Chemical Engineering Journal 451 (2023) 138478

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Thermophilic-mesophilic temperature phase anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure: Effect of hydraulic retention time on mesophilic-methanogenic stage

Leonor Sillero, Rosario Solera *, Montserrat Pérez

Department of Environmental Technologies, IVAGRO, Faculty of Marine and Environmental Sciences (CASEM), University of Cádiz, Pol. Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain

ARTICLE INFO

Keywords:
Temperature-Phase Anaerobic co-Digestion
Sewage sludge
Wine vinasse
Poultry manure
Biomethane
Hidraulic retention time

ABSTRACT

The present study investigated the influence of the hydraulic retention time (HRT) in temperature-phase anaerobic co-digestion (TPAcD) for methane production. The reactors were started-up with a mixing ratio of 49.5:49.5:1 of sewage sludge, wine vinasse and poultry manure. The TPAcD was operated at thermophilic temperatures in the first stage and mesophilic temperatures in the second stage. The thermophilic stage operated with a constant HRT of 5 days, while the methanogenic stage was optimized under the HRT of 15, 12, 10, 8, 5, 4, and 3 days. The best results were obtained for an HRT of 12 days in the methanogenic stage, 56.35 % of volatile solids (VS) biodegradation was achieved, with a methane yield of 391 mL CH_4/gVS_{added}. Regarding the whole TPAcD process (acidogenic following by methanogenic), the vS and total volatile fatty acids reached, respectively, 93.13 % and 97.43 % of removal efficiency. The microbial population revealed that *Eubacteria* was higher than the *Archaea* at the HRT with the highest methane yield, and the microbial activity increased proportionally to the organic loading rate, which in turn was related to methane production. Due to the strong pathogen reduction in the TPAcD, the digestate obtained can be classified as class A biosolids in all HRT evaluated, being a promising alternative for its application as agricultural fertilizer. Finally, the presented TPAcD process can be an environmeltally friendly alternative for the management of sewage sludge, wine vinasse, and poultry manure in an integrated biorefinery for the recovery of bioenergy and fertilizer, advocating a sustainable approach for the circular economy transition.

1. Introduction

Increasing and constant urbanization causes large amounts of sewage sludge (SS) to be generated in urban areas. These sludges are characterised by a high organic load, high concentration of pathogens and other toxics such as heavy metals [1], which makes them difficult and costly to manage. As a consequence, it is increasingly necessary to develop adequate sludge removal techniques [2,3]. A recurring waste in southern Spain is wine vinasse (WV) from its distillation for the production of Jerez brandy. The discharge of this waste can cause great environmental problems due to its low pH around 3.5 and its high organic load with values of 42 g/L of Chemical Oxygen Demand (COD). These characteristics make wine vinasse an optimal substrate to be mixed with sewage sludge and managed by anaerobic co-digestion, with the consequent improvement in biogas production [4–6]. Another waste that presents major environmental problems is poultry manure (PM) from the poultry farm [7]. This industry is experiencing an increase due to the increase in the export of poultry meat to other countries. In southern Spain, turkey fattening industries generate a large amount of poultry droppings that need to be treated. This waste has a high organic

Abbreviations: SS, Sewage Sludge; WV, Wine Vinasse; PM, Poultry manure; TPAD, Temperature-Phase Anaerobic Digestion; TPAcD, Temperature-Phase Anaerobic co-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 Ammoniacal 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; PUA, Propionate-utilizing acetongs; BUA, Butyrate-utilizing acetongs; AUM, Acetate-utilizing methanogens; HUM, Hydrogen-utilizing methanogens; HAUM, Hydrogen/Acetate-utilizing methanogens.

* Corresponding author.
E-mail address: rosario.solera@uca.es (R. Solera).

https://doi.org/10.1016/j.cej.2022.138478
Received 15 June 2022; Received in revised form 28 July 2022; Accepted 2 August 2022
Available online 4 August 2022

load with Total Chemical Oxygen Demand (TCOD) values of 300 g/L and high ammonia content with values around 20 g/L, making it susceptible to generating contamination in the environment. In general, animal manures, such as poultry manure, contain high concentrations of ammonia causing toxicity and inhibition of anaerobic digestión [8].

Anaerobic co-digestion is of great importance when treating these wastes, since it reduces them and in turn avoids greenhouse gases emissions in their treatment [9]. It has been reported that the co-digestion of sewage sludge with other organic wastes is an effective method to improve the production of biomethane, achieving dilution of toxic compounds, increased organic load, greater stability in the digestate, greater reduction of greenhouse gases and economic and energy savings by sharing equipment [10–12]. Therefore, a sustainable way to manage them would be to mix poultry manure with sewage sludge and wine vinasse for anaerobic co-digestion of the three substrates together. Due to the characteristics of each one, the mixture offers prospects for improvement in biogas production since a nutrients balance is achieved in the substrate.

There are numerous technologies to improve anaerobic digestion [13]. Recently, Temperature Phase Anaerobic Digestion (TPAD) systems are considered more efficient than single stage systems. With the use of TPAD technology, very important advantages are obtained such as greater organic reduction, increased biogas production, increased elimination of volatile solids (VS), greater elimination of pathogens in the effluent by combining high and low temperatures and less formation of foams and volatile fatty acids (VFA) in the effluent [2,14]. It also allows to manage the pH in each stage, and the system is capable of better resisting organic shock loads, presenting greater stability in the process [15,16]. TPAD is a two-stage anaerobic digestion process consisting of thermophilic and mesophilic digesters connected in series in order to incorporate the advantages of thermophilic and mesophilic anaerobic digestion (Fig. 1). In the first stage, hydrolysis, solubilization and acidogenesis occur. Methane production would occur in the second stage. Normally it operates in hydraulic retention times (HRT) of 2 to 5 days in the first stage and 10 to 40 days in the second stage, using shorter solid retention times in the first stage in reference to the second stage [17–21]. TPAD processes have been studied for different substrates, the most common being sewage sludge [14,17], although other substrates such as sunflower oil cake waste were also found [22]. Recently it has been highlighted its use mixed with other substrates that improve the characteristics, obtaining better results of vS removal and higher biogas yield. Some examples of co-digestion in TPAcD would be using sewage sludge and sugar beet pulp [23], sewage sludge and municipal organic waste [24] or sewage sludge and wine vinasse [5], or food waste and garden waste [25].

HRT strongly affects the performance of the anaerobic digestion process and biogas production yields. As the HRT decreases, the organic loading rate (OLR) increases [26]. It is necessary to study the influence of HRT for each anaerobic digestion system carried out and for each substrate or substrates used as feed. Knowledge of the optimal HRT is crucial to understand each process and to establish the best operational conditions, thus obtaining the best methane yields and allowing a broader vision for successful future industrial scale-up.

After anaerobic digestion, large amounts of biosolids with a high nutrient content are produced. These biosolids could be applied to the soil as fertilizers or agronomic amendments. This method is very beneficial for the environment, but there is concern about some components that can harm the environment, human and animal health. According to EPA standards, these biosolids could be classified as class A if an adequate reduction in pathogen content is achieved and the safety of their application to the soil is guaranteed. [14,27]. 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 [27–29]. 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 [14,30–32]. With this method it would be possible to close the process emitting zero waste.

The objective of this study was to investigate the effect of decreasing the HRT in the temperature-phase anaerobic co-digestion (TPAcD) of sewage sludge, wine vinasse and poultry manure during the optimisation of the second mesophilic-methanogenic stage. For this purpose, it was necessary to determine the best operating conditions and to achieve the best results in terms of vS removal efficiency and methane yield produced, as well as to check the stability of the process without the detriment of the high ammoniacal nitrogen concentrations present in the poultry manure. The evolution of the microbial populations involved in the methanogenic stage was also studied. Finally, the concentration of

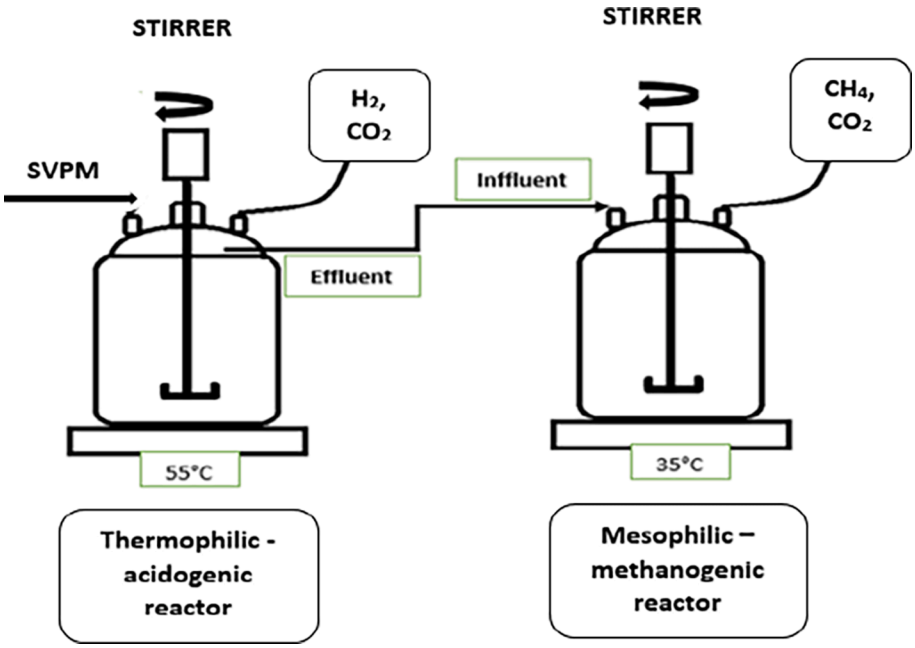

Fig. 1. Diagram of the reactors connected in series in the TPAcD process and operating conditions.

pathogens in the effluent was analysed to check its possible classification as a class A biosolid. In summary, this study aims to test the feasibility of a TPAcD system carried out with three very different substrates, in which the valorisation of different wastes would take place, with the consequent obtaining of high added value products such as bioenergy and agronomic amendment. The feasibility of this process would allow great environmental solutions on a local scale and the obtaining of benefits for producers, within the concept of the circular economy.

2. Material and methods

2.1. Characterization of the substrates and digester operation.

The substrates used in this study were sewage sludge (SS), wine vinasse (WV) and poultry manure (PM). The sewage sludge was supplied by the Guadalete municipal wastewater treatment plant and the wine vinasse by the González Byass winery, both in Jerez de la Frontera, Cádiz, Spain. The poultry manure was collected from an agricultural farm called Marta Aragon S.L., in Chiclana de la Frontera, Cádiz, Spain. Previous studies in the research group addressed the task of determining the best proportion of these substrates to configure the reactor feed. To this end, the biodegradation potential of the mixture with different proportions was studied, carrying out biochemical hydrogen potential (BHP) and biochemical methane potential (BMP) tests, of the mixture of sewage sludge and vinasse [4] and of the mixture of sewage sludge, vinasse and different proportions of poultry manure, reaching the conclusion that the best proportion was SS:WV:PM (49.5:49:5:1) [6,33].

The most relevant characteristics of the substrates used in the anaerobic co-digestion studied were shown in Table 1, as well as the mixture of both used as feed for the acidogenic reactors and the characteristics of the influent used to feed the methanogenic reactors.

As can be seen in Table 1, the substrates used have very different characteristics. It was worth mentioning the high values of TS, vS TCOD, SCOD, TAN and alkalinity of poultry manure, which make their individual handling very difficult. For this reason, it was proposed to combine it for obtaining a combined SSWVPM feed by mixing sewage sludge and wine vinasse (50:50) with 10 g/L of poultry manure, achieving C/N ratios for feeding values close to 30, the optimal value recommended for anaerobic co-digestion by many authors [34–38]. Moreover, the characteristics of the acidic effluent from the first stage of TPAcD operating at 5 days of HRT were also shown in Table 1. The 5-day HRT of the acidogenic reactors was established as a consequence of an optimisation study of these reactors fed with SSWVPM, which showed the best biohydrogen productions and yields at this HRT.This effluent was used as feed for the methanogenic reactors of the second TPAcD stage. It was characterized by a high organic load and a high concentration of TVFA, optimal for the methanogenic stage.

Methanogenic reactors operated at different HRTs (15, 12, 10, 8, 5, 4 and 3 days). Table 2 shows the different organic load rates (OLR) expressed as gVS L^{-1} d^{-1} for each HRT tested, as well as the daily flow rate supplied, expressed as mL/d.

Table 1
Characterization of the substrates individually, mixture of substrates for feeding and acidogenic influent.

Parameters	SS	WV	PM	SS:WV:PM	ACIDIFIED SS:WV:PM
Ph	6.45 ± 0.06	3.25 ± 0.14	9.36 ± 0.12	4.85 ± 0.16	5.55 ± 0.08
TCOD (g/L)	56.11 ± 0.04	41.02 ± 0.15	292.31 ± 1.53	57.07 ± 0.21	47.81 ± 1.26
SCOD (g/L)	14.31 ± 0.13	39.99 ± 0.19	163.78 ± 0.63	36.38 ± 0.18	37.16 ± 1.46
TS (g/L)	41.17 ± 0.11	22.63 ± 0.19	462.12 ± 0.72	34.84 ± 0.07	27.95 ± 2.01
vS (g/L)	35.84 ± 0.19	19.96 ± 0.18	384.01 ± 0.89	28.35 ± 0.11	22.98 ± 1.29
TVFA (mgAcH/L)	2693 ± 71	1347 ± 38	n.d.	1896 ± 54	6004 ± 164
C/N	45.15 ± 0.78	112.00 ± 3.26	3.04 ± 0.86	30.57 ± 1.01	17.36 ± 0.68
TAN (g/L)	0.23 ± 0.02	0.27 ± 0.04	23.54 ± 1.12	1.98 ± 0.03	2.26 ± 0.16
Alkalinity (g/L)	1.35 ± 0.21	0.00 ± 0.00	36.42 ± 0.18	0.54 ± 0.02	0.69 ± 0.12

2.2. TPAcD reactors and operating conditions

Continuously stirred tank reactors (CSTR) connected in series were used for this test and equipped with stainless steel blades driven by motors programmed for stirring at 40 rpm. Each reactor has three liters of capacity with two liters of working volume. They were deposited on a heating plate programmed at 55 °C for those in thermophilic conditions and at 35 °C for mesophilic stage. The head of the reactor has an outlet for the gas generated, which is collected in a 5-liter capacity Tedlar bag. Another outlet houses the temperature probe and others allow the feeding and outlet of effluents.

A daily manual feeding was carried out, consisting of withdrawing the adequate amount of effluent from the methanogenic reactor and replacing the same amount with the effluent from the acidogenic reactor. For this, a 120 mL syringe was used. Subsequently, the acidogenic reactor was fed with the mixture of sewage sludge, wine vinasse and poultry manure.

In this study, a temperature phase anaerobic co-digestion process (TPAcD) was carried out, performing a first acid thermophilic stage with 5-day HRT (considered optimal in previously carried out studies), and a second mesophilic stage with different HRTs (15, 12, 10, 8, 5, 4 and 3 days) in order to determine the optimal conditions in each case. The feed provided consisted of sewage sludge and wine vinasse (50:50) with 10 g/L of poultry manure. To carry out anaerobic digestion in the temperature phase, it was important to determine the pH and temperature at each stage, due to the different rates of bacterial growth. Therefore, a temperature of 55 °C and a pH around 5.5 was recommended in the thermophilic acidogenic digester to achieve hydrogen production. For the methanogenic digester, temperature of 35 °C and a neutral pH around 7.5 were selected [4,19,20,33,39].

2.3. Analytical methods

An initial substrates and feeds characterization 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 ammoniacal nitrogen (TAN), ammonia, alkalinity and carbon / nitrogen ratio (C/N ratio).

For the determination of TS, vS TCOD and SCOD the Standard Methods APHA-AWWA-WPFC [40] 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 [40,41]. 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 [40]. Alkalinity, total ammoniacal nitrogen and ammonia were measured using the HANNA

Table 2
Operating conditions for the methanogenic digester.

HRT (days)	15	12	10	8	5	4	3
OLR (gVS L^{-1} d^{-1})	2.78	3.45	4.14	5.17	8.26	10.34	13.75
Flow rate (mL/d)	120	150	180	225	360	450	600

multiparameter photometer (HI83399), following the standard APHA-AWWA-WPFC methods [40]. 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 determined by gas chromatographic separation (SHIMADZU GC-2010). H_2, CH_4, CO_2 and O_2 were analysed by thermal conductivity detector (TCD) using a Supelco Carboxen 1010 plot column. Gas samples were taken using a 1 mL Dynatech Gastight gas syringe.

2.4. Microbial analysis

The evolutions of the main bacterial groups in the tests were analyzed. Microorganisms were counted using the fluorescent in situ hybridization (FISH) technique [8,42–45] at the end of each HRT carried out and with the reactor operating under stable conditions. The main steps of the FISH technique using oligonucleotide probes directed at 16S rRNA were cell fixation, permeabilization and hybridization with the probe chosen for each case. The concentration of formamide varies according to the probe used, for this the concentration of formamide in the hybridization buffer was selected according to Montero et al. (2019) [46]. Samples were visually examined and cells counted using an Axio Imager upright epifluorescence microscope (Zeiss) equipped with a 100 W mercury lamp and a 100x oil objective lens. Six microbial groups were directly determined: *Eubacteria, Archaea,* butyrate-using acetogens (BUA), propionate-using acetogens (PUA), acetate-using methanogens (AUM), hydrogen-using methanogens (HUM), and hydrogen/acetate-utilizing methanogens (HAUM). The probes used for counting microorganisms were detailed in the table 3.

The total population was calculated as the sum of *Eubacteria* and *Archaea*, since they represent the majority of the microorganisms that can be found in anaerobic digesters. The percentages of each population were determined with respect to the total population. The HUM were calculated as the difference between the *Archaea* and the AUM. Acetogens were represented as the sum of PUA and BUA. Methanogenic activity was calculated as the volume ratio of generated CH_4 (L) and the number of *Archaea* (cells) contained in the digester [39,41,47,49].

2.5. Effluent classification as biosolid class a

Pathogenic microorganisms were determined in the feed of the acidogenic reactors and in the stable effluents of each TPAcD tested in the methanogenic reactors, to quantify total coliforms, Escherichia coli (E-Coli) and Salmonella. The samples were analysed at the beginning and at the end of the test and the concentration of pathogens in the effluent and the degree of pathogen reduction after the anaerobic co-digestion process in the thermophilic-mesophilic TPAcD were determined. The determination of total coliforms, E-Coli [Method 9222H] and Salmonella [Method 9260B] was performed according to standard methods [40]. Total coliforms were calculated through E-Coli, as it represents approximately 90 % of them [5]. For the possible classification of the effluent as class A biosolids, the specifications of The United States Environmental Protection Agency (US EPA) and the Regulation (EU) 2019/1009 of the European Parliament and the Council of June 5, 2019 were followed, which set the limit of pathogens that could be present in the effluent for its classification as class A biosolids and therefore for its application as a safe agricultural fertilizer.

3. Results and discussion

3.1. Characterization of the methanogenic effluents in each HRT test

The stable effluent of each HRT tested in the methanogenic reactors were analyzed. Tests were performed in triplicate so the results were shown as the average of these. Table 4 show the average values of the parameters easured at the end of each HRT tested. As can be seen, the pH in all cases remained above 7.4, optimal values for the methanogenic stage. The TCOD, SCOD, TS, vS and alkalinity followed the same trend, increasing the concentration as the HRT decreased. The concentration of TAN found in the effluents increased as the HRT decreased, until reaching a HRT of 4 and 3 days, where the concentration of TAN started to decrease. This tends to occur at very short HRT, where at high OLR, the rate of ammonia generation was not sufficient for TAN accumulation to occur [50,51]. With respect to TVFA, it followed a tendency to increase as the HRT decreased, reaching a maximum concentration of 1368 mg/L for a 3-day HRT. Acetic acid oscillated throughout the process, increasing at the beginning, decreasing in the middle HRT (8 and 5 days), and then increasing in the 4 and 3-day HRT, reaching values close to 500 mg/L. Butyric acid remained in very low quantities in all the effluents of the different HRT analysed, with a slight increase in the 3-day HRT. And finally, propionic acid remained in low concentrations for high HRT, until descending to HRT of 8 days, where a progressive increase can be observed as the HRT descends further, reaching values of 411 mg/L for HRT of 3 days.

3.2. pH

Fig. 2 shows the pH values for the different HRTs tested in the methanogenic reactors. The pH was a fundamental parameter to study the monitoring of the anaerobic degradation process [39]. The acidogenic digester was maintained at a pH of 5.42 ± 0.08 throughout the process without the need to resort to an external agent to correct it. At all times, in the methanogenic reactors, pH values ranged between 7.3 and 7.98, which were optimal for the methanogenic microorganisms, indicating that a balance was reached between the metabolic activities of the different microbial groups [52,53]. A slight drop in pH can be observed when the HRT switch occurs from 4 to 3 days. When the organic load increases, a punctual imbalance occurs between the metabolic activities of the microbial groups, but the pH was quickly recovered without the need to resort to external agents. All this means that the methanogenic reactors operated under stable conditions, that the anaerobic co-digestion of SSWVPM does not affect the efficiency of the TPAcD process and that, in addition, the system has a high buffer capacity.

3.3. Removal efficiencies

Table 5 shows, firstly, the removal efficiencies expressed as % removal of TCOD, SCOD, TS and vS for the acidogenic fermentation stage at 5 days of HRT. Subsequently, the percentage values of the removal efficiencies of the methanogenic reactors at the different HRTs tested were shown.

Table 3
Oligonucleotide probes used in this assay, target groups of each of them and hybridization conditions.

	Probe sequences (from 5́ a 3́)	Objective	Formamida (%)	T(°C)	Time (h)	Ref.
EUB338	GCTGCCTCCCGTAGGAGT	*Eubacteria*	20	46	1.5	[39,47]
ARC915	GTGCTCCCCCGCCAATTCCT	*Archaea*	35	46	1.5	[39,47]
SYMBAC824	GTACCCGCTACACCTAGT	*Syntrophobacter* spp. *(PUA)*	10	46	2	[41]
SYNM700	ACTGGTXTTCCTCCTGATTTCTA	*Syntrophomonadaceae (BUA)*	30	52	2	[41]
MSAE825	TCGCACCGTGGCCGACACCTAGC	*Methanosaetaceae (AUM)*	20	46	1.5	[39,44]
MBAC1174	TACCGTCGTCCACTCCTTCCTC	*Methanobacteriaceae (HUM)*	35	46	1.5	[41,44,46]
MS821	CGCCATGCCTGACACCTAGCGAGC	*Methanosarcinae (HAUM)*	40	46	2	[43,48]

Table 4
Average values of the parameters measured during each HRT tested.

Parameters	HRT (Days) Methanogenic stage 15	12	10	8	5	4	3
pH	7.71 ± 0.06	7.74 ± 0.08	7.76 ± 0.05	7.54 ± 0.10	7.42 ± 0.08	7.38 ± 0.04	7.34 ± 0.09
TCOD (g/L)	14.09 ± 0.78	16.05 ± 0.68	17.33 ± 0.93	27.54 ± 1.52	33.39 ± 2.04	36.65 ± 3.04	38.91 ± 2.51
SCOD (g/L)	6.51 ± 1.01	6.24 ± 0.84	6.48 ± 0.54	10.66 ± 0.85	16.11 ± 0.87	16.60 ± 0.48	16.97 ± 0.96
TS (g/L)	15.26 ± 1.33	16.73 ± 1.21	17.41 ± 1.44	20.21±1.37	18.56 ± 2.31	19.99 ± 2.21	21.05 ± 2.19
vS (g/L)	9.53 ± 1.14	9.11 ± 1.58	9.94 ± 0.87	13.02 ± 0.91	13.96 ± 1.21	15.57 ± 1.87	16.25 ± 1.43
Alkalinity (g/L)	4.67 ± 0.12	5.80 ± 0.11	5.74 ± 0.08	5.88 ± 0.13	6.70 ± 0.22	7.70 ± 0.12	8.10 ± 0.13
Ammonia (g/L)	2.32 ± 0.04	2.31 ± 0.04	2.84 ± 0.02	3.01 ± 0.05	2.52 ± 0.08	1.71 ± 0.07	1.45 ± 0.04
TAN (g/L)	4.51 ± 0.05	5.28 ± 0.10	5.15 ± 0.09	5.46 ± 0.11	6.31 ± 0.14	4.53 ± 0.02	3.97 ± 0.07
TVFA (mgAcH/L)	154.54 ± 0.68	195.26 ± 1.12	245.90 ± 1.34	248.47 ± 1.18	274.24 ± 1.52	757.56 ± 2.31	1368.24 ± 2.02
Acetic Ac. (mg/L)	118.82 ± 0.74	132.24 ± 0.99	170.48 ± 1.22	81.79 ± 1.04	98.61 ± 0.79	423.79 ± 1.84	467.45 ± 3.18
Propionic Ac. (mgAcH/L)	n.d.	28.97 ± 0.34	14.08 ± 0.29	151.82 ± 0.55	161.50 ± 0.68	329.75 ± 1.77	410.69 ± 1.33
Butyric Ac. (mgAcH/L)	10.36 ± 0.24	4.12 ± 0.13	5.52 ± 0.23	6.75 ± 0.18	5.62 ± 0.41	16.52 ± ± 0.29	133.35 ± 2.54

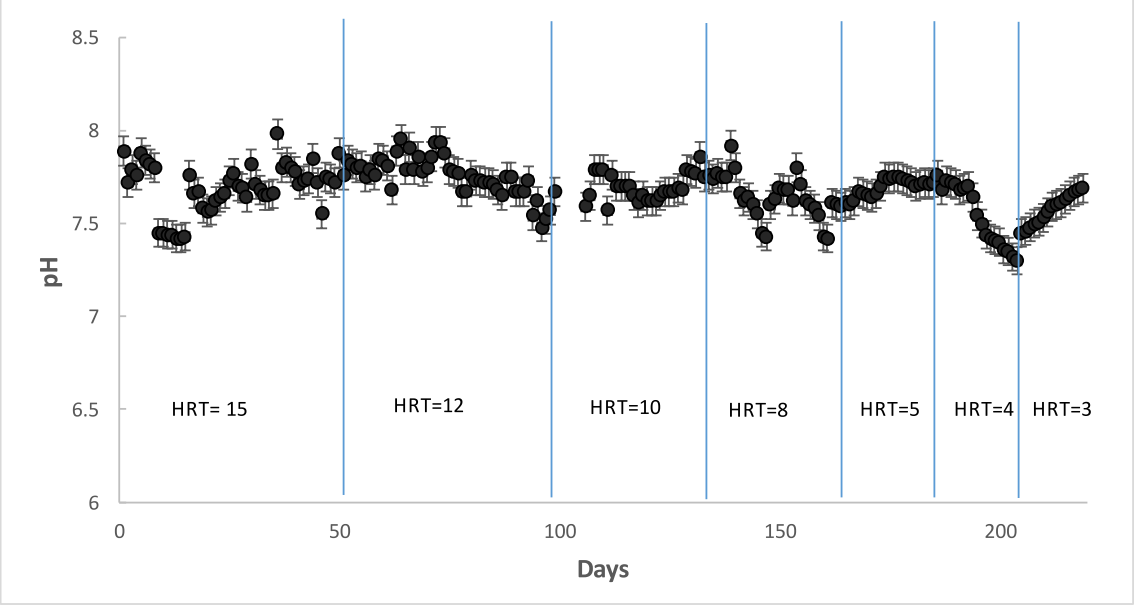

Fig 2. Evolution of pH in the methanogenic reactors, for each HRT tested.

Table 5
Average removal efficiency expressed as % removal of TCOD, SCOD, TS and vS for acidogenic reactors at 5-day HRT, and for methanogenic reactors at each HRT tested (15, 12, 10, 8, 5, 4 and 3 days).

Reactor	HRT	%REMOVAL TCOD	SCOD	TS	VS
Acidogenic Stage	**5**	5.66 ± 0.36	−71.24 ± 8.01	27.17 ± 1.22	33.32 ± 2.35
Methanogenic Stage	**15**	58.87 ± 3.18	72.02 ± 1.98	47.18 ± 3.82	56.72 ± 4.36
	12	60.95 ± 3.01	77.66 ± 1.22	47.78 ± 2.34	59.81 ± 1.74
	10	59.07 ± 2.71	72.32 ± 2.22	46.66 ± 3.46	56.35 ± 5.03
	8	45.97 ± 2.38	65.57 ± 2.31	46.22 ± 1.87	50.55 ± 2.85
	5	41.56 ± 3.10	61.04 ± 2.56	37.71 ± 4.12	48.54 ± 3.85
	4	37.13 ± 4.21	58.09 ± 5.28	35.44 ± 3.54	39.86 ± 3.66
	3	36.26 ± 3.88	55.12 ± 4.32	35.21 ± 4.67	37.87 ± 5.37

For the acidogenic stage, very low % elimination of TCOD were collected, and even negative for SCOD. This was due to the solubilization of organic matter, which may explain the negative values obtained for SCOD at this stage. In addition to the hydrolyzation of the insoluble fraction of TCOD that was transformed into soluble compounds and VFA before its conversion into methane [39]. Regarding the elimination of TS and vS values of 27 % and 33 % elimination, respectively, were recorded in the first stage. These values were very typical of this stage, being able to find similar records in the acidogenic fermentation stage with food residues, achieved a 29 % removal of vS [15]. Thus, Malinowski et al. (2020) [25] registered total COD removal values between 2 and 27 % and volatile solids removal values between 14 and 39 %, which coincides with the results observed in this study. For the second methanogenic stage of TPAcD, the highest elimination values were presented. For 12-day HRT, the values recorded were higher compared to other HRTs tested. Elimination percentage values around 61 % of TCOD, 78 % of SCOD, 48 % of TS and 60 % of vS were reached. For this same HRT, the highest methane yield values were recorded, which will be analyzed in the following sections. From an HRT of 8 days onwards, elimination yields decrease drastically (Table 5). The growth of the microbial

population to degrade organic matter depends on the optimum organic loading rate provided [54]. For a THR of 12 days the maximum population of the microorganisms was reached coinciding with the maximum purification efficiency, as will be seen in the following sections.

The best vS elimination results in the TPAcD system were obtained by operating at HRTs of 5 days in the first stage (33.32 %) and 12 days in the second stage (59.81 %), achieving overall vS reduction percentages of 93.13 %. The results of this study were better than those obtained in TPAcD sludge monodigestion systems (37.60 % vS removal) [55]. However, in sludge co-digestion studies, higher vS removal percentages were achieved. Thus, in the co-digestion of sludge and organic waste in TPAcD systems performed by Borowski (2015), he obtains 52,10 % and 77 % vS removal in the methanogenic stage and in the overall system, respectively [24]. In the tests conducted by Tena et al., (2021) who recorded 38.17 % vS removal in the second methanogenic stage and a total of 53.19 % removal in the whole TPAcD process for the co-digestion of sludge and wine vinasse [5]. With these values, it can be stated that a third co-substrate, such as poultry manure, improves vS removal by 43 % compared to co-digestion of sewage sludge and wine vinasse for a TPAcD process. This was because the mixing of different wastes increases biodegradability and dilutes the toxics in the mixture, and in turn, the TPAcD system was more stable and presented better energy recovery results and scrubbing efficiencies for highly biodegradable wastes [56].

3.4. Evolution of VFA, Alkalinity, TAN and ammonia.

The VFA were then measured, totally and individually, to check their evolution in each reactor and for each HRT tested. The concentrations of TAN and ammonia were also analyzed and the possible relationships that could cause instability in some of the tested cases were studied. The initial concentration of TVFA in the feed was 2928.64 mgAcH/L. In the TPAcD system, in thermophilic acid reactors there was an increase in TVFA with respect to the feed, going from 2928.64 mg/L to 6004.24 mg/L (Table 1). These TVFAs were introduced into the methanogenic reactors to conclude the anaerobic digestion process. These TVFA were easily eliminated, obtaining values above 95.80 % elimination in all HRTs tested, even 97.43 % for HRT of 15 days. The main composition of the TVFA was mainly acetic acid, propionic acid and butyric acid as can be seen in Fig. 3. It can be seen how the proportion of propionic acid was similar after acid fermentation, however, the composition of butyric acid increases considerably. This fact was very important in the anaerobic digestion process, since butyric acid and hydrogen production, in this first stage, would be produced from acetic acid, which would help to understand the metabolic pathways used by microorganisms in acidogenic reactors [57]. As a consequence, acetic acid decreases its proportion but would continue to be dominant together with butyric acid.

The composition of TVFAs was a very important characteristic for a possible use in the production of biomethane. The composition of butyric acid and acetic acid was beneficial for the second methanogenic stage [58,59]. Although the TVFA concentration in the effluent of the methanogenic reactors was quite small and similar up to 5-day HRT, as can be seen in Fig. 4, it was observed that as HRT decreases to 4 and 3 days, the accumulation of TVFA, mainly due to propionic and acetic acids. Acetic acid remained relatively constant for high HRTs, however, it tripled in concentration for shorter HRTs (4 and 3 days). For a 15-day HRT, propionic acid was non-existent, and it appears in the effluent when the HRT drops to 10 days, although it does so in small quantities. But for HRTs less than 8 days, its concentration was dominant and skyrocketed compared to the rest of the compounds. Therefore, at shorter HRTs, propionic acid tends to accumulate. This trend was also observed by Jiang et al., (2022), which observed that the concentration of VFA, especially propionic acid, increased as the OLR increased, causing instability in the system [60]. As a consequence, the efficiency of the reactors decreases a little due to the concentration of propionic acid and TAN, but the concentration of propionic acid did not exceed in any case the inhibition values reported in the literature (900 mg/L) [25].

Finally, if the TVFA/Alk ratio was observed, the stability of the system can be verified. It has been reported that values below 0.4 represent the sufficient buffer capacity of the system [61], however, other authors consider the threshold value to be 0.3, registering instability in the reactor if this value increases [59]. Although values below 0.1 were preferable, indicating that the system was strong enough [53,62,63]. In this study, the ratio was calculated for all reactors and each HRT tested. As can be seen in Fig. 4, in the methanogenic reactors, mean values lower than 0.1 were obtained for all the HRTs tested, except for a 3-day HRT, where a higher value of 0.17 was obtained, but within the values considered optimal to confirm the stability and robustness of the TPAcD system.

In this way, inhibition has been observed in ranges between 1.5 and 14 gTAN/L [64–68]. It was known that an adequate concentration of TAN provides benefits for microorganisms, > 500mgTAN/L [66], 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. Due to the addition of poultry manure, high TAN values were recorded in all HRT tests, always above 4 g/L, reaching values > 6 g/L for 5-day HRT. For shorter HRTs, a decrease in TAN was observed as a consequence of a shorter time for accumulation to occur.

In short, the TPAcD system was more sensitive to inhibition by propionic acid at high concentrations of ammonia and TAN. According

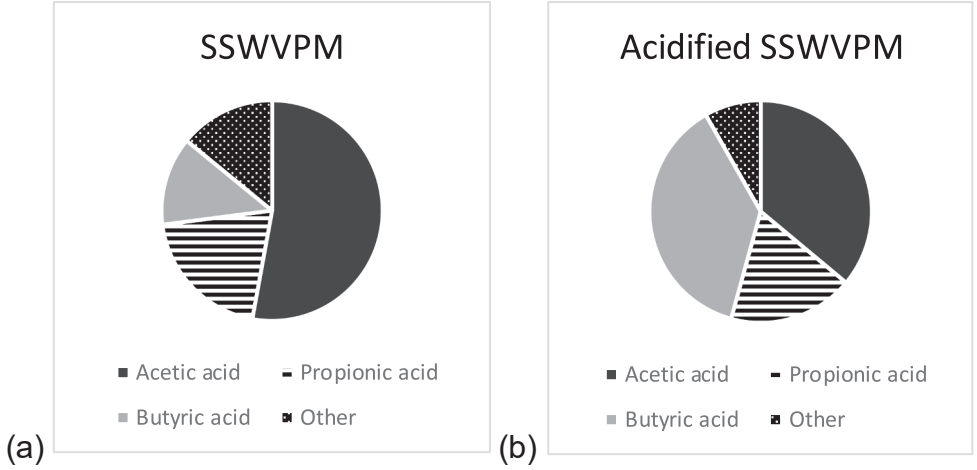

Fig. 3. Proportion of acetic acid, butyric acid and propionic acid for feed (a)) and acidified SSWVPM (b)).

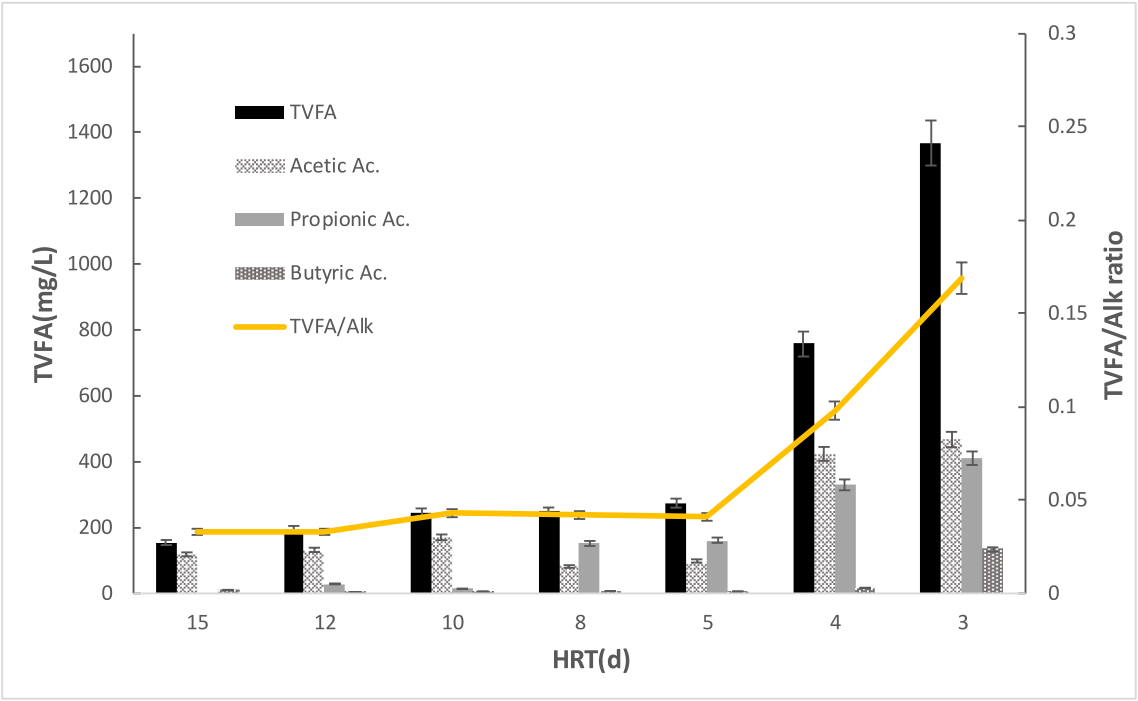

Fig. 4. TVFA in each HRT tested, and concentration of acetic acid, propionic acid, butyric acid and TVFA/Alk ratio.

to Calli et al. (2005), at high concentrations of ammonia (1.5–3 g/L) [8] methanogens were inhibited causing accumulation of propionic acid and acetic acid, and a decrease in biomethane production [64,66]. With these results, it can be affirmed that the anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure in temperature phase (TPAcD) was much more stable at HRT of 12 and 10 days, with an insignificant propionic concentration that did not affect the development of the process.

3.5. Methane yield

Fig. 5 shows the average methane yield obtained for each HRT carried out, as well as the average volume of biogas recorded and the percentage of methane in its composition.

The best methane generation results were obtained when the TPAcD system operates at 5 days in the first stage and 12 in the second stage with a methane yield of 391.15 $mLCH_4/gVS_{added}$. Under these conditions, the maximum removal efficiency, in terms of TS, vS and COD, was also obtained. As for the daily methane production, it increased as the organic load contributed to the system increased, reaching values of 2.64 L per day for 3-day HRT. At the same time, the methane content of the biogas produced decreased to 43 %. Therefore, the trend in methane content tends to decrease as HRT decreases. This means that a lower vS input to the digesters was needed to obtain the highest yield values for the 15 and 12-day HRTs.

Anaerobic monodigestion of sewage sludge achieves methane yields between 140 and 160 mL/gVS [69,70]. With the addition of vinasse and poultry manure for anaerobic co-digestion, these methane yield values can be tripled.

Many authors consider the TPAcD system to be more favorable compared to a single-stage process [18,24,59,71–73] with values in the methanogenic stage similar to those obtained in this study. In the particular case of anaerobic tri-digestion in TPAcD systems, the anaerobic tridigestion of palm oil mill effluents, sewage sludge and food waste, higher yields were obtained than those of bidigestion or monodigestion [74]. In the study of anaerobic tri-digestion of cattle slurry, manure and pasture silage as substrates, it was obtained the methane yields of 215 $mLCH_4/gVS_{added}$ operating an optimum HRT of 4 days in the first stage and 10 days in the second stage [55].

3.6. 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 feed supplied to the acidogenic reactors (SSWVPM), and the stable effluents from the methanogenic reactors were measured.

In the first place, the presence of *Salmonella* was found in the feed of the reactors. However, an absence was recorded in the effluents of all the reactors. This suggests that the anaerobic co-digestion of the three considered substrates in TPAcD system was effective in the elimination of *Salmonella* in all the HRTs considered.

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.

As can be seen in Table 6 and Fig. 6, although the concentration of coliforms was high in the feeds, after undergoing anaerobic co-digestion, the values drop drastically, falling below the limit required to be classified as Class A biosolids by current European legislation and the U.S. EPA .

Thus, all the values recorded were below 945 (CFU)/gTS. The combination of the thermophilic phase and the mesophilic phase reduces the number of pathogens sufficiently. The content of pathogens in methanogenic effluents increases as the HRT decreases. It was expected that by shortening the total processing time, the number of pathogens would increase. However, clearance was>96,70 % for all HRTs. This indicates that anaerobic co-digestion was an efficient practice in the elimination of pathogens in a TPAcD system, mainly due to its effect of dilution of contaminants offered by co-digestion, high temperatures were key to eliminate pathogens in the first stage of the TPAcD that occurs in acidogenic reactors. Therefore, it was possible to classify the effluents as class A biosolids in all the HRTs tested, where anaerobic co-digestion of 3 substrates (S, V and PM) was practiced. The final product

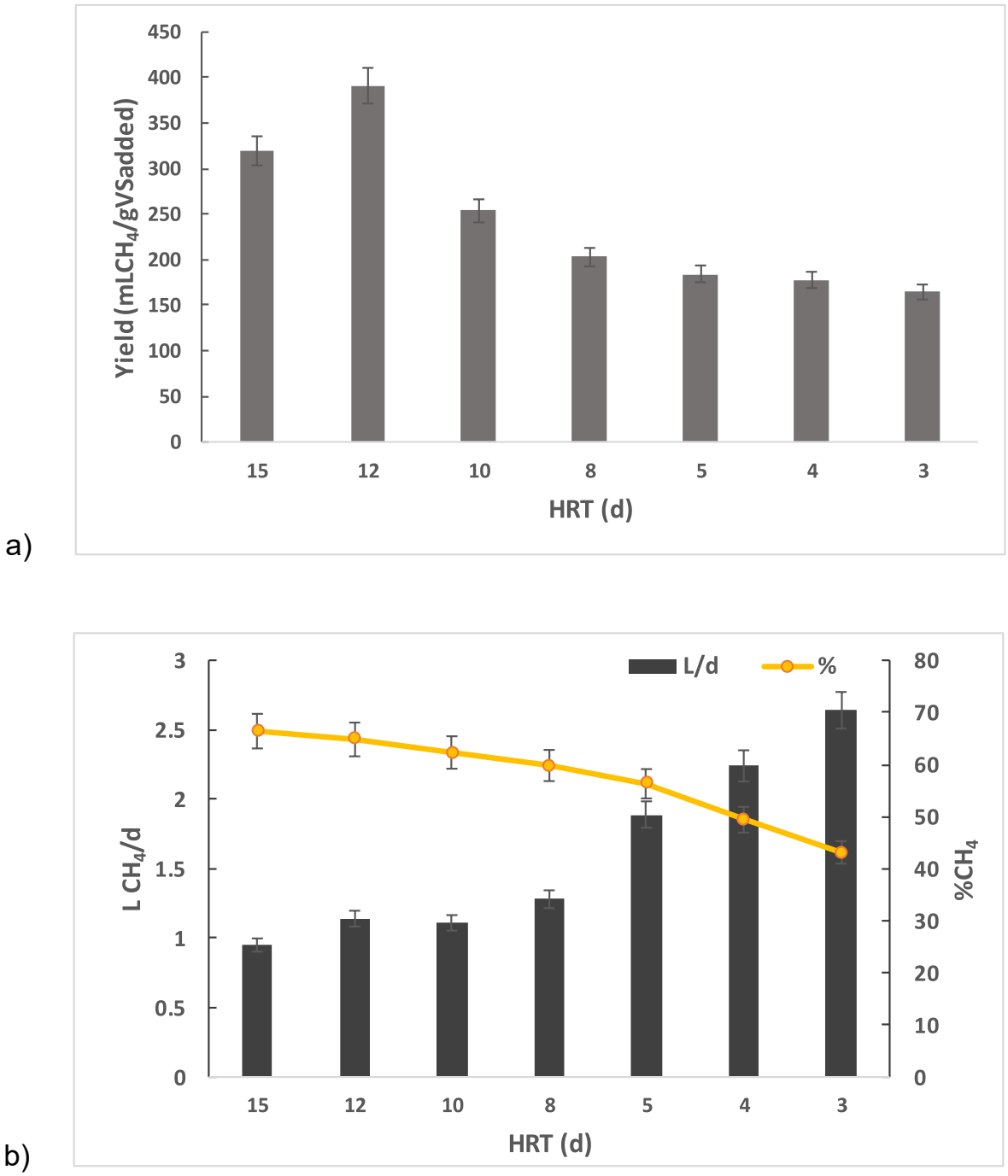

Fig. 5. a) Methane yield for the second stage of TPAcD, at each HRT tested for each one. b) Average volume of daily biogas produced, expressed in L/d, and average percentage of CH_4 in the biogas studied.

Table 6
Concentration of E-Coli and Total Coliforms expressed in (CFU)/gTS, recorded in the reactor feed.

Feed	*E-Coli* (CFU/gTS)	*Total Coliform* (CFU/gTS)
SSWVPM	16,500	28,700

of anaerobic co-digestion 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 [5].

3.7. Microbial population dynamics

The concentrations of microorganisms in the digester effluent were determined for each HRT tested. The analysis of the microbial population was performed at the end of each stable period of operation, when the microorganisms were adapted to the different organic loading conditions. Table 7 shows a summary of the percentages of the main groups of microorganisms involved in the methanogenic stage. As can be seen, the total population of microorganisms remained relatively constant for the tested HRTs, with the highest concentration for the 12 and 10 day HRTs. During these HRTs, the maximum values of methane yield and vS removal were recorded. Subsequently, the total population decreases

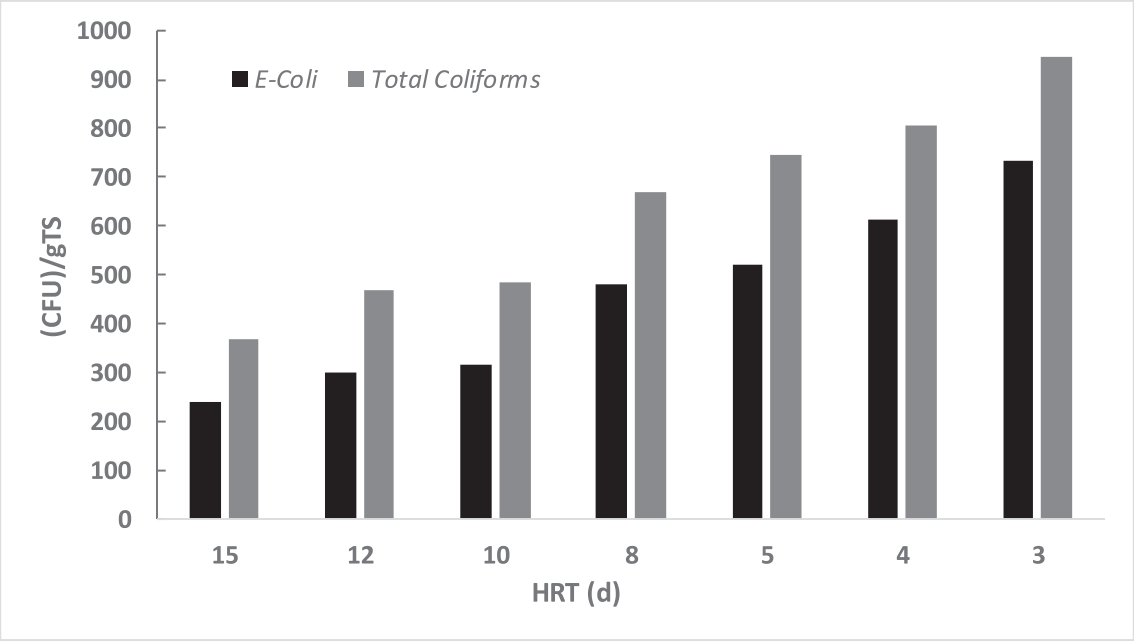

Fig. 6. Concentration of *E-Coli* and *total coliforms* in the methanogenic effluents at the different HRTs tested.

Table 7
Characterisation of the different populations in the digester effluent at each of the tested HRTs.

HRT	D	15	12	10	8	5	4	3
Total Population	10^9 cells/mL	5.60 ± 0.58	7.34 ± 0.97	6.44 ± 0.33	4.83 ± 0.29	4.53 ± 0.41	3.95 ± 0.44	3.87 ± 0.38
Eubacteria	%	49.78 ± 2.28	74.26 ± 3.22	78.2 ± 3.56	83.04 ± 2.14	76.24 ± 2.05	66.74 ± 2.05	35.37 ± 2.43
Archaea	%	50.22 ± 1.26	25.74 ± 1.89	21.8 ± 1.38	16.96 ± 1.20	23.76 ± 2.01	33.26 ± 1.99	64.63 ± 2.06
PUAs	%	37.31 ± 1.76	20.59 ± 1.28	18.78 ± 1.78	33.11 ± 1.26	37.18 ± 2.01	35.61 ± 1.55	7.31 ± 1.66
BUAs	%	12.58 ± 0.81	14.87 ± 1.13	16.56 ± 1.34	10.20 ± 5.06	12.17 ± 2.18	15.52 ± 2.05	51.28 ± 1.97
HUMs	%	7.96 ± 1.37	14.55 ± 1.56	13.04 ± 1.49	11.50 ± 1.07	16.95 ± 1.25	19.20 ± 2.57	47.20 ± 2.22
AUM s	%	10.70 ± 1.11	5.39 ± 1.63	5.53 ± 1.43	3.58 ± 1.26	2.91 ± 1.18	3.71 ± 0.35	5.57 ± 1.75
HAUMs	%	31.56 ± 2.14	5.80 ± 1.78	3.23 ± 0.99	1.88 ± 0.45	10.35 ± 1.12	11.85 ± 0.84	11.95 ± 0.87
Microbial Activity	10^{-10} LCH_4/cells	1.69 ± 0.14	1.55 ± 0.17	1.72 ± 0.15	2.62 ± 0.22	4.17 ± 0.19	5.67 ± 0.77	6.82 ± 0.13

from the 8-day HRT to values of 3.87E10^9 cells/mL. With respect to microbial activity, a progressive increase was recorded as the OLR increased, with values ranging from 1.69E10^{-10} LCH_4/cells for the 15-day HRT to 6.82E10^{-10} LCH_4/cells for the 3-day HRT. These results were consistent with those recorded for methane production for each HRT tested, showing the same increasing trend (Fig. 5b)).

The percentages of *Eubacteria* and *Archaea* varied as OLR increased. For the longest and shortest HRT tested (15 and 3 days) the percentage of *Archaea* was higher than *Eubacteria*. For the rest of the HRT, *Eubacteria* presented a higher ratio than *Archaea*, with *Eubacteria:Archaea* ratios of 74:26, 78:22, 83:17 and 76:24, for HRT 12, 10, 8 and 5 days, respectively, which correspond to the maximum methane yield values. Similar results have been reported by Zahedi et al. (2013) in which they observed an increase in the proportion of Eubacteria as OLR increased [41]. This was due to the fact that by shortening the HRT in the methanogenic reactor, the feed flow rate (acidogenic effluent flow rate) increases, so the contribution of the population of Eubacteria to the methanogenic reactor was greater. For OLR > 13.75 gVS $L^{-1}d^{-1}$, washout of microorganisms occurs, causing instability of the system. In addition, a higher proportion of *Eubacteria* than *Archaea* would be related to a higher stability of the process [23,39,41,75]. As for the acetogens, the proportion of BUA was always higher than the proportion of PUA, except for 3-day HRT, where this trend was reversed. This may be due to the high values of butyric acid in the effluent, and that for very short HRT, with a high OLR, the VFA cannot be consumed at the rate at which they were supplied to the system [75].

The results for the *Archaea* population (Table 7, Fig. 7) showed that the average values of the HUM/AUM ratio were always greater than one, except for HRT of 15 days. Increasing HUM versus AUM has been shown to improve the stability of anerobic co-digestion. This was mainly due to the large amount of butyric acid in the substrate and the short HRTs that were tested. The HUM must rapidly consume the hydrogen generated to prevent it from accumulating, as acetogens and AUM do not grow well in the presence of hydrogen [41]. On the other hand, the process of acetoclastic methanogenesis was dominated by the ratio between AUM and HAUM, which depends on the substrate and the operating conditions [76]. During the studied process, the population of *Methanosarcinae* was greater for HRT of 15 days, decreasing progressively for HRT of 12, 10 and 8 days and increasing after 5 days of HRT. This increase in shorter HRT was mainly due to the fact that HAUM were more tolerant to high concentrations of VFA, in particular acetic acid, and have a high growth rate, which could lead to an increase in their competitiveness with AUM in the system [72]. In this way, it was known that AUM have a slower growth rate at higher acetic acid concentration (which occurred at shorter HRT), giving way to higher concentration of HAUM, which were found to be favoured under these conditions [25,76,77]. This was possible because HAUM can follow different metabolic pathways (acetoclastic and hydrogenotrophic) [78]. The metabolic pathway established by the microbial population for anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure in TPAcD was

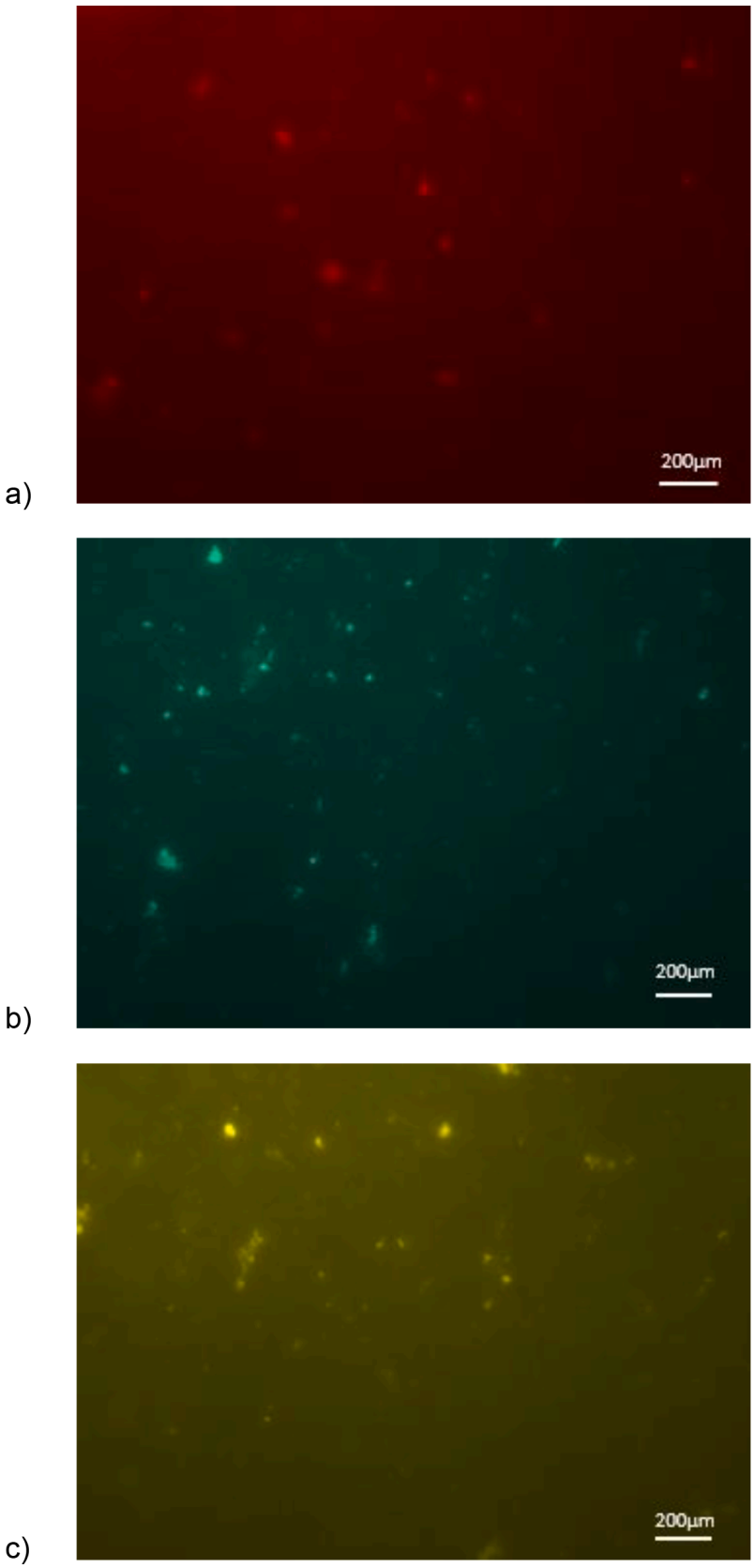

Fig. 7. Population of different microorganisms for 12-day HRT, observed in microoscopy. a) AUM population, b) HAUM population, c) HUM population.

hydrogenotrophic, with HUM dominating during all HRTs tested, except for 15 days, and increased HAUM concentration in shorter HRTs. This led to an increase in the ratio of *archaea* to *eubacteria*, reaching ratios of 35:65 for 3-day HRT.

3.8. Prospects

This study is the prelude to breaking down the obstacles to the implementation of TPAcD systems on a local scale to properly manage the transport of waste to the treatment plant, taking into account the seasonality of its generation. Determining the best conditions for the generation of biogas and agricultural fertilizer would improve the needs of local producers and farmers, solving an environmental problem and obtaining great benefits. The bioenergy produced could self-supply the treatment plant and the agricultural fertilizer would replace mineral fertilizers, avoiding major environmental pollution problems, closing the circle of nutrient recovery. The feasibility of this system shows the opportunity for socio-economic, industrial and environmental improvement, reducing greenhouse gases emissions.

4. Conclusions

The effect of HRT on the methanogenic stage of a TPAcD technology for the co-digestion of sewage sludge, wine vinasse and poultry manure was studied. The best results in the methanogenic stage were obtained for a HRT of 12 days, being:

- Maximum methane yield with 391 $mLCH_4/gVS_{added}$.
- High percentage of vS removal (56 %) in the second stage, and considering the entire TPAcD process, the percentage amounts to 93 %.
- Elimination>96.70 % of VFA in the effluent, with a negligible proportion of proprionic acid.
- No inhibition in biogas production was observed at high concentrations of TAN (>4.5 g/L).
- The *Eubacteria* population was higher than the *Archaea* in the HRTs with the highest methane yield, and the proportion of PUA was higher than that of BUA. While in the *Archaea* population, methanogenesis was dominated by HUM. Microbial activity increased as OLR increased.
- All effluents complied with US EPA standards and could be classified as Class A biosolids.

Therefore, the thermophilic-acidogenic, mesophilic-methanogenic TPAcD system can be considered as a suitable alternative for the management of different wastes with HRT (5/12) at each stage respectively. This system was a sustainable and environmentally friendly technology, managing to solve an environmental problem of organic waste accumulation, and to obtain high added value products such as biofertiliser and bioenergy, transferring to a circular economy model.

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

The authors do not have permission to share data.

Acknowledgment

This study has been funded by the Project Management of agro-food waste and sludge within the framework of the circular economy: energy and fertilizer production through anaerobic co-digestion in a pilot plant (P18-RT-1348) of the Andalusian Research, Development and Innovation Plan (PAIDI 2020). The authors would like to thank the poultry farm Marta Aragón, S.L. for their collaboration.

References

[1] V. Riau, M.A. De la Rubia, M. Pérez, Assessment of solid retention time of a temperature phased anaerobic digestion system on performance and final sludge characteristics, J. Chem. Technol. Biotechnol. 87 (2012) 1074–1082, https://doi.org/10.1002/jctb.3709.
[2] Y. Qin, A. Higashimori, L.J. Wu, T. Hojo, K. Kubota, Y.Y. Li, Phase separation and microbial distribution in the hyperthermophilic-mesophilic-type temperature-phased anaerobic digestion (TPAD) of waste activated sludge (WAS), Bioresour. Technol. 245 (2017) 401–410, https://doi.org/10.1016/j.biortech.2017.08.124.
[3] V. Ripoll, C. Agabo-García, M. Perez, R. Solera, Improvement of biomethane potential of sewage sludge anaerobic co-digestion by addition of "sherry-wine" distillery wastewater, J. Clean. Prod. 251 (2020), https://doi.org/10.1016/j.jclepro.2019.119667.
[4] M. Tena, B. Luque, M. Perez, R. Solera, Enhanced hydrogen production from sewage sludge by cofermentation with wine vinasse, Int. J. Hydrogen Energy. 45 (2020) 15977–15984, https://doi.org/10.1016/j.ijhydene.2020.04.075.
[5] M. Tena, M. Perez, R. Solera, Benefits in the valorization of sewage sludge and wine vinasse via a two-stage acidogenic-thermophilic and methanogenic-mesophilic system based on the circular economy concept, Fuel. 296 (2021), 120654, https://doi.org/10.1016/j.fuel.2021.120654.
[6] L. Sillero, R. Solera, M. Perez, Biochemical assays of potential methane to test biogas production from dark fermentation of sewage sludge and agricultural residues, Int. J. Hydrogen Energy. (2022), https://doi.org/10.1016/j.ijhydene.2022.02.080.
[7] S. Khoufi, A. Louhichi, S. Sayadi, Optimization of anaerobic co-digestion of olive mill wastewater and liquid poultry manure in batch condition and semi-continuous jet-loop reactor, Bioresour. Technol. 182 (2015) 67–74, https://doi.org/10.1016/j.biortech.2015.01.092.
[8] B. Calli, B. Mertoglu, B. Inanc, O. Yenigun, Effects of high free ammonia concentrations on the performances of anaerobic bioreactors, Process Biochem. 40 (2005) 1285–1292, https://doi.org/10.1016/j.procbio.2004.05.008.
[9] L.C. Ampese, W.G. Sganzerla, H. Di Domenico Ziero, A. Mudhoo, G. Martins, T. Forster-Carneiro, Research progress, trends, and updates on anaerobic digestion technology: A bibliometric analysis, J. Clean. Prod. 331 (2022), https://doi.org/10.1016/j.jclepro.2021.130004.
[10] N. Alemahdi, H. Che Man, N. Abd Rahman, N. Nasirian, Y. Yang, Enhanced mesophilic bio-hydrogen production of raw rice straw and activated sewage sludge by co-digestion, Int. J. Hydrogen Energy. 40 (2015) 16033–16044, https://doi.org/10.1016/j.ijhydene.2015.08.106.
[11] A.P. da Cunha, M.C. Cammarota, I. Volschan, Anaerobic co-digestion of sewage sludge and food waste: Effect of pre-fermentation of food waste in bench- and pilot-scale digesters, Bioresour. Technol. Reports. 15 (2021), 100707, https://doi.org/10.1016/j.biteb.2021.100707.
[12] Y. Wang, Z. Wang, Q. Zhang, G. Li, C. Xia, Comparison of bio-hydrogen and bio-methane production performance in continuous two-phase anaerobic fermentation system between co-digestion and digestate recirculation, Bioresour. Technol. 318 (2020), 124269, https://doi.org/10.1016/j.biortech.2020.124269.
[13] W.G. Sganzerla, L.C. Ampese, T.A.C. Parisoto, T. Forster-Carneiro, Process intensification for the recovery of methane-rich biogas from dry anaerobic digestion of açaí seeds, Biomass Convers. Biorefinery. (2021), https://doi.org/10.1007/s13399-021-01698-1.
[14] V. Riau, M.Á. De la Rubia, M. Pérez, Temperature-phased anaerobic digestion (TPAD) to obtain class A biosolids: A semi-continuous study, Bioresour. Technol. 101 (2010) 2706–2712, https://doi.org/10.1016/j.biortech.2009.11.101.
[15] S. Paudel, Y. Kang, Y.S. Yoo, G.T. Seo, Effect of volumetric organic loading rate (OLR) on H2 and CH4 production by two-stage anaerobic co-digestion of food waste and brown water, Waste Manag. 61 (2017) 484–493, https://doi.org/10.1016/j.wasman.2016.12.013.
[16] K. Feng, Q. Wang, H. Li, Y. Zhang, Z. Deng, J. Liu, X. Du, Effect of fermentation type regulation using alkaline addition on two-phase anaerobic digestion of food waste at different organic load rates, Renew. Energy. 154 (2020) 385–393, https://doi.org/10.1016/j.renene.2020.03.051.
[17] X. Wang, C. Gao, X. Qi, Y. Zhang, T. Chen, Y. Xie, A. Zhang, J. Gao, Enhancing sludge fermentation and anaerobic digestion by mechanical cutting pretreatment, J. Water Process Eng. 40 (2021), 101812, https://doi.org/10.1016/j.jwpe.2020.101812.
[18] H. Chen, S. Chang, Dissecting methanogenesis for temperature-phased anaerobic digestion: Impact of temperature on community structure, correlation, and fate of methanogens, Bioresour. Technol. 306 (2020), 123104, https://doi.org/10.1016/j.biortech.2020.123104.
[19] K. Postawa, J. Szczygieł, M. Kułażyński, Heuristic methods in optimization of selected parameters of Two-Phase Anaerobic Digestion (TPAD) model, Fuel. 281 (2020), https://doi.org/10.1016/j.fuel.2020.118257.
[20] C. Amodeo, S. Hattou, P. Buffiere, H. Benbelkacem, Temperature phased anaerobic digestion (TPAD) of organic fraction of municipal solid waste (OFMSW) and digested sludge (DS): Effect of different hydrolysis conditions, Waste Manag. 126 (2021) 21–29, https://doi.org/10.1016/j.wasman.2021.02.049.
[21] J. Fernández-Rodríguez, M. Pérez, L.I. Romero, Semicontinuous Temperature-Phased Anaerobic Digestion (TPAD) of Organic Fraction of Municipal Solid Waste

(OFMSW). Comparison with single-stage processes, Chem. Eng. J. 285 (2016) 409–416, https://doi.org/10.1016/j.cej.2015.10.027.
[22] M.A. De La Rubia, F. Raposo, B. Rincón, R. Borja, Evaluation of the hydrolytic-acidogenic step of a two-stage mesophilic anaerobic digestion process of sunflower oil cake, Bioresour. Technol. 100 (2009) 4133–4138, https://doi.org/10.1016/j.biortech.2009.04.001.
[23] R. Montañés, M. Pérez, R. Solera, Anaerobic mesophilic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of pH control, Chem. Eng. J. 255 (2014) 492–499, https://doi.org/10.1016/j.cej.2014.06.074.
[24] S. Borowski, Temperature-phased anaerobic digestion of the hydromechanically separated organic fraction of municipal solid waste with sewage sludge, Int. Biodeterior. Biodegrad. 105 (2015) 106–113, https://doi.org/10.1016/j.ibiod.2015.08.022.
[25] C. Malinowsky, W. Nadaleti, L. Rech, R. Bayard, A. Borges, D.C. Junior, A. Jo, Start-up phase optimization of two-phase anaerobic digestion of food waste : Effects of organic loading rate and hydraulic retention time 296 (2021), https://doi.org/10.1016/j.jenvman.2021.113064.
[26] S. Sivaprakasam, K. Balaji, Effect of HRT on biogas yield in treating dairy industry wastewater using Upflow Anaerobic Sludge Fixed Film reactor, Mater. Today Proc. 43 (2020) 1443–1448, https://doi.org/10.1016/j.matpr.2020.09.182.
[27] X. Wang, N. Andrade, J. Shekarchi, S.J. Fischer, A. Torrents, M. Ramirez, Full scale study of Class A biosolids produced by thermal hydrolysis pretreatment and anaerobic digestion, Waste Manag. 78 (2018) 43–50, https://doi.org/10.1016/j.wasman.2018.05.026.
[28] E.P.A.R. Viii, Biosolids Management Handbook Topic United States Regulations and Practical Experience on Biosolids Reuse and Disposal 1 . 2-1, (n.d.).
[29] A.B. Rubin, Standards for the use or disposal of sewage sludge, Fed. Regist. 64 (1999) 42552–42573.
[30] U. Europea, Reglamento (UE) 2019/1009 del Parlamento Europeo y del Consejo de 5 de junio de 2019, D. Of. La Unión Eur. 2019 (2019) 1–114.
[31] V. Riau, M.Á. de la Rubia, M. Pérez, Temperature-phased anaerobic digestion (TPAD) to obtain Class A biosolids. A discontinuous study, Bioresour. Technol. 101 (2010) 65–70, https://doi.org/10.1016/j.biortech.2009.07.072.
[32] E. Lloret, M.J. Salar, J. Blaya, J.A. Pascual, Two-stage mesophilic anaerobic-thermophilic digestion for sludge sanitation to obtain advanced treated sludge, Chem. Eng. J. 230 (2013) 59–63, https://doi.org/10.1016/j.cej.2013.06.066.
[33] L. Sillero, R. Solera, M. Perez, Anaerobic co-digestion of sewage sludge, wine vinasse and poultry manure for bio-hydrogen production, Int. J. Hydrogen Energy. (2021), https://doi.org/10.1016/j.ijhydene.2021.11.032.
[34] S. Zahedi, D. Sales, L.I. Romero, R. Solera, Hydrogen production from the organic fraction of municipal solid waste in anaerobic thermophilic acidogenesis: Influence of organic loading rate and microbial content of the solid waste, Bioresour. Technol. 129 (2013) 85–91, https://doi.org/10.1016/j.biortech.2012.11.003.
[35] J. Zheng, M. Gao, Q. Wang, J. Wang, X. Sun, Q. Chang, Y. Tashiro, Enhancement of L-lactic acid production via synergism in open co-fermentation of Sophora flavescens residues and food waste, Bioresour. Technol. 225 (2017) 159–164, https://doi.org/10.1016/j.biortech.2016.11.055.
[36] W.O. Meneses-Quelal, B. Velázquez-Martí, J. Gaibor-Chávez, Z. Niño-Ruiz, Biochemical potential of methane (BMP) of camelid waste and the Andean region agricultural crops, Renew. Energy. 168 (2021) 406–415, https://doi.org/10.1016/j.renene.2020.12.071.
[37] M. Hakimi, R. Shamsuddin, R. Pendyala, A.A. Siyal, H. AlMohamadi, Co-anaerobic digestion of chicken manure with the addition of Cymbopogan citratus, Mentha piperita and Citrus sinensis as fly deterrent agents: Biogas production and Kinetic study, Bioresour. Technol. Reports. 15 (2021), 100748, https://doi.org/10.1016/j.biteb.2021.100748.
[38] I. Syaichurrozi, Biogas production from co-digestion Salvinia molesta and rice straw and kinetics, Renew. Energy. 115 (2018) 76–86, https://doi.org/10.1016/j.renene.2017.08.023.
[39] M. Tena, M. Perez, R. Solera, Effect of hydraulic retention time on hydrogen production from sewage sludge and wine vinasse in a thermophilic acidogenic CSTR: A promising approach for hydrogen production within the biorefinery concept, Int. J. Hydrogen Energy. 46 (2021) 7810–7820, https://doi.org/10.1016/j.ijhydene.2020.11.258.
[40] S. Methods, Standard Methods for the Examination of Water and Wastewater, Water Res. 16 (2012) 1495–1496, https://doi.org/10.1016/0043-1354(82)90249-4.
[41] S. Zahedi, D. Sales, L.I. Romero, R. Solera, Optimisation of the two-phase dry-thermophilic anaerobic digestion process of sulphate-containing municipal solid waste: Population dynamics, Bioresour. Technol. 148 (2013) 443–452, https://doi.org/10.1016/j.biortech.2013.09.002.
[42] H.D. Ariesyady, T. Ito, S. Okabe, Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester, Water Res. 41 (2007) 1554–1568, https://doi.org/10.1016/j.watres.2006.12.036.
[43] F. Kara Murdoch, R.W. Murdoch, G.C. Gürakan, F.D. Sanin, Change of microbial community composition in anaerobic digesters during the degradation of nonylphenol diethoxylate, Int. Biodeterior. Biodegrad. 135 (2018) 1–8, https://doi.org/10.1016/j.ibiod.2018.09.002.
[44] B. Montero, J.L. Garcia-Morales, D. Sales, R. Solera, Evolution of microorganisms in thermophilic-dry anaerobic digestion, Bioresour. Technol. 99 (2008) 3233–3243, https://doi.org/10.1016/j.biortech.2007.05.063.
[45] S. Zahedi, R. Solera, J.L. García-Morales, H. Ennouri, D. Sales, Evaluation of the effect of glycerol supplementation on the anaerobic digestion of real municipal solid waste in batch mode, Fuel. 193 (2017) 15–21, https://doi.org/10.1016/j.fuel.2016.12.024.
[46] B. Montero, J.L. García-Morales, D. Sales, R. Solera, Analysis of methanogenic activity in a thermophilic-dry anaerobic reactor: Use of fluorescent in situ hybridization, Waste Manag. 29 (2009) 1144–1151, https://doi.org/10.1016/j.wasman.2008.08.010.
[47] S. Zahedi, D. Sales, L.I. Romero, R. Solera, Dark fermentation from real solid waste. Evolution of microbial community, Bioresour. Technol. 151 (2014) 221–226, https://doi.org/10.1016/j.biortech.2013.10.063.
[48] L. Raskin, J.M. Stromley, B.E. Rittmann, D.A. Stahl, Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens, Appl. Environ. Microbiol. 60 (1994) 1232–1240, https://doi.org/10.1128/aem.60.4.1232-1240.1994.
[49] C. Agabo-García R. Solera M. Pérez First approaches to valorizate fat, oil and grease (FOG) as anaerobic co-substrate with slaughterhouse wastewater: Biomethane potential, settling capacity and microbial dynamics Chemosphere. 259 (2020). 10.1016/j.chemosphere.2020.127474.
[50] L. Sillero, R. Solera, M. Perez, Improvement of the anaerobic digestion of sewage sludge by co-digestion with wine vinasse and poultry manure : Effect of different hydraulic retention times, Fuel. 321 (2022), 124104, https://doi.org/10.1016/j.fuel.2022.124104.
[51] W. Chuenchart, M. Logan, C. Leelayouthayotin, C. Visvanathan, Enhancement of food waste thermophilic anaerobic digestion through synergistic effect with chicken manure, Biomass and Bioenergy. 136 (2020), 105541, https://doi.org/10.1016/j.biombioe.2020.105541.
[52] S. Zahedi, R. Solera, M. Pérez, An eco-friendly way to valorize winery wastewater and sewage sludge: Anaerobic co-digestion, Biomass and Bioenergy. 142 (2020) 1–9, https://doi.org/10.1016/j.biombioe.2020.105779.
[53] S. Zahedi, M. Rivero, R. Solera, M. Perez, Mesophilic anaerobic co-digestion of sewage sludge with glycerine: Effect of solids retention time, Fuel. 215 (2018) 285–289, https://doi.org/10.1016/j.fuel.2017.11.007.
[54] M. Guo, Y. Jiang, J. Xie, Q. Cao, Q. Zhang, A. Mabruk, C. Chen, Bamboo charcoal addition enhanced the nitrogen removal of anammox granular sludge with COD: Performance, physicochemical characteristics and microbial community, J. Environ. Sci. (China) 115 (2022) 55–64, https://doi.org/10.1016/j.jes.2021.07.010.
[55] L.J. Wu, T. Kobayashi, Y.Y. Li, K.Q. Xu, Comparison of single-stage and temperature-phased two-stage anaerobic digestion of oily food waste, Energy Convers. Manag. 106 (2015) 1174–1182, https://doi.org/10.1016/j.enconman.2015.10.059.
[56] G. Srisowmeya, M. Chakravarthy, A. Bakshi, G. Nandhini Devi, Improving process stability, biogas production and energy recovery using two-stage mesophilic anaerobic codigestion of rice wastewater with cow dung slurry, Biomass and Bioenergy. 152 (2021), 106184, https://doi.org/10.1016/j.biombioe.2021.106184.
[57] P.T. Couto, F. Eng, W. Naessens, I. Nopens, M. Zaiat, R. Ribeiro, Modelling sugarcane vinasse processing in an acidogenic reactor to produce hydrogen with an ADM1-based model, Int. J. Hydrogen Energy. 45 (2020) 6217–6230, https://doi.org/10.1016/j.ijhydene.2019.12.206.
[58] M. Dooms, H. Benbelkacem, P. Buffière, High solid temperature phased anaerobic digestion from agricultural wastes: Putting several reactors in sequence, Biochem. Eng. J. 130 (2018) 21–28, https://doi.org/10.1016/j.bej.2017.11.011.
[59] F. Valentino, G. Munarin, M. Biasiolo, C. Cavinato, D. Bolzonella, P. Pavan, Enhancing volatile fatty acids (VFA) production from food waste in a two-phases pilot-scale anaerobic digestion process, J. Environ. Chem. Eng. 9 (2021), 106062, https://doi.org/10.1016/j.jece.2021.106062.
[60] M. Jiang, W. Qiao, Y. Wang, T. Zou, M. Lin, R. Dong, Balancing acidogenesis and methanogenesis metabolism in thermophilic anaerobic digestion of food waste under a high loading rate, Sci. Total Environ. 824 (2022), 153867, https://doi.org/10.1016/j.scitotenv.2022.153867.
[61] R. Montañés Alonso, R. Solera del Río, M. Pérez García, Thermophilic and mesophilic temperature phase anaerobic co-digestion (TPAcD) compared with single-stage co-digestion of sewage sludge and sugar beet pulp lixiviation, Biomass and Bioenergy. 93 (2016) 107–115, https://doi.org/10.1016/j.biombioe.2016.05.028.
[62] R. Montañés, M. Pérez, R. Solera, Mesophilic anaerobic co-digestion of sewage sludge and a lixiviation of sugar beet pulp: Optimisation of the semi-continuous process, Bioresour. Technol. 142 (2013) 655–662, https://doi.org/10.1016/j.biortech.2013.05.017.
[63] M. Perez, R. Rodriguez-Cano, L.I. Romero, D. Sales, Anaerobic thermophilic digestion of cutting oil wastewater: Effect of co-substrate, Biochem. Eng. J. 29 (2006) 250–257, https://doi.org/10.1016/j.bej.2006.01.011.
[64] J. Ariunbaatar, E. Scotto Di Perta, A. Panico, L. Frunzo, G. Esposito, P.N.L. Lens, F. Pirozzi, Effect of ammoniacal nitrogen on one-stage and two-stage anaerobic digestion of food waste, Waste Manag. 38 (2015) 388–398, https://doi.org/10.1016/j.wasman.2014.12.001.
[65] A. Gomes, D.O. Paranhos, O. Fernando, H. Adarme, G. Fernandes, S. De Queiroz, S. Francisco, D. Aquino, E. Engineering, G. Program, P. Ufop, O. Preto, M. Gerais, Bioresource Technology Methane production by co-digestion of poultry manure and lignocellulosic biomass : Kinetic and energy assessment, Bioresour. Technol. 300 (2020), 122588, https://doi.org/10.1016/j.biortech.2019.122588.
[66] J. Wang, W. Wan, Kinetic models for fermentative hydrogen production: A review, Int. J. Hydrogen Energy. 34 (2009) 3313–3323, https://doi.org/10.1016/j.ijhydene.2009.02.031.
[67] R.M. Potdukhe, N. Sahu, A. Kapley, R. Kumar, Bioresource Technology Reports Co-digestion of waste activated sludge and agricultural straw waste for enhanced biogas production, Bioresour. Technol. Reports. 15 (2021), 100769, https://doi.org/10.1016/j.biteb.2021.100769.

[68] W. Li, H. Khalid, Z. Zhu, R. Zhang, G. Liu, C. Chen, E. Thorin, Methane production through anaerobic digestion : Participation and digestion characteristics of cellulose, hemicellulose and lignin, Appl. Energy. 226 (2018) 1219–1228, https://doi.org/10.1016/j.apenergy.2018.05.055.

[69] X. Liu, M. Du, Q. Lu, D. He, K. Song, Q. Yang, A. Duan, D. Wang, How Does Chitosan A ff ect Methane Production in Anaerobic Digestion ?, (2021). https://doi.org/10.1021/acs.est.1c04693.

[70] X. Liu, Y. Wu, Q. Xu, M. Du, D. Wang, Q. Yang, Mechanistic insights into the effect of poly ferric sulfate on anaerobic digestion of waste activated sludge, Water Res. 189 (2021), 116645, https://doi.org/10.1016/j.watres.2020.116645.

[71] D. Akgul, M.A. Cella, C. Eskicioglu, Influences of low-energy input microwave and ultrasonic pretreatments on single-stage and temperature-phased anaerobic digestion (TPAD) of municipal wastewater sludge, Energy. 123 (2017) 271–282, https://doi.org/10.1016/j.energy.2017.01.152.

[72] W. Lv, W. Zhang, Z. Yu, Evaluation of system performances and microbial communities of two temperature-phased anaerobic digestion systems treating dairy manure, Bioresour. Technol. 143 (2013) 431–438, https://doi.org/10.1016/j.biortech.2013.06.013.

[73] B. Ruffino, G. Campo, A. Cerutti, G. Scibilia, E. Lorenzi, M. Zanetti, Comparative analysis between a conventional and a temperature-phased anaerobic digestion system: Monitoring of the process, resources transformation and energy balance, Energy Convers. Manag. 223 (2020), 113463, https://doi.org/10.1016/j.enconman.2020.113463.

[74] B. Tonanzi, A. Gallipoli, A. Gianico, D. Montecchio, P. Pagliaccia, S. Rossetti, C. M. Braguglia, Elucidating the key factors in semicontinuous anaerobic digestion of urban biowaste: The crucial role of sludge addition in process stability, microbial community enrichment and methane production, Renew. Energy. 179 (2021) 272–284, https://doi.org/10.1016/j.renene.2021.07.049.

[75] M. Tena, M. Perez, R. Solera, Effect of hydraulic retention time on the methanogenic step of a two-stage anaerobic digestion system from sewage sludge and wine vinasse: Microbial and kinetic evaluation, Fuel. 296 (2021), 120674, https://doi.org/10.1016/j.fuel.2021.120674.

[76] M.B. Kurade, S. Saha, E. Salama, S.M. Patil, S.P. Govindwar, Bioresource Technology Acetoclastic methanogenesis led by Methanosarcina in anaerobic co-digestion of fats, oil and grease for enhanced production of methane, Bioresour. Technol. 272 (2019) 351–359, https://doi.org/10.1016/j.biortech.2018.10.047.

[77] K. Feng, Q. Wang, H. Li, X. Du, Y. Zhang, Microbial mechanism of enhancing methane production from anaerobic digestion of food waste via phase separation and pH control, J. Environ. Manage. 288 (2021), 112460, https://doi.org/10.1016/j.jenvman.2021.112460.

[78] A. Khan, S. Akbar, V. Okonkwo, C. Smith, S. Khan, A. Ali, F. Adnan, U. Zeeshan, S. Ahmed, M. Badshah, Bioresource Technology Enrichment of the hydrogenotrophic methanogens for, in-situ biogas up-gradation by recirculation of gases and supply of hydrogen in methanogenic reactor, Bioresour. Technol. 345 (2022), 126219, https://doi.org/10.1016/j.biortech.2021.126219.