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Co-digestion of two-phase olive-mill waste and cattle manure: Influence of solids content on process performance

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ABSTRACT

The solids content is a key parameter in the development of anaerobic digestion as it can determine the proper operation and performance of the process. The influence of the total solids content on the mesophilic anaerobic co-digestion of two-phase olive-mill waste (2POMW) and cattle manure (CM) was investigated. Four different total solids (TS) concentrations, in a 75:25 mixture of 2POMW:CM, were studied in batch reactors of 2 L capacity: 10%TS (R10), 15%TS (R15), 20%TS (R20) and 28.6%TS (Reactor non-diluted). The methane yields and the organic matter removal efficiency for the reactor with 10 and 15% TS were significantly higher than in the reactors with a higher solids content (R20 and Rnd). The hydrolytic and acidogenic phases were not adversely affected by the total solid content since the concentration of volatile fatty acids (VFAs) increased as TS percentage increased. However, a clear effect on the methanogenic phase was observed, which led to the accumulation of VFAs in the reactors R15, R20 and Rnd. Experimental results have shown that the best conditions correspond to the reactor containing 10% TS. The volatile solids and VFA removal in reactor R10 were 57.5% and 93.7% respectively. Moreover, the methane yield and the specific methane production were 35.80 $LCH_4/kgVS_{added}$ and 82.51 $LCH_4/kgVS_{removed}$ respectively.

1. Introduction

Anaerobic co-digestion can be defined as the simultaneous biological treatment of two, or more, organic biodegradable wastes to obtain a renewable energy source (biogas) and a digestate susceptible to being used as agricultural fertilizer. This type of treatment is a feasible alternative for the management of food-industry waste with a high content of organic matter [1,2]. The combination of substrates with different characteristics usually enhances the biogas yield from anaerobic digestion due to an improved nutrient balance in the mixture (C/N ratio) and dilution of the concentration of toxic compounds [3].

In anaerobic digestion, complex metabolic relationships occur among different populations of microorganisms whose growth rates vary depending on environmental conditions [4,5]. Certain inhibitory substances - such as ammonium, sulfates, heavy metals or organic compounds - can slow the metabolic activity of any of these microbial populations. Moreover, there is also a wide variety of specific inhibitors of each waste [6], which are capable of inhibiting the activity of anaerobic microorganisms [7,8]. For example, phenolic compounds are characteristic of the by-products of olive oil extraction. It has been demonstrated the inhibition of acetoclastic methanogenesis by phenolic compounds in olive-mill wastewater [9]. High concentrations of volatile fatty acids (VFAs) can also produce the inhibition of anaerobic digestion. In this sense, it has been reported in the literature a decrease in the population of methanogenic microorganisms by propionic acid concentrations above 900 mg/L [10,11]. Furthermore, there are environmental factors (temperature, pH, etc.) and operational variables (organic loading rate, mixing, etc.) that can alter the activity of methanogenic microorganisms and thus potentiate the inhibitory effect of the VFAs.

Two-phase olive-mill waste (2POMW) is a by-product of the olive oil extraction process in which a horizontal centrifuge is used to separate the oil fraction from this waste. The modern continuous olive oil production systems can be classified into “*continuous three-phase systems*” and “*continuous two-phase systems*”. In the three-phase process, water is added to the oily paste obtained from grinding and the separation by

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centrifugation generates three elements: oil, "*orujo*" (solid residue) and "*alpechín*" (liquid residue of vegetation water and added water). On the other hand, in the two-phase systems, no water is added to the mixture, obtaining only by centrifugation the 2POMW (solid residue and vegetation water). The two-phase system saves water, making it cheaper and more environmentally friendly, making it the most widely used system in the olive oil industry. As it is previously described, the 2POMW is a semi-solid, slightly acidic waste with high solid content (28.6–50.4%) and very high organic matter content (60.3–98.5%) [12]. This waste contains compounds such as lignin, hemicellulose, cellulose, fats, water-soluble carbohydrates and proteins. It also has a high C/N ratio (28.2–72.9) [13] and a fraction of soluble phenolic compounds whose composition depends on the fruit (type, maturity degree, etc.), climatic conditions and processing technique [12]. The by-products of the olive oil extraction process have an antibacterial activity and phytotoxic character due to the presence of these phenolic compounds [14]. Approximately 800 kg of 2POMW per ton of processed olives are generated by using the two-phase system [13]. According to the International Olive Council [15], the Spanish olive oil production in 2020/2021 was 1.3 million tons which corresponds to 45% of global production and, hence, around five million tons of 2POMW were generated [16].

On the other hand, the Spanish Ministry of Agriculture, Fisheries and Food [17] has reported that bovine population reached around 6.7 million livestock units in 2021; this implies a cattle manure (CM) production of about 73.5 million tons. The CM contains the feces and urine from the animals, used bedding, sand and sediments. Some of these materials are slowly biodegradable or recalcitrant substances and therefore the biodegradability of the manure and the biogas production are low [18,19]. The composition of manure depends on factors as the type of livestock farm, feed and environmental conditions [20]. The CM is also characterized by a high organic nitrogen content, a low C/N ratio (15.5) and high pH values [21]. A high proportion of the volatile solids (VS) corresponds to cellulose, hemicelluloses and lignin. The cellulose and hemicelluloses are readily converted to methane gas by anaerobic microorganisms but lignin will not be degraded during anaerobic digestion, since it is a hardly biodegradable compound [22].

The concentration of total solids (TS) is an important variable in the anaerobic digestion process. Based on the TS content of the waste, the process can be distinguished between dry anaerobic digestion that occurs at TS concentrations higher than 15% and wet anaerobic digestion with TS concentrations between 0.5% and 15% [23]. Some of the advantages of dry anaerobic digestion versus wet digestion are: reduced reactor volume, lower energy requirements and lower water consumption [24]. However, high TS content may result in organic overloading of the reactors, causing lower biogas yield. Chen et al. (2014) [25] observed inhibition of methanogenesis in co-digestion of food waste with green waste when TS content was increased above 25%. Fernández et al. (2008) [26] studied the influence of initial TS content on the biodegradation of the organic fraction of municipal solid waste (OFMSW), and a low methane yield was obtained due to increasing the solid proportion from 20% to 30%. Motte et al. (2013) [27] studied the effects of solids content, inoculation ratio and particle size on methane production and found that the main parameter governing the methane production was TS content. Moreover, high solids content influences the rheological properties of the medium, affecting the mixing and homogeneity of reactor content and limiting mass transfer, which diminish the methane production [28].

Based on the above, the main aim of the present work was to study the effect of TS concentration on anaerobic co-digestion of a mixture of 2POMW and CM (75:25 ratio). TS contents between 10% and 28.6% were used to evaluate the process performance of wet and dry anaerobic digestion. The methane yield and the organic matter removal efficiency were used as measures of process efficiency to determine the optimum TS content.

2. Materials and methods

2.1. Batch digesters

The experiments were carried out in four stainless steel digesters of 3 L total volume and 2 L working volume [16]. The top of the reactor has several openings with different functions: a sampling port, a biogas outlet, an inlet for a temperature sensor probe and the input of a mechanical stirring rod (20 r.p.m.). Each reactor was hermetically sealed to maintain anaerobic conditions during the digestion process and was fitted with a heating jacket to maintain the mesophilic temperature (35 °C). The biogas produced during fermentation was collected in a 5 L Tedlar® bag.

2.2. Physicochemical characteristics of substrates and inoculum

Table 1 shows the composition and characteristics of the 2POMW, CM and inoculum. The parameters were determined in triplicate analysis.

Two-phase olive-mill waste was collected from an olive oil mill (Cooperativa Nuestra Señora de los Remedios) located in Olvera, Cádiz (Spain). The CM was obtained from a semi-intensive livestock farm of dairy cattle in El Puerto de Santa Maria (Cádiz, Spain). Together with the animal feces, the collected CM also contained residues of straw use as bedding material and traces of soil. Both substrates were homogenized at the laboratory and stored at −4 °C to preserve their original characteristics.

Mesophilic anaerobic effluent from a laboratory digester, adapted to co-digestion of both wastes, was used as inoculum. This reactor was operated with a hydraulic retention time (HRT) of 15 days and organic loading rate (OLR) of 5.16 gVS/L_Rd. The daily biogas production was 0.36 L/L_Rd with a methane content of 76% and volatile solids (VS) removal yield of 37.8%.

Table 1
Characteristics of the substrates used (2POMW and CM) and the inoculum.

Parameter	Units	2POMW	CM	Inoculum
pH [a]	–	5.41 (±0.04)	7.84 (±0.02)	8.07 (±0.02)
EC	μS/cm	1549.67 (±10.21)	1100.67 (±19.40)	–
Moisture	%	70.13 (±1.12)	83.97 (±0.17)	94.79 (±0.05)
Total solids (TS)	g/kg	298.65 (±11.21)	160.29 (±1.72)	52.14 (±0.48)
Volatile solids (VS)	g/kg [b]	267.70 (±10.24)	132.14 (±1.69)	33.39 (±0.38)
sCOD	g O_2/kg [b]	98.63 (±0.01)	43.14 (±0.41)	10.13 (±0.15)
DOC	g C/L	40.37 (±0.05)	14.63 (±0.09)	3.95 (±0.11)
TVFA	mgHAc/L	1248.92 (±2.05)	1893.18 (±1.32)	133.22 (±1.15)
Total phenols	(g/L)	1.62 (±0.005)	–	–
Organic matter	%	90.31 (±0.20)	82.44 (±0.17)	64.05 (±0.14)
C	%	52.38	47.81	37.15
N	%	1.27	2.88	4.65
C/N	–	41.23	16.61	7.99
Total Alkalinity	g$CaCO_3$/L	4.05 (±0.27)	21.90 (±0.04)	–
TKN	g/kg [b]	3.79 (±0.10)	4.62 (±0.00)	2.42 (±0.51)
Ammonia	gNH_3-N/kg [b]	0.12 (±0.004)	1.38 (±0.02)	–

Each value represents the mean of 3 replicates (±standard deviation).
EC: Electric conductivity; sCOD: Soluble chemical oxygen demand; DOC: Dissolved organic carbon; TKN: Total Kjeldahl Nitrogen; TVFA: Total Volatile Fatty Acids.

[a] water extract 1:10.

[b] Expressed in fresh weight.

2.3. Experimental procedure

Previous studies investigating the influence of mixture ratio of 2POMW and CM on anaerobic co-digestion indicated a high biogas production for the 75:25 ratio (2POMW:CM) [16]. On the basis of these results, four mixtures of the co-substrates at the 75:25 ratio with TS percentages of 10, 15, 20 and 28.6% respectively were analyzed in order to study the performance of wet and dry anaerobic co-digestion. The TS content of each mixture was adjusted to the desired level with deionized water. The reactors were named as R10, R15, R20 and Rnd (Reactor non-diluted). The reactors were filled with the co-substrate mixture up to 80% of effective volume (1600 mL) and were completed with 400 mL of the inoculum described above. The reactors were flushed with nitrogen gas to obtain anaerobic conditions before the start of the experiment. The initial characteristics of the mixtures are shown in Table 2. The pH was adjusted between 7.5 and 8.0 by using a solution of Na_2CO_3 (2.8 M).

During anaerobic digestion period (115 days), biogas production and composition were daily analyzed.

2.4. Analytical methods

The following parameters were measured by triplicate for the waste characterization and the assay monitoring: total solids (TS), total volatile solids (VS), pH, total Kjeldahl nitrogen (TKN), Soluble chemical oxygen demand (sCOD), dissolved organic carbon (DOC), alkalinity, volatile fatty acids (VFAs) and ammonia (NH_3-N). All parameters were determined following Standard Methods [29].

The TS, VS, pH and TKN were directly determined from the samples. The others parameters were measured over samples previously lixiviated (10 g of sample in 100 mL of distilled water during 30 min). Subsequently, the samples were filtered through a 0.47 μm glass-fiber filter for the determination of sCOD and DOC.

Samples for total phenols measurements were centrifuged, filtered and analyzed by liquid chromatography using 0.2 mM syringic acid as internal standard, according to the methodology described in the literature [30].

The DOC was determined by combustion/non-dispersive infrared gas analysis method using a total organic carbon analyzer (Shimadzu® TOC-5000).

For VFAs determination, the following procedure was used: samples from leaching were filtered through a 0.22 μm Teflon filter, acidified with a solution 1:2 (v/v) of phosphoric acid, spiked with phenol as internal standard and, finally, analyzed in a gas chromatograph (Shimadzu® GC-2010) equipped with a flame ionization detector and a capillary column filled with Nukol (polyethylene glycol modified by nitroterephthalic acid). The temperatures of the injection port and detector were 200 and 250 °C, respectively. Nitrogen was the carrier gas at 42.1 mL/min. In addition, hydrogen and synthetic air were used as gas chromatograph flame ionization. Total acidity (TVFA) was calculated by the addition of individual VFA levels, taking into account the molecular weights of the different VFAs in order to expressing this parameter as acetic acid concentration.

The biogas produced was collected in a Tedlar® bag and the volume was quantified using a high-precision gas meter (Ritter® Drum-type Gas Meters, 0.1 mbar). Gas volumes were expressed at standard temperature and pressure conditions. The main components of biogas (methane, carbon dioxide and hydrogen) were determined by gas chromatography (Shimadzu® GC-14 B) with a stainless steel column packed with Carbosive SII and a thermal conductivity detector (TCD). The injected sample volume was 1 mL and the operational conditions were as follows: 7 min at 55 °C; ramped at 27 °C/min until 150 °C; detector temperature: 255 °C; injector temperature: 100 °C. The carrier was helium and the flow rate used was 30 mL/min.

Table 2
Initial composition of co-digestion assays (R10, R15, R20 and Rnd). Mixtures are based on fresh weight.

Reactor	TS_0 (g/kg)	VS_0 (g/kg)	TVFA[a]	Initial C:N ratio
R10	84.22 (±3.71)	69.13 (±3.28)	382.6	27:1
R15	130.22 (±0.67)	112.37 (±1.16)	498.5	28:3
R20	165.65 (±1.04)	144.83 (±1.15)	858.1	29.0
Rnd	226.13 (±1.10)	199.47 (±1.61)	1094.1	29.6

[a] As acetic acid (mg/L).

2.4.1. Determination of non-solubilized carbon (NSC), dissolved acid carbon (DAC) and acidogenic substrate as carbon (ASC)

To evaluate the performance of the anaerobic digestion phases and quantify levels of hardly biodegradable compounds in the reactors, trends of non-solubilized carbon (NSC), dissolved acid carbon (DAC) and acidogenic substrate as carbon (ASC) were determined. The NSC is the fraction of the organic carbon that has not been solubilized in the hydrolytic stage. DAC represents the fraction of soluble organic carbon in acid form, i.e. the fraction corresponding to VFAs. The ASC is the fraction of solubilized organic matter that has not been transformed into VFAs.

The NSC and ASC were determined according to equations (1) and (3) proposed by Fdez-Güelfo et al. (2012) [31]. The DAC was calculated according to equation (4), where A_iH, represents the concentration of each individual VFA measured by gas chromatography; n_i, is the number of carbon atoms of each A_iH; MW_i, is the molecular weight of each A_iH. The total organic carbon (TOC) was calculated from equation (2) as suggested by Navarro et al. (1993) [32].

$$NSC = TOC - DOC \tag{1}$$

$$TOC = VS\hat{A}\cdot 0.51 \tag{2}$$

$$ASC = DOC - DAC \tag{3}$$

$$DAC = \sum_{i=2}^{i=7}[A_iHn_i12/MW_i] \tag{4}$$

3. Results and discussion

The increased solids content implies an increase of the organic matter available to the microorganisms. For this reason, an increased biogas production can be expected in reactors with higher TS concentrations. However, hardly biodegradable organic matter and potentially inhibitory compounds can also increase in these reactors. Fdez-Güelfo et al. (2012) [31] established several indirect parameters (NSC, DAC and ASC) to evaluate the performance of the anaerobic digestion processes. Those parameters has been also used to evaluate the inhibition degree of the hydrolytic phase in the production of bio-hydrogen by dark fermentation of organic solid waste [33] and the acidogenic anaerobic co-digestion of OFMSW and mixed sludge [34]. In those papers, these parameters were also used to provide additional information about the evolution of the hardly biodegradable organic matter.

3.1. Evolution of organic matter concentration

To determine the evolution of the organic matter concentration in the reactors, several analytical parameters were measured: volatile solids (VS), dissolved organic carbon (DOC), soluble chemical oxygen demand (sCOD) and volatile fatty acids (VFAs). It should be noted that only VS is related to the total organic matter in the reactor while DOC, sCOD and VFAs are related to the solubilized fraction of total organic matter.

As it was previously commented, several indirect parameters (NSC, DAC and ASC) have also been proposed to analyze the performance of the different stages in the anaerobic digestion process. These new parameters were used in order to analyze the evolution of the organic matter concentration in particulate and soluble fractions.

The temporary evolution of VS removal is shown in Fig. 1. The initial organic matter content, expressed in term of VS, was proportional to the TS percentage. As it can be seen, all reactors presented a high reduction of initial VS content during the first 3 days with values of about 23–27%. These percentages represent a VS removal of 41–59% with respect to the end of the test. Thus, in the first days of the assay, a significant organic matter removal was observed.

3.1.1. Particulate organic matter

The temporary evolution of particulate organic matter, expressed in terms of NSC, is shown in Fig. 2. The initial concentrations were 25.6, 43.5, 55.5 and 78.3 g/kg for reactors R10, R15, R20 and Rnd, respectively. In the first 3 days of the test, the levels of NSC removal were around 30–37%. In this same period of time, an increase in VFAs concentration (Fig. 3) was observed. Moreover, the main components of biogas in this period were carbon dioxide and hydrogen (Fig. 5). These facts indicate an adequate development of the hydrolytic and acidogenic phases regardless of the TS content in the reactors. From day 9, a gradual decrease in NSC in reactors with lower TS content (R10 and R15) was observed. NSC removal in R15 was higher than in R10 due to the greater availability of potentially hydrolyzable organic matter. A latency period was observed in the evolution of NSC for R20 and Rnd from day 9 to days 16 and 43, respectively. During this period, VS removal did not occur (Fig. 1) due to the accumulation of VFAs that inhibited hydrolysis and acidogenesis of organic matter. After the latency period, NSC levels progressively decreased to reach stable values at the end of the study. This behavior was similar for R10 and R15, which did not exhibit a latency period.

At the end of the experimental period (day 115), the VS removal efficiency for R10, R15, R20 and Rnd was 57.5, 56.8, 49.6 and 43.2%, respectively. These values correspond to final VS concentrations in each reactor of 29.4, 48.6, 73.0 and 113.4 g/kg, respectively. In addition, these values are related to the accumulation of hardly biodegradable compounds in the reactors that can be quantified by the NSC. Thus, the final NSC concentrations (Table 3), expressed as g/kg, show a linear correlation against the TS percentage in the reactors ($\text{NSC} = 1.5 \cdot \text{TS} - 6.925$; $R^2 = 0.995$).

Furthermore, the NSC concentration at the end of the test represented approximately 60% of TOC in the reactors (NSC/TOC ratio), corresponding mainly to insoluble lignocellulosic compounds, which are characteristic of both substrates [35,13].

3.1.2. Dissolved organic matter

The VFAs were produced by the acidogenic microorganisms using the organic matter previously solubilized in the hydrolytic stage. The evolution of the VFAs (acetic acid, propionic acid and n-butyric acid) in the reactors is shown in Fig. 3. As it can be seen, initially (0–15 days) the hydrolytic and acidogenic stages occurred with a high generation of VFAs. During this period, the increase in VFAs levels was proportional to the TS content in the reactors, indicating that the acidogenic phase was not negatively influenced by the increase in the soluble organic matter.

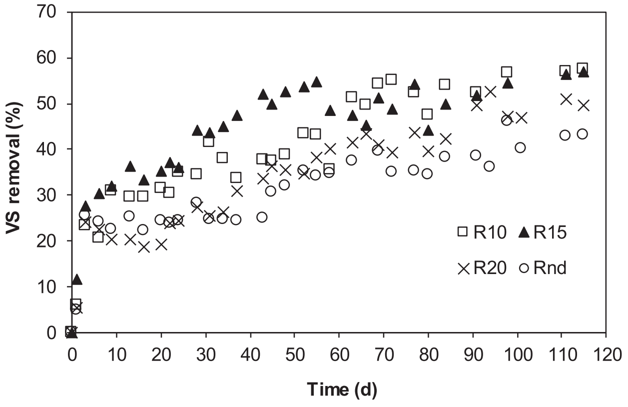

Fig. 1. Temporary evolution of volatile solids (VS) removal.

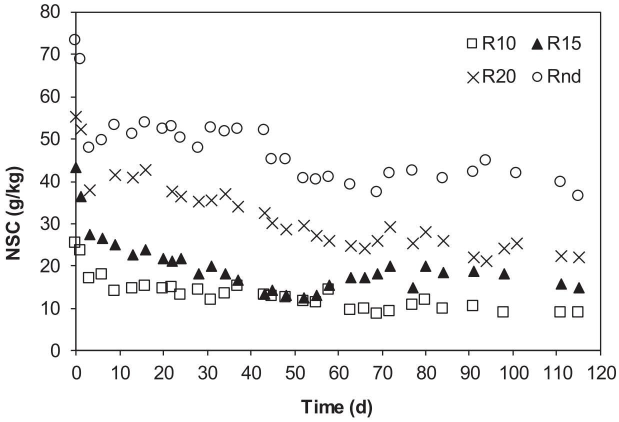

Fig. 2. Temporary evolution of non-soluble carbon (NSC) in the effluents of reactors.

As it can be seen in Fig. 3, during the first 55 days the maximum acetic acid concentrations were reached in each reactor (7.20, 9.76, 15.67 and 16.61 g/L for R10, R15, R20 and Rnd respectively).

However, as the TS percentage increases, the acetogenesis of n-butyric acid slows down. As it can be seen, as the TS percentage increases, the decrease in the n-butyric concentration is delayed and begins approximately on days 20, 30, 35 and 70. These decreases in the n-butyric concentrations coincide with an increase in the acetic acid concentration and biogas production.

As for propionic acid, it can be observed its accumulation from day 10 in all reactors, reaching higher final concentration values as the TS content increases (0.08, 1.39, 1.66 and 2.58 g/L for R10, R15, R20 and Rnd respectively).

These facts show that acetogenesis from long-chain fatty acids ($n > 5$) is not affected by an increase in TS content. However, the increase in TS content partially inhibits the acetogenesis from n-butyric by making it slower and inhibits the acetogenesis of propionic resulting in its final accumulation.

This acid accumulation can be due to the low growth rate of the microorganisms responsible for their degradation (HPr: $\mu_{max} = 0.008$ h^{-1}; HBu: $\mu_{max} = 0.013$ h^{-1}) and the thermodynamics of chemical reactions in a mesophilic regime (HPr: $\Delta G^{\circ} = +76.1$ kJ; HBu: $\Delta G^{\circ} = +48.1$) [36,37]. The partial inhibition of butyric acid degradation can be caused by the presence of hydrogen [38]. The maximum concentrations of n-butyric reached were 1.08, 1.81, 3.06 and 5.86 g/L for R10, R15, R20 and Rnd, respectively (Fig. 3).

The maximum levels of propionic acid were 1.04, 1.57, 2.17 and 2.83 g/L for R10, R15, R20 and Rnd, respectively (Fig. 3) and these concentrations can be related to the biodegradation of phenol compounds, such as polyphenols, characteristic of 2POMW [12]. The phenol metabolism and the generation of propionic acid as final product were studied by Pullammanappallil et al. (2001) [39]. The estimated initial concentrations of polyphenols in the reactors were 0.27, 0.41, 0.54 and 0.77 gTSP/L (total soluble phenols) for R10, R15, R20 and Rnd, respectively. A comparative study between the maximum propionic acid concentration and the initial concentration of total polyphenols (both concentration expressed as g/L) in the reactors showed a linear correlation between both variables ($[\text{Propionic acid}]_{\text{MAX}} = 3.572 \cdot [\text{Total polyphenols}]_0 + 0.120$; $R^2 = 0.989$), which was not observed for other VFAs. These results confirmed the metabolic relationship between propionic acid and the phenolic compounds of 2POMW. Moreover, the elimination of propionic acid did not occur during the test period, except

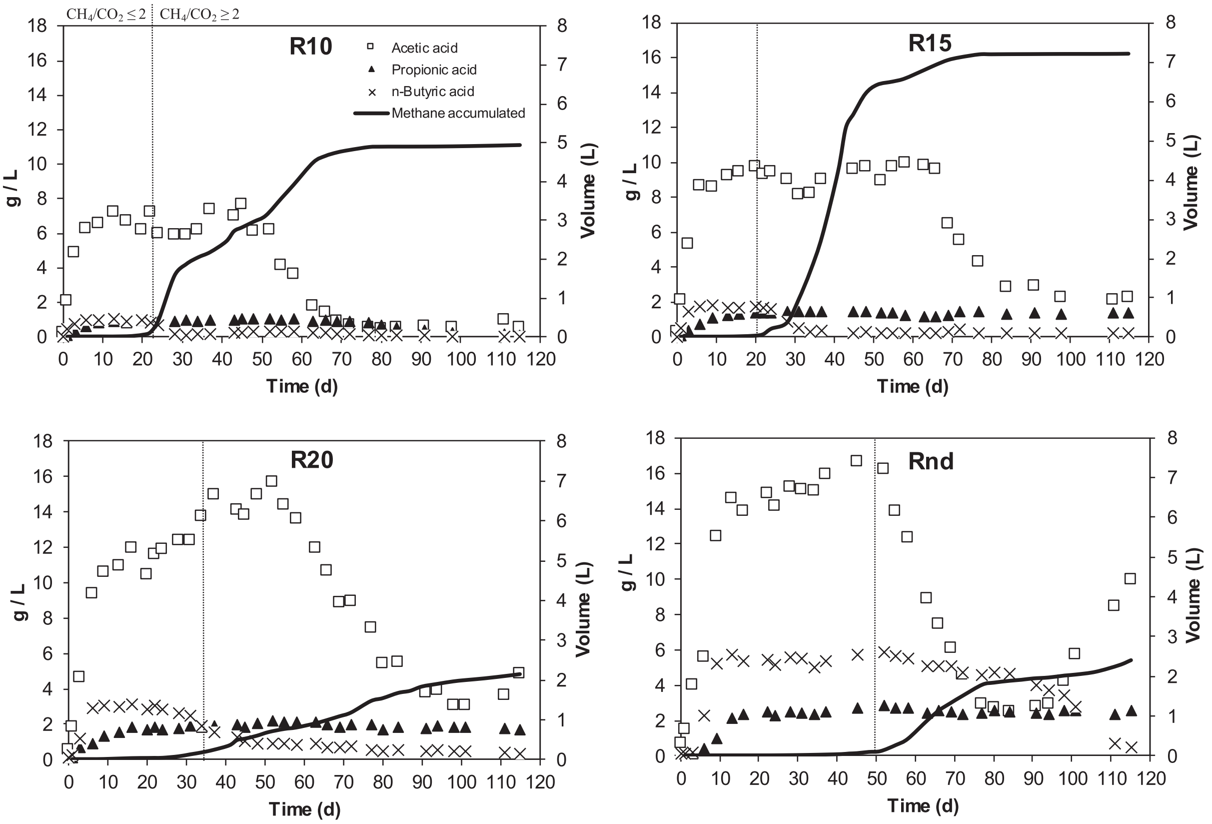

Fig. 3. Comparative evolution of individual VFAs (acetic, propionic and n-butyric acids) and accumulated methane production.

Table 3
Values of the indirect parameters (TOC, NSC, ASC and DAC) at the end of the assay. Units in g/kg fresh weight.

Reactor	TOC	NSC	NSC/TOC	ASC	DAC
R10	14.9	8.9	0.60	5.8	0.3
R15	24.8	15.0	0.60	7.8	1.9
R20	37.2	22.2	0.60	11.9	3.2
Rnd	57.8	36.6	0.63	15.4	5.8

for R10, due to the lower metabolic cost of acetic acid and butyric acid that were present until the end of the experimental period.

At the end of the experimental period, concentrations around 0.23 g/L of long-chain fatty acids (n-valeric acid and caproic acid) in the R15, R20 and Rnd reactors were observed. This accumulation can be due to inhibition by the presence of high levels of acetic, propionic and butyric acids in the reactors. The removal efficiency of TVFA, based on the maximum levels reached, was 93.7%, 65.6%, 59.2% and 48.7% for reactors R10, R15, R20 and Rnd, respectively. An inverse linear trend was observed between removal efficiency of VFAs and TS content of the initial mixture.

Additionally, analysis of parameters such as ASC and DAC provided information about the evolution of the soluble organic matter [31]. The evolutions of ASC and DAC are shown in Fig. 4. As it can be seen, during the first 6–9 days, ASC decreased while DAC increased, indicating that the solubilized organic matter was transformed into VFAs.

Subsequently, ASC levels remained relatively constant, despite the fluctuations observed for reactors with higher TS contents (R20 and Rnd), as a result of the heterogeneity of the samples. Moreover, DAC concentrations remained constant with average levels during this period of 3.5, 4.9, 7.5 and 10.2 g/kg for R10, R15, R20 and Rnd, respectively. Thereafter, the decrease of DAC can be related to acetic acid degradation in the reactors. However, ASC levels remained constant, indicating the presence of hardly biodegradable compounds in the substrate. Thus, the ASC at the end of the test was a measurement of the non-biodegradable soluble organic fraction. The final ASC concentrations in each reactor are shown In Table 3. It can be noted that there is a linear correlation between final ASC concentrations and the initial TS content, both expressed as g/kg ($[ASC]_{FINAL} = 0.071 \cdot [TS] - 0.472$ $R^2 = 0.975$).

3.2. Evolution of biogas

The methanogenic microorganisms produce methane from two major metabolic pathways: by reduction of carbon dioxide with hydrogen (H_2-utilizing methanogenic archaea) and by fermentation of acetic acid to produce methane and carbon dioxide (acetoclastic archaea) [22].

Fig. 3 shows the accumulated methane production together with the VFAs evolution (acetic acid, propionic acid and n-butyric acid). Fig. 5 shows the biogas composition obtained in the different reactors. As it can be seen, the TS concentration affects the biogas production. A lag phase of 6–9 days for biogas production was observed for R10, R15 and R20, while for Rnd the delay in biogas production lasted for 20 days. This lag phase could be caused by a decrease in pH, due to the generation of VFAs, as well as by the presence of polyphenols at the beginning of the assay.

In Figs. 3 and 5, a value of 2 for the ratio $CH_4:CO_2$ has been marked with vertical dotted dashed lines to differentiate between the two metabolic pathways for the methane production.

After the initial lag period, the methane production comes mainly from hydrogenotrophic activity ($CH_4:CO_2 < 2$) as confirmed by the low levels of hydrogen and the absence of VFAs degradation. Montero, et al. (2008) [40] observed a higher proportion of hydrogenotrophic methanogens in reactors with high TS content when methane percentage did

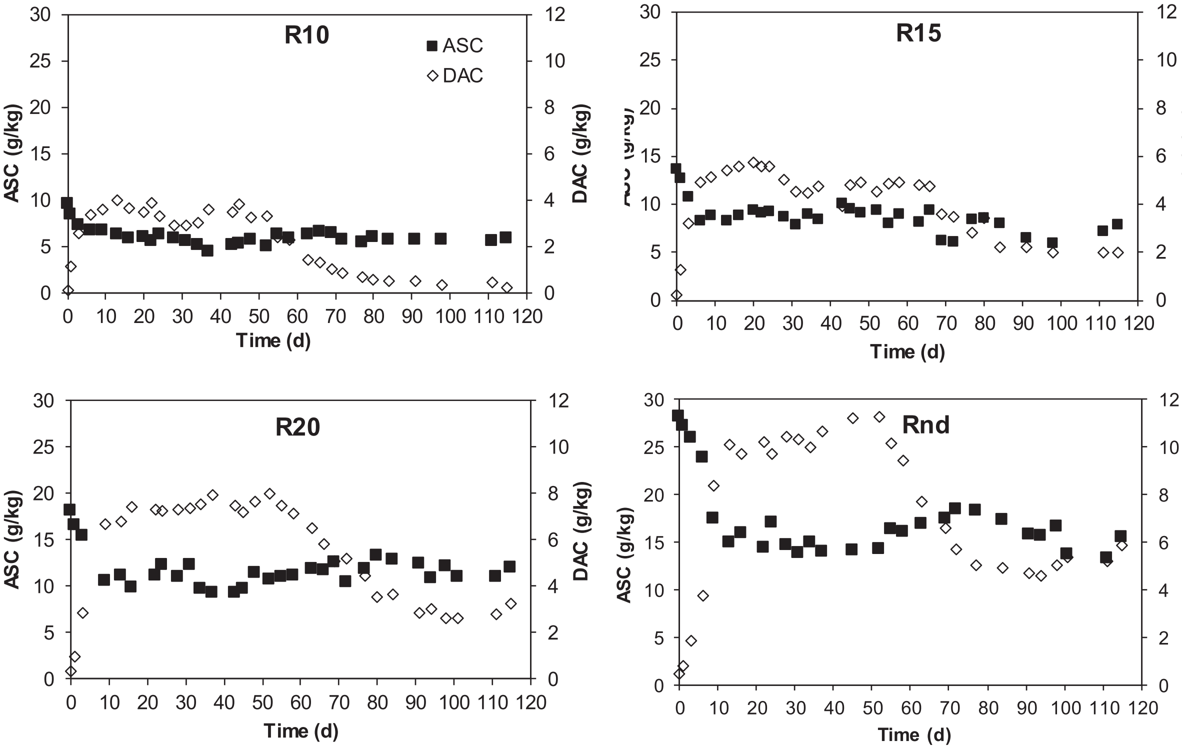

Fig. 4. Temporary evolution of acidogenic substrate as carbon (ASC) and dissolved acid carbon (DAC) in the reactors effluents.

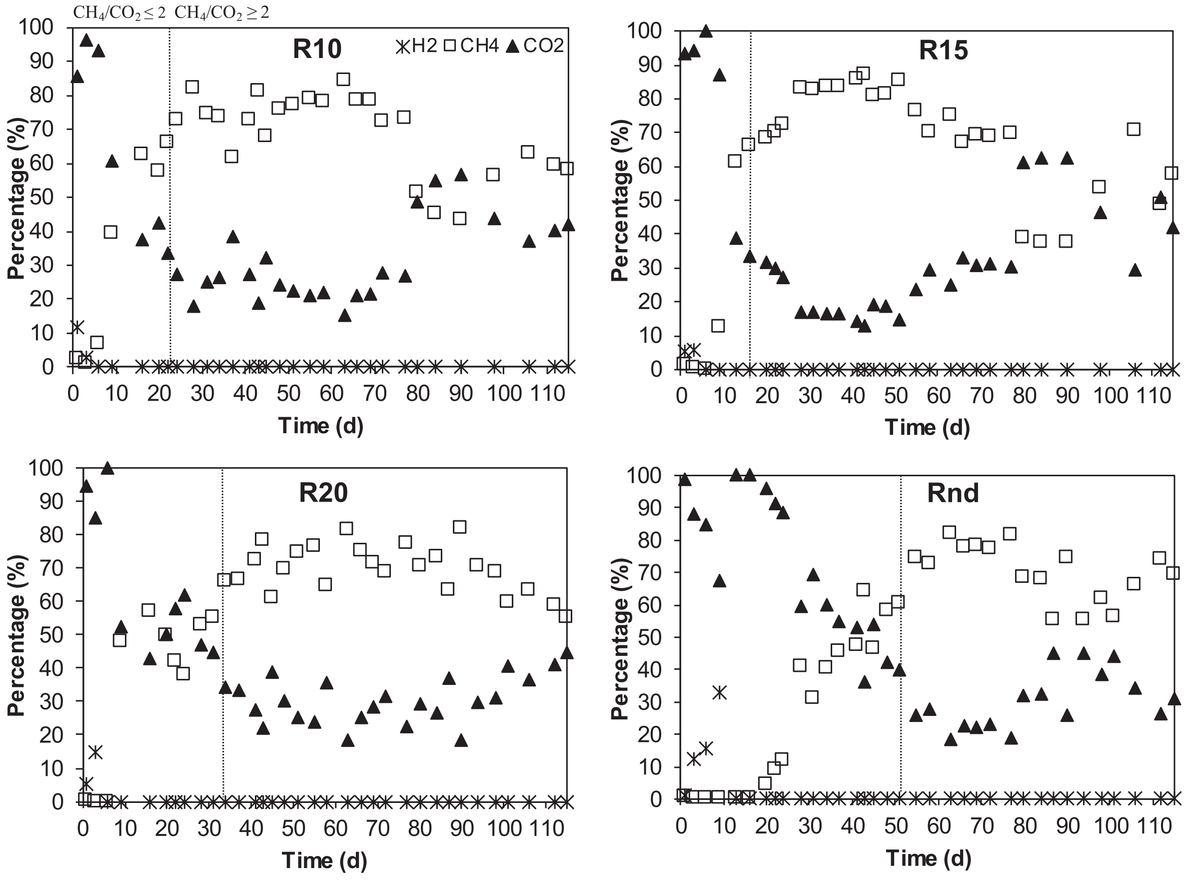

Fig. 5. Temporary evolution of biogas composition.

not exceed 35–40%. Furthermore, the increase in the TS content in the reactors resulted in a longer duration for the hydrogenotrophic methanogenic phase. Thereafter, methane production related to the activity of both methanogenic microbiota (acetoclastic and hydrogenotrophic) (CH_4:$CO_2 \geq 2$) was observed from 22, 16, 34 and 55 days for R10, R15, R20 and Rnd, respectively, with a methane percentage of 65–70%. This stage was coupled with butyric acid degradation and subsequent consumption of acetic acid accumulated in the reactors.

The methane production and the specific methane production at the end of the experimental period are shown in Table 4. Comparing the reactors with lower TS content (R10 and R15), it can be observed that a higher methane production was obtained in R15 (7.21 L) in accordance with its higher content of potentially biodegradable organic compounds. However, the specific methane production (based on both VS added and removed) were higher in R10, with values of 35.80 $LCH_4/kgVS_{added}$ and 82.51 $LCH_4/kgVS_{removed}$. The main reason was the degradation of the whole of the VFAs generated, in comparison with the rest of reactors for which unmetabolized VFAs were determined at the end of the study.

In the reactors with higher TS content (R20 and Rnd), the accumulated volume of methane was lower than in R10 and R15 despite the higher concentration of available organic matter. This can be due to the inhibition of acetoclastic methanogenesis, mainly by organic overloading. In dry anaerobic digestion of OFMSW (with 20% and 30% TS content), Fernandez et al. (2008) [26] obtained lower methane production and organic matter removal in the reactor with higher TS content. Moreover, another possible problem related to the low methane yields in the reactors with higher TS content could be the rheological conditions of these mixtures. Abbassi-Guendouz et al. (2012) [28] observed a physical limitation related to the liquid/gas mass transfer for total solids content over 30% in the anaerobic digestion of cardboard.

4. Conclusions

The results of this study showed the critical influence of the TS concentration on the process performance of the batch mesophilic anaerobic co-digestion of 2POMW:CM (75:25 v/v). According to the previous results, the following main conclusions may be stablished:

Methane yields and the organic matter removal efficiency were significantly lower in the reactors with a higher TS content (R20 and Rnd).

An initial lag phase in methane production was observed at the beginning of the assays which was directly related to the TS content in the reactors. Data indicated that the mixture with a TS content of 10% (R10) presents a shorter lag phase, a higher methane yield and a higher VFAs removal efficiency.

On the basis of VS added, for R10 the methane yield ($LCH_4/kgVS_{added}$) was increased by 13% and 81% compared to R15 and the average obtained for reactors with higher TS content (R20 and Rnd). Concretely, 35.80 $LCH_4/kgVS_{added}$ was obtained by this reactor.

On the other hand, based on VS removed, for R10 the specific methane yield ($LCH_4/kgVS_{removed}$) was increased by 8% and 63% with respect to R15 and the average obtained for R20 and Rnd. A production of 82.51 $LCH_4/kgVS_{removed}$ was reached by this digester.

In general, the hydrolytic and acidogenic phases were not adversely affected by the TS content since the VFAs concentration increased as TS content increased. However, a clear effect on the methanogenic phase was observed, which led to the accumulation of VFAs in the reactors R15, R20 and Rnd. Experimental results have shown that the best conditions correspond to the R10. In this reactor, the VS and VFA removal were 57.5% and 93.7% respectively.

CRediT authorship contribution statement

J.A. Rubio: Investigation, Writing – original draft, Visualization. **L.A. Fdez-Güelfo:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization. **L.I. Romero-García:** Conceptualization, Methodology. **A.C. Wilkie:** Conceptualization, Methodology. **J.L. García-Morales:** Conceptualization, Methodology, Project administration, Funding acquisition.

Table 4
Methane production and specific methane production in co-digestion assays.

	Units	R10	R15	R20	Rnd
Total methane production	LCH_4	4.95 (±0,02)	7.21 (±0,04)	2.16 (±0,01)	2.40 (±0,01)
Specific methane production	$LCH_4/kgVS_{added}$	35.80 (±0,18)	32.10 (±0,16)	7.45 (±0,04)	6.01 (±0,03)
	$LCH_4/kgVS_{removed}$	82.51 (±0,41)	74.74 (±0,37)	20.43 (±0,10)	19.04 (±0,09)

Each value of volatile solids (VS) represents the mean of 3 replicates, for the volumetric determination of biogas the accuracy of the precision gas-meter (0.5%) was considered for the calculation of final standard deviation (±).

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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