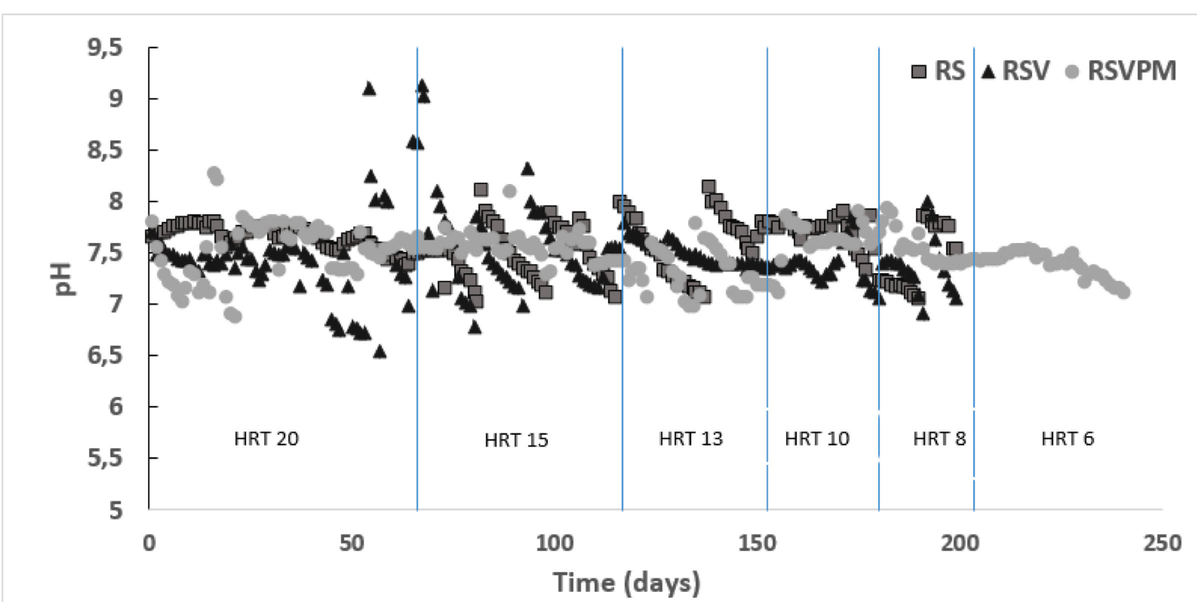

Fig. 3. Evolution of the pH throughout the test, for the different systems analyzed, as the HRT decreased.

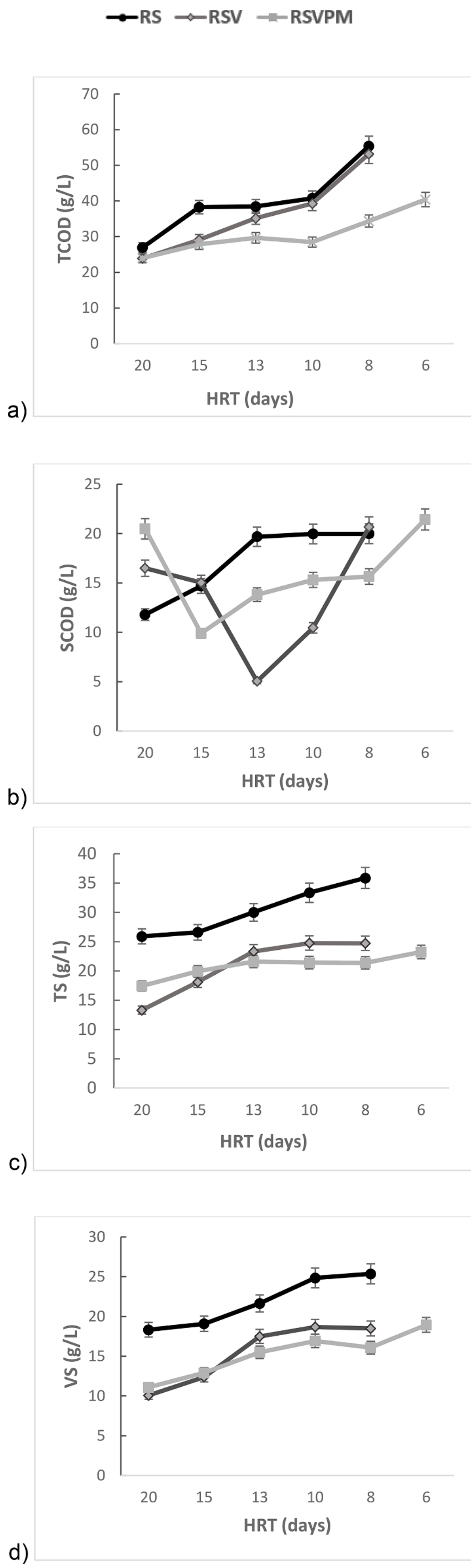

Fig. 4. Average of the values recorded in the effluent in each HRT tested, in the different systems. The parameters measured were: a) TCOD evolution, b) SCOD evolution, c) TS evolution and d) VS evolution.

than in that of RSV and RSVPM. This means that the quality of the effluent was higher after anaerobic codigestion of SV and SVPM than after anaerobic digestion of S. This was mainly due to two reasons, on the one hand, to the difference in the substrate used in each case, and on the other hand, to the different biodegradation that occurred in each system. With respect to anaerobic codigestion using vinasse, it should be noted that it provides a high SCOD content to the mixture and with respect to the different biodegradation that occurs in each system, it would be necessary to check the performance of the different systems by comparing organic matter removal values, which will be discussed in the next section [39,53].

The VFAs were then measured, in total and individually, to check their evolution in each reactor and for each HRT tested. The concentrations of TAN were also analyzed and the possible relationships that caused instability in some of the cases tested were studied.

The highest values of total acidity were recorded for HRT of 20 days. For HRT between 15 and 10 days, their concentration tends to decrease, however for HRT of 8 days, they increase again in all cases except in RSVPM. In the case of 3-substrate anaerobic co-digestion (SVPM), the most stable values were recorded again for each HRT tested, in a range between 200 and 763 mg/L. It should be noted that the VFA did not exceed the inhibitory values published in the literature in any case [33,39,54–56]. In addition, values >90% of VFA removal were achieved for all experiments, for RS (HRT between 15 and 8 days), for RSV (HRT between 15 and 10 days) and for RSVPM (HRT between 15 and 6 days). This indicates that the anaerobic co-digestion in the mesophilic range of these three substrates (SVPM) creates a stable system, which is not inhibited by short HRT due to VFA accumulation [52]. On the other hand, mesophilic range anaerobic co-digestion has been shown to help mitigate the damaging effects and system instability that occurs due to VFA accumulation [48].

In Fig. 5 a) it can be seen how RS registers the highest TAN values throughout the process (4.5–6.9 g/L). Anaerobic co-digestion systems show lower values than RS (2.71–5.34 g/L). The application of nitrogenous waste such as PM helps to optimize the C/N ratio, but it is a concern when it comes to keeping the co-digestion process stable due to the accumulation of TAN. For RSVPM the recommended TAN concentration was exceeded (4 g/L)[52], although no inhibition was observed in the production of methane for this reason. In the three systems carried out, the same trend was followed, registering a strong decrease in the concentration of TAN for the shorter HRTs tested. This was caused by high OLR and low HRT, because the ammonia generation rate was not sufficient to accumulate in the system compared to the HRT [52]. It was known that an adequate concentration of TAN provides benefits for microorganisms,> 500mgTAN/L [57], but the concentration limit of TAN was not fixed and must be studied for each case since it depends on the substrates used, the conditions of operation and the degree of acclimatization of the microorganisms. In this way, inhibition has been observed in ranges between 1.7 and 14 gTAN/L [56], between 3 and 6 gTAN/L [58], or for values above 3.8 gTAN/L [57], or above 1.5 gTAN/L [30,59].

With respect to alkalinity, for the RS system it remained high throughout the process and for all the HRTs tested, with values around 7 g/L (Fig. 5 b)). For the RSV case, the alkalinity grew strongly as the HRT decreased, with initial values of 3.38 g/L that double in HRT of 8 days. RSVPM registered lower and more stable values throughout the process, oscillating in a range between 2.92 and 3.48 g/L, except for a 6-day HRT, where its concentration increased to values of 6.15 g/L. According to the literature, alkalinity values were recommended in an anaerobic digester between 2000 and 4000 mg/L [51,52]. For RSVPM, the measured alkalinity was within the recommended values except for the shorter HRTs. This was due to the fact that at high organic loads the buffering capacity of the system decreases on the one hand, and that co-digestion with poultry manure produces a high amount of alkalinity above 4000 g/L [52].

The VFA/Alk ratio was monitored to clarify at which HRT the

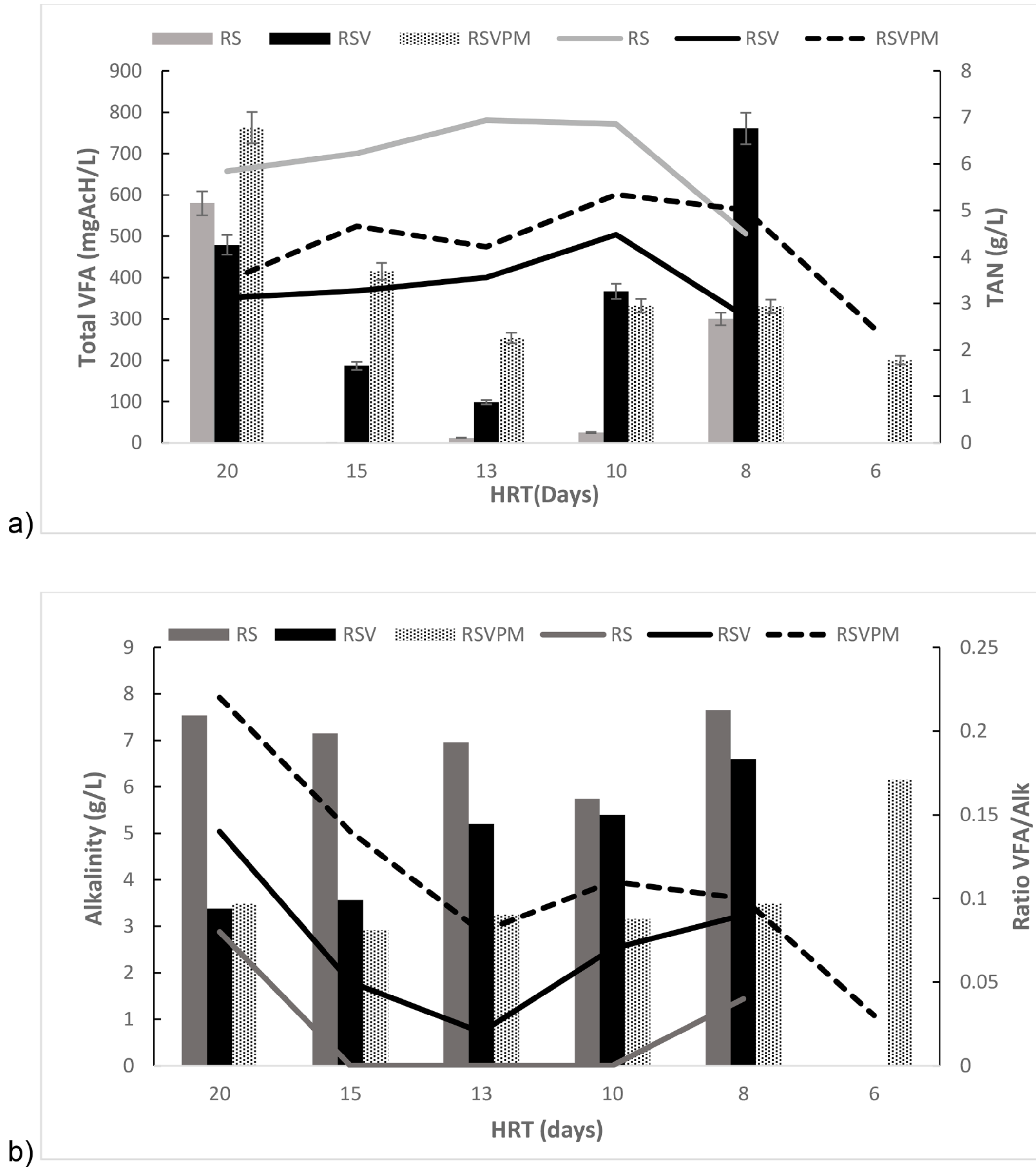

Fig. 5. Average of values recorded during the test, for the different systems analyzed at the different HRT tested: a) evolution of VFA and TAN, b) evolution of alkalinity and VFA/Alk ratio.

anaerobic co-digestion could operate without risk to the process. Fig. 5 b), shows the average values for this parameter in each HRT tested for each experiment. Throughout the process, the values remained below 0.4, indicating favourable operating conditions [7]. RS and RSV for HRT between 15 and 8 days remained with values below 0.1, however RSVPM presented higher values in all cases between (0.03 and 0.22). For a 13-day HRT, this parameter was below 0.1, obtaining a value of 0.08. This fact coincides with the maximum removal % of VS and the maximum methane yield for the same HRT. According to several authors, values around 0.1 were recommended to ensure a high buffering capacity of the system. [7,22,42].

3.2. Removal efficiencies

The degradation of the different parameters related to the organic matter in the anaerobic digestion of S, and anaerobic co-digestion of SV and SVPM, at the different HRTs were shown in Fig. 6.

The highest percentages of TCOD removal (Fig. 6 a)) were recorded for the anaerobic co-digestion systems, well above the values obtained for the anaerobic digestion of S. For the anaerobic co-digestion of SVPM, the following values were observed highest values for all HRTs tested, except for the longest HRT corresponding to 20 days. For 15-day HRT, the maximum value of 51% TCOD removal was reached.

For SCOD removal (Fig. 6 b)), the values recorded were also much higher for anaerobic co-digestion systems, and again, the highest removal percentages were recorded for RSVPM, reaching their maximum for a 13-day HRT with values of 62% SCOD removal. For an HRT of 13 days, this system showed higher organic matter decomposition efficiency, while as OLR increased, its capacity to assimilate feed declined.

For TS removal (Fig. 6 c)), the anaerobic co-digestion of SVPM once again obtained the highest % removal at 13-day HRT with values of 50% TS removal. However, the anaerobic co-digestion SV registered the lowest percentages of removal, leaving in this case the anaerobic digestion of S with intermediate TS removal values between the two anaerobic co-digestion systems.

For SV removal (Fig. 6 d)) the trend was similar to TCOD and SCOD removal, again registering the highest removal percentages for SVPM

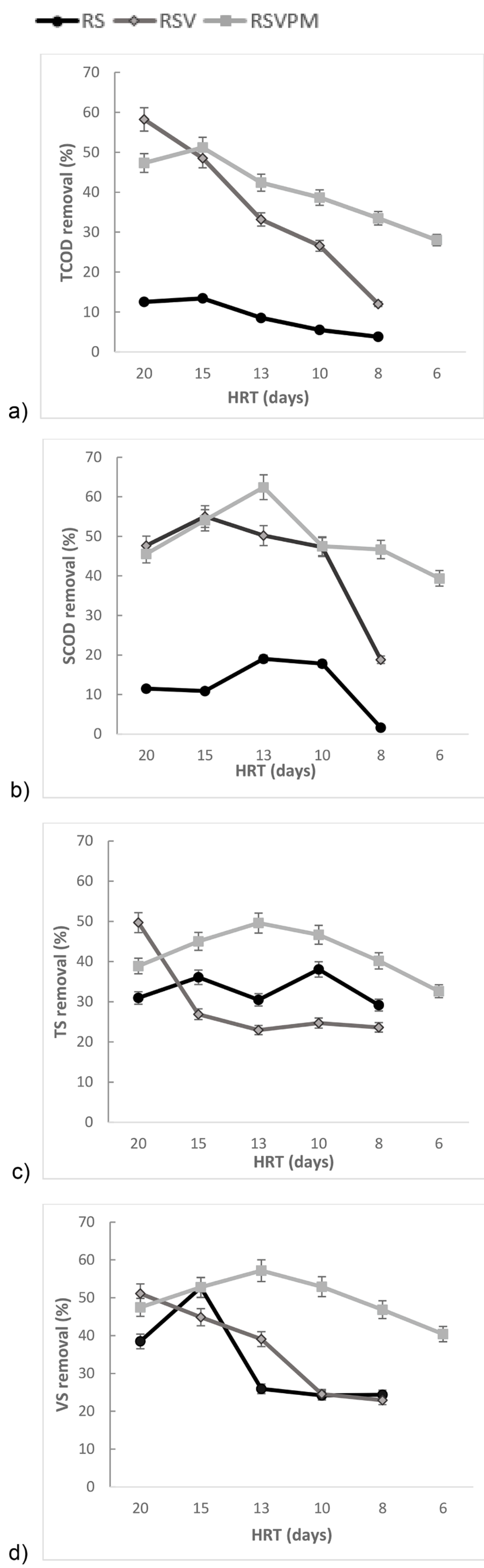

Fig. 6. Recorded mean removal values (%), for the different systems in the tested HRTs a) TCOD removal, b) SCOD removal, c) TS removal and d) VS removal.

anaerobic co-digestion with a maximum of 57% for 13-day HRT. For long HRTs of 20 and 15 days, the anaerobic digestion of S and anaerobic co-digestion of SV also present high VS removal values, of 38% and 58%, respectively. But as the HRT decreases, these values drop to 23% VS removal for 8-day HRT. However, RSVPM recorded values between 40% and 57% throughout the process, reaching a drop in HRT for up to 6 days without suffering inhibition.

It was known that hydrolysis, the first phase of anaerobic digestion, was the limiting step in this process and that the sewage sludge contains hardly biodegradable organic matter [4,60,61]. This would explain why as the HRT decreases, higher concentrations of COD, TS and VS were recorded and therefore the elimination percentage of these parameters decreases. In general, the highest removal percentages were recorded in the RSVPM. The 13-day HRT was sufficient to remove the maximum organic matter. These data support the idea that anaerobic co-digestion with poultry manure helps to achieve better results in purification efficiencies. The same trend was observed for the co-digestion of food waste and chicken manure [52].

In summary, the increase in OLR or decrease in HRT causes a decrease in removals in all parameters. However, this decrease occurs in a smoother way when it comes to the anaerobic co-digestion of SVPM. The co-digestion of three substrates maintained the most stable and high removal percentages throughout the process at the different HRTs tested. In addition, it reached the maximum % removal at a HRT of 13 days. This was crucial, since VS is considered to be the main organic precursor for methane production [62] and in this case, the maximum % SV removal coincides with the maximum methane yield observed for RSVPM in a 13-day HRT, as will be discussed in the next section. Finally, it should be noted that no organic matter removal rate reached values of 70%. This was because the microorganisms did not use all the non-solubilized material and not all the soluble organic carbon in the waste can be biologically degraded [39].

Therefore, it can be affirmed that the anaerobic co-digestion of 3 substrates makes the process more stable and it can be lowered to shorter HRTs than with the anaerobic bi-digestion and mono-digestion of sewage sludge. Similar values were observed by Gaur et al. (2017) [62] in anaerobic co-digestion of sludge and cow dung with food waste, who recorded a maximum VS elimination of 56.58% and Cabbai et al. (2016) [63], in anaerobic co-digestion of OFMSW and sewage sludge, who registered 67.3% of removal of VS.

3.3. *Methane yields*

Methane production from sewage sludge digestion only, co-digestion of sewage sludge and wine vinasse, and sewage sludge, wine vinasse and poultry manure were compared. Methane yield in mL/gVS added and daily production in L/d were monitored. The methane yield depends on several factors, such as the feed supplied to the reactor, the HRT and the operating temperature. The average of the methane yield and the production generated daily for each test in each HRT tested were shown in Fig. 7 a) and b), respectively.

To compare the energy efficiency of systems with different raw materials, it is necessary to do so in terms of methane values per gram of organic material [39]. As expected, the RS reactor presented the lowest values in terms of methane yield regarding the rest of the studies, (Fig. 7 a)). RS presented the lowest performance for a HRT of 20 days. The yield increased as the HRT decreased until reaching values close to 130 $mLCH_4/gVS_{added}$ for an HRT of 10 days. Subsequently, for shorter HRTs than 10 days, the methane yield decreased. RSV recorded ascending methane yield values as the HRT decreased, reaching a maximum value of 210 $mLCH_4/gVS_{added}$ at 13-day HRT and continuing to decrease afterwards in the shorter HRTs tested. Finally, RSVPM followed the same trend, increasing their performance as the HRT decreases to reach their maximum for HRT of 13 days, with values of 261 $mLCH_4/gVS_{added}$. Subsequently, for the shorter HRTs tested, the methane yield decreased in the reactors. Although during all the HRTs tested, the yield was higher

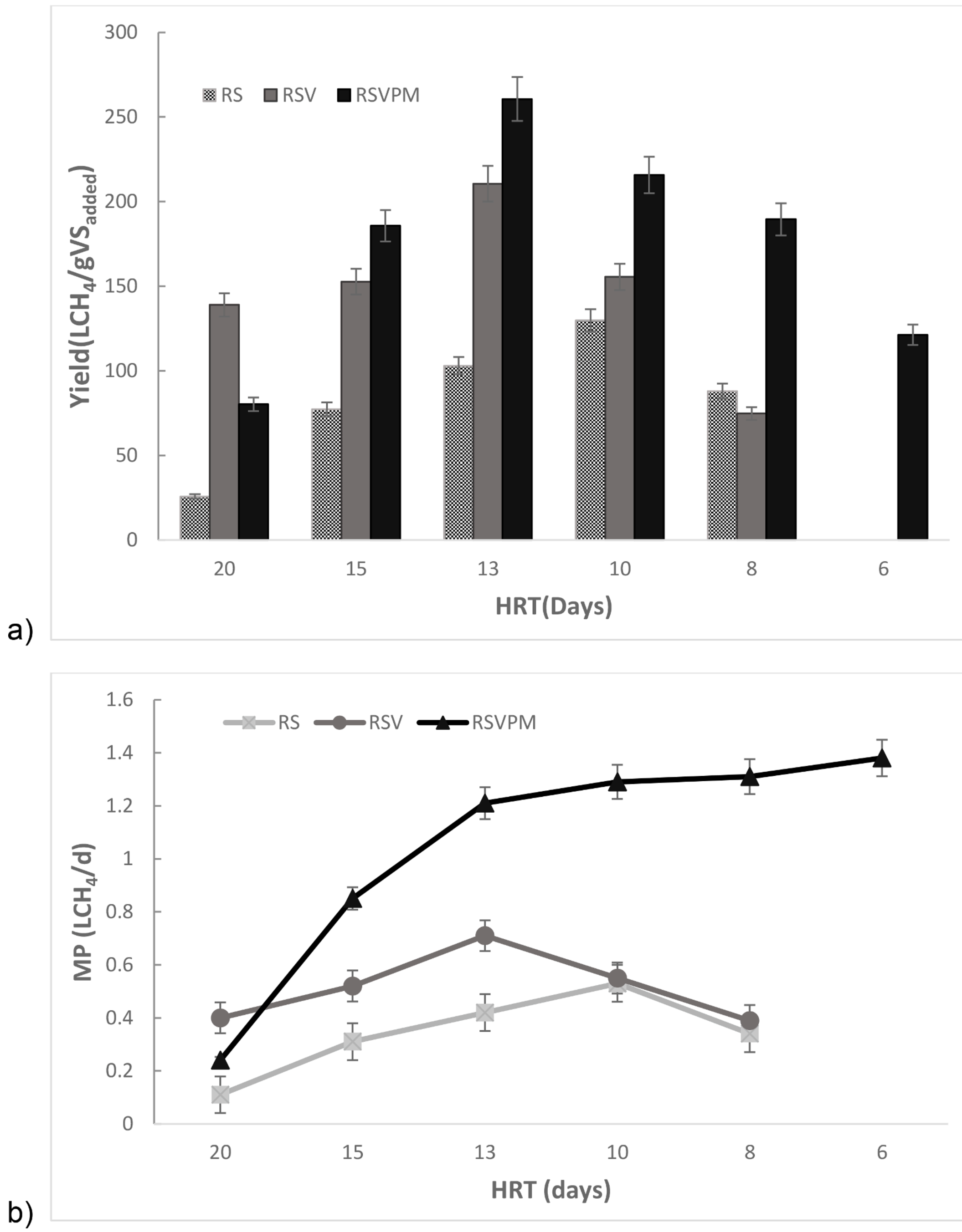

Fig. 7. a) Methane yield for each system in the different tested HRTs ($mLCH_4/gVS_{added}$), b) Average methane production expressed (L/d) in each stable HRT for the three tested systems.

for RSVPM, at an HRT of 20 days this did not occur, with RSV presenting better results. This could be due to the addition of PM, requiring more time for the microorganisms to acclimatize to higher concentrations of TAN, and thus produce biogas regularly. An improved performance of anaerobic co-digestion compared to sludge mono-digestion is clearly observed. This means that the added substrates were optimal for the improvement of co-digestion. PM and V provided greater biodegradability and soluble organic material, increasing the amount of energy produced per gram of residue.

If the results of the daily production of methane were analyzed (Fig. 7 b)), it can be observed that as the OLR increases or the HRT decreases, the daily production of biogas increases, up to an HRT of 13 days. Thereafter, methane production was progressively inhibited in RS and RSV, at HRT of 10 and 13 days, respectively. This was mainly due to imbalances in pH, since at lower HRT, the system tended to acidify, causing less conversion of organic matter to methane by microorganisms. This fact corresponds to a lower purification efficiency in terms of TCOD and VS, as well as an increase in VFA and TAN. Under the same operating conditions, biogas production in RSVPM was much higher than in RS and RSV, mainly due to the presence of more easily biodegradable compounds. At the beginning of the experiment, when the HRT was lowered, the response of the RSVPM system was immediate in terms of increased biogas production. However, when 13-day HRTs were tested, at lower HRTs the increase in biogas production was not as noticeable. For HRT of 13, 10, 8 and 6 days, the daily liters of methane increased very slightly from 1.21 to 1.38 L/d. The system does not suffer from inhibition, but at higher OLRs, a greater production of biogas is not observed, obtaining its maximum performance in HRT of 13 days. Although the pH remained stable over time, the methane yield fell from HRT 13 days. (Fig. 7 b)). The concentration of TAN and VFA has a strong influence on biogas production. This inconvenience must be controlled even if the pH remains stable [39,64].

Accordingly, the anaerobic co-digestion favors the production of methane obtaining better yields, reaching values of 210 and 261 $mLCH_4/gVS_{added}$ for RSV and RSVPM respectively. Similar values were found in the literature on the yields obtained in anaerobic digestion of sludge, with yields of $0.23LCH_4/gVS$ in HRT of 10 days [5], and with the anaerobic co-digestion of cattle manure and animal waste, obtaining $257mLCH_4/gVS_{added}$ for a HRT of 25 days [65], or the anaerobic co-digestion of sludge with municipal organic waste reaching 333 $mLCH_4/kgVS$ [54].

In summary, for all the HRTs tested, RSVPM presents the best methane yields, reaching its maximum for a 13-day HRT with values of $261mLCH_4/gVS_{added}$. The addition of a third co-substrate, in adequate proportions, to the anaerobic co-digestion of SV, such as PM, favors the production of methane and makes the process more stable at high OLR. These results were consistent with the higher value of VS removal, a decrease in TAN and VFA concentration at 13-day HRT for RSVPM.

3.4. Class a biosolid listing

To carry out the classification as class A biosolid according to the US EPA and Regulation (EU) 2019/1009 of the European Parliament and the Council of June 5, 2019, the different feeds provided to the reactors (sewage sludge, sewage sludge + wine vinasse, sewage sludge + wine vinasse + poultry manure) and the stable effluents from the reactors where they presented the best methane yield results were analyzed.

In the first place, regarding the analysis for the detection of *Salmonella*, the presence of *Salmonella* was found in the feed of the reactors. However, an absence was recorded in the effluents of all reactors. This suggests that in all the cases considered it was effective in eliminating Salmonella for the stable HRTs. The concentrations of *E. coli* and Total Coliforms in each feed and in the effluents are shown in Table 2 and Fig. 8, respectively. The feedstock corresponding to each reactor and the effluents for the HRT were analyzed where the highest methane yields were achieved. Total coliforms determined include fecal coliforms and E-Coli.

As can be seen in table 2, none of the feeds meets the necessary legal requirements according to European legislation and the US EPA to be classified as class A biosolids. After undergoing anaerobic co-digestion, these values drop drastically, placing them below the limit required to be classified as class A biosolids by current European legislation and the US EPA, as can be seen in Fig. 8. It should be noted that vinasse dilutes the initial concentration of these micro-organisms as the input comes mainly from sludge and poultry manure.

In Fig. 8. it can be seen how the RS does not achieve sufficient elimination of pathogens in the effluent, remaining above 1000 (CFU)/gTS. For the RSV and RSVPM systems, pathogen inactivation was sufficient. All values were below the limit required for classification as class A biosolids in cases of anaerobic co-digestion. Total coliform elimination percentages of 95.64% for RSV and 96.89% for RSVPM were achieved. Regarding E. Coli, it was possible to eliminate 96.61% for RSV and 96.86% for the effluent of the RSVPM reactor, both remaining below the limit established by European legislation. This indicates that anaerobic co-digestion was an efficient practice in the elimination of pathogens, mainly due to its effect of diluting contaminants, without the need to operate in thermophilic ranges, which helps to contribute to a circular economy by reducing the energy cost to develop this technology. The final product of anaerobic codigestion was suitable for use as an agronomic fertilizer in accordance with European legislation and the US EPA. This fact contributes to reducing the environmental impact produced by chemical fertilizers [21] and manages to close the cycle of the circular economy of this study.

Table 2
Concentration of *E. Coli* and Total Coliforms expressed as (CFU)/gTS, registered in the feed of the reactors.

Feed	*E-Coli* (CFU/gTS)	*Total Coliform* (CFU/gTS)
S	20,600	41,950
SV	6050	8650
SVPM	14,500	29,400

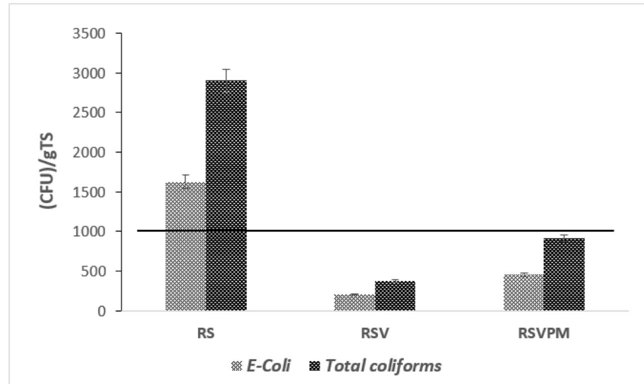

Fig. 8. Total *E-Coli* and *total coliforms* concentration in the effluent of the three systems studied.

4. Conclusions

The results obtained in this study show, in the first place, that the elimination percentages of VS were higher for RSVPM in all the HRTs tested. For a 13-days HRT, values of 51% and 57% removal, respectively, were achieved. In all tests, these percentages decrease as the HRT falls below 13 days. RSVPM showed the best values in terms of methane yield for HRT of 13 days ($261mLCH_4/gVS_{added}$), slightly decreasing for the rest of HRT tested. VFA removal was>90% in the optimal HRTs. Regarding the pathogen removal results, anaerobic co-digestion was able to remove enough pathogens to be classified as class A biosolids.

Therefore, it can be concluded that the anaerobic tri-digestion of sewage sludge, wine vinasse and poultry manure substrates was more beneficial than the bi-digestion of sewage sludge and wine vinasse and the mono-digestion of sewage sludge. Allowing to operate at shorter HRT, achieving better purification efficiency, an increase in methane yield and the possibility of being able to classify the effluent as class A biosolid. Thus, anaerobic co-digestion with agri-food substrates would be an attractive solution that could be implemented in WWTPs to valorize biowaste by obtaining biogas and agronomic amendment, within the framework of the circular bioeconomy, maximizing greenhouse gas mitigation efficiency.

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.

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