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Successful and stable operation of anaerobic thermophilic co-digestion of sun-dried sugar beet pulp and cow manure under short hydraulic retention time

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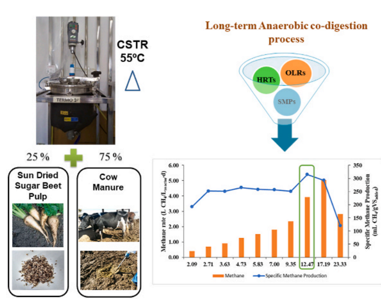
HIGHLIGHTS

- High methane productivities can be reached even at 4 and 5 days of HRT.
- OLR as high as 17.19 $gVS/L_{reactor}$·d has been successfully assimilated.
- The optimum SMP was reached at 5-day HRT (315 mL CH_4/g VS_{added}).
- $TVFA/VS_{added}$ could be useful for the process stability analysis.
- The acetoclastic methanogens wash out occurred at 3-day HRT.

GRAPHICAL ABSTRACT



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ABSTRACT

This work consists of a long-term (621 days) experimental study about biogas production from sun dried sugar beet pulp and cow manure. Thermophilic (55 °C) anaerobic co-digestion was performed in semi-continuous reactors, testing ten hydraulic retention times (30-3 days) (HRTs) and organic loading rates (2–24 $gVS/L_{reactor}$•d) (OLRs). Results showed that the best global system performance (regarding stability, biogas production, and organic matter removal) was achieved at an HRT as short as 5 days (OLR of 12.47 $gVS/L_{reactor}$•d) with a biogas yield of 315 mL/gVS_{added}. The gradual OLR increase allowed system control and time-appropriate intervention, avoiding irreversible process disturbances and maintaining admissible acidity/alkalinity ratios (<0.8) for HRTs ranging from 30 to 4 days. The accumulation of acetic acid was the main cause of the process disturbance observed at short HRTs. It was deduced that for the HRT of 3 days, the methane productivity was mainly owing to the hydrogen-utilizing methanogens pathway. This research clearly shows how an adequate combination of agro-industrial wastes and livestock manure could be processed by anaerobic co-digestion in short HRTs with great efficiency and stability and deepens in the understanding of the start-up, stability and optimization of the co-digestion.

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Abbreviations

AD	Anaerobic digestion
CM	Cow manure
DOC	Dissolved organic carbon (g C/kg)
SD-ESBP	Sun dried exhausted sugar beet pulp
HRT	Hydraulic retention time (d)
MPR	Methane production rate($L/L_{reactor}$·d)
OLR	Organic loading rate ($gVS/L_{reactor}$·d)
PM	Pig manure
sCOD	Soluble chemical oxygen demand (g/kg)
SMP	Specific methane production ($mLCH_4/gVS_{added}$)
tCOD	Total chemical oxygen demand (g/kg)
TKN	Total Kjeldahl nitrogen (gN/kg)
TS	Total solids (%)
TVFA	Total acidity (g HAc/L)
VFAs	Volatile fatty acids (g HAc/L)
VS	Volatile solids (%)

1. Introduction

Worldwide energy production is originated mainly from fossil sources (International Energy Agency-IEA, 2020). Being exhaustible and non-renewable, a shift towards bioenergy production from renewable sources such as residual biomass is of utmost importance and urgency. The agro-industrial sector is one of the fields that generates a large amount of waste and by-products, in most cases poorly valorized and dumped in landfills, contributing to environmental pollution and the emission of greenhouse gases (GHG) (Dar et al., 2021; Rahman et al., 2019).

Furthermore, the huge generation of livestock manure in the world carries several environmental pollution risks, whether through the emission of uncontrolled gases or through infiltration into groundwater (Basumatary et al., 2021; Glanpracha and Annachhatre, 2016; Zhang et al., 2017).

According to EurObserv'ER, biogas production increased slightly in 2019 to 16.6 Mtoe (0.7% more than in 2018). The Renewable Energy Directive (2018/2001) establishes a new legal framework for the development of bioenergy, by introducing criteria for sustainability and GHG reduction. The analyzes show that the contribution of biogas could increase from 16 Mtoe in 2015 to 30 Mtoe by 2030 (EurObserv'ER, 2020).

Anaerobic digestion (AD) in the agricultural sector would help reduce greenhouse gases, according to the Methane Strategy of the European Commission, of October 2020, in which it proposes as a climate objective for 2030, to reduce 55% of methane emissions (European Comission, 2020).

AD is a sustainable proven technological process for organic waste treatment and bioenergy production as biomethane, which has similar properties to natural gas and has a wide application potential (Prussi et al., 2021). Nevertheless, AD might face some challenges hindering a successful process due to feedstock characteristics (Basumatary et al., 2021; Dar et al., 2021). In this context, the use of a single feedstock such as carbonaceous substrates and agro-wastes or, nitrogenous substrates from the livestock industry, could inhibit the AD process due to acidification by the generation of volatile fatty acids (VFAs) or increased ammonia, respectively, for each type of feedstock (Meng et al., 2020; Rahman et al., 2019; Zhang et al., 2017).

Anaerobic co-digestion refers to the simultaneous digestion of two or more substrate with different and complementary characteristics, aiming to balance the nutritional requirement of microorganisms during the biomethanation process (Almomani and Bhosale, 2020; Mawson et al., 1991; Pagés-Díaz et al., 2015; Rahman et al., 2019).

Thermophilic AD has been reported to influence intra and extracellular environment of bacterial communities involved in the AD process and their growth kinetics by accelerating the hydrolysis of macromolecules and the conversion rates of intermediates released in the process (Basumatary et al., 2021; Fdez-Güelfo et al., 2011; Shin et al., 2019). In fact, the usual hydraulic retention time (HRT) for thermophilic bioreactors is shorter than mesophilic ones. Nevertheless, when the hydrolysis/acidogenesis rates are pronounced, the system acidification may occur due to VFAs accumulation and methanogenic archaea are inhibited in overloaded systems (Aboudi et al., 2020; Gómez-Quiroga et al., 2019).

The configuration of the AD process by combining co-digestion of complementary substrates (stability, buffering, nutrients balance, etc.) and thermophilic conditions (short HRT, kinetics increase, etc.) might be of interest for a successful process design with substrates with such complex characteristics in individual anaerobic treatments.

In the literature, there are several studies on the co-digestion of agricultural and livestock residues, but scarce studies could be found on the valorization of tubers wastes and by-products, such as by-products of the sugar beet industry. Sugar beet pulp is commonly used as an ingredient in the manufacture of animal feed.

Among the works carried out with sugar beet by-products, there are some studies about the treatment of different parts of this substrate (root and leaves, wet pulp, or molasses) and different operating conditions (Aboudi et al., 2015a; Fang et al., 2011; Suhartini et al., 2014). However, thermophilic co-digestion of sun dried beet pulp with cow manure (CM) has not yet been studied. Aboudi et al. (2015a, 2016a) have investigated the mesophilic co-digestion of a mixture of sugar beet pulp and molasses with CM and pig manure (PM). Lehtomäki et al. (2007) studied the mesophilic anaerobic co-digestion of stems and leaves of sugar beet with CM at only one hydraulic retention time (HRT) of 20 days. Fang et al. (2011) studied the mesophilic co-digestion of sugar beet pulp, with desugared molasses and CM, at one HRT of 20 days and different loading rates. Therefore, it is worth mentioning that scarce studies investigated the AD of sun-dried exhausted sugar beet pulp, which is an economic and sustainable strategy to preserve and store this residual biomass for long periods after production campaigns because its generation is large and seasonal. Furthermore, this natural pretreatment could affect its characteristics and biodegradation. It should be borne in mind that the pretreatment which a given agri-food waste receives after its generation affects its composition and behavior in anaerobic treatment (Liu et al., 2020). To the best of the authors' knowledge, anaerobic co-digestion of sun-dried exhausted beet pulp with animal manure has not been studied in semi-continuous thermophilic assays. In this context, this research aims to investigate the thermophilic anaerobic co-digestion of the above-mentioned co-substrates in long-term operation semi-continuous reactors studying both different organic loading rates and HRTs, giving insight into biogas production optimization and the system stability.

2. Material and methods

2.1. Feedstock and start-up of the anaerobic digester

The characteristics of the substrates and inoculum used in this study are shown in Table 1. The sun dried exhausted sugar beet pulp (SD-ESBP) was provided from the industrial sugar production plant belonging to "Azucarera" (a Spanish AB Sugar-UK company) located in Jerez de la Frontera (Cádiz, Spain) without any previous pre-treatment, except the natural desiccation by prolonged exposure of the fresh pulp to the sun. The process of extracting sugar from the beet results in a wet pulp as a by-product, which is subsequently pressed and exposed to the sun till drying, avoiding excessive costs of artificial drying (Koppar and Pullammanappallil, 2008). The humidity of SD-ESBP was 12% and it was stored at 20 °C until use. On the other hand, fresh CM was manually collected periodically (every 3 months) from a farm located in El Puerto

Table 1
Characteristics of the co-substrates and the inoculum source used in this study.

Parameters	Units	CM	SD-ESBP	Inoculum source
pH	–	7.28 ± 0.48	5.73 ± 0.59	7.84 ± 0.09
Total solids	(%)	23.65 ± 4.12	88.42 ± 2.18	9.50 ± 0.32
Volatile solids	(%)	13.77 ± 0.68	80.97 ± 4.07	3.02 ± 0.24
Alkalinity[a]	($gCaCO_3$/kg)	16.11 ± 8.15	2.14 ± 1.01	34.27 ± 2.26
TVFA[a]	(g/kg)	4.73 ± 2.45	73.52 ± 12.24	8.81 ± 0.97
sCOD[a]	(g/kg)	28.87 ± 8.15	235 ± 25.40	34.63 ± 2.93
tCOD[a]	(g/kg)	73.79 ± 12.44	554 ± 29.90	–
DOC[a]	(g/kg)	6.79 ± 1.12	74.65 ± 6.5	–
N–NH4$^+$	(g N/kg)	0.72 ± 0.24	0.28 ± 1.32	–
TKN[a]	(g N/kg)	20.86 ± 8.14	14.65 ± 3.11	–
C/N ratio	–	17.38 ± 4.50	31.79 ± 6.17	–

[a] g/kg (wet basis).

de Santa María (Cádiz, Spain). In the farm facilities, the cowshed area from where the manure was collected had a slope that acted as natural runoff of urine.

The CM presented a humidity of 78% and, hence, it was frozen at −20 °C to prevent its degradation during storage. The values of characterization are shown in Table 1.

An effluent from a thermophilic (55 °C) semi-continuous anaerobic reactor for the single digestion of SD-ESBP (without any co-substrate) was used as inoculum source for the start-up of the co-digestion process. When used, this reactor had been working in steady conditions at a HRT of 30 days for more than 130 days. Besides, this reactor showed a specific methane production (SMP) of 108 L/kgVS_{added}, a methane production rate (MPR) of 0.27 L/$L_{reactor}$·d, a VS removal of 57.3%, and a total acidity concentration of 9 g/L.

2.2. Experimental design

The feeding for the semi-continuous reactor was an optimized mixture of SD-ESBP and CM with a proportion of 25:75 (w/w), respectively, according to previous studies of Gómez-Quiroga et al. (2020). The total solids content of the feeding was adjusted to 8% (8.38 g SD-ESBP/100 g mixture) with distilled water, to avoid rheological problems in the anaerobic digestion process (Aboudi et al., 2017).

A 10 L (22 cm external diameter) semi-continuous stirred tank reactor, built-in stainless steel, was used. A temperature of 55 °C was maintained in the reactor, which was controlled through a water-heating jacket connected to a circulating water bath. The stirring was established at 12 rpm and it was performed by an external mechanical motor coupled to a stirring rod with U-type anchor blades (17.5 cm diameter) inside the reactor (20 cm internal diameter).

The reactor was fed in a semi-continuous mode and the feeding was provided one time per day.

A series of decreasing HRTs have been tested to determine the optimum and the critical conditions. The optimal condition has been considered as those in which the maximum specific methane productivity is reached (mLCH_4/gVS_{added}). The critical condition is that in which the decrease in HRT produces a disturbance of the system and the increase in the OLR, with respect to the previous HRT, does not produce the corresponding increase in the methane production rate MPR (LCH_4/$L_{reactor}$·d). Moreover, in the critical condition, a significant decrease in SMP is observed compared to that obtained in optimal HRT. HRTs and OLRs used in this study are shown in Table 2.

A 30-day HRT was used for the start-up of the experiment in order to maintain the HRT used in the inoculum reactor. The inoculum to substrate ratio of the semi-continuous digester was set as 1/1 by feeding a 50% of the working volume by inoculum and 50% with the SD-ESBP:CM mixture.

Table 2
OLRs, HRTs and semi-continuous experimental run times.

OLR (gVS/$L_{reactor}$·d)	HRT (days)	Duration of the experiment (days)
2.09 ± 0.02	30	142
2.71 ± 0.12	20	141
3.63 ± 0.16	15	77
4.73 ± 0.02	12	64
5.83 ± 0.17	10	63
7.00 ± 0.01	8	26
9.35 ± 0.41	6	38
12.47 ± 1.29	5	22
17.19 ± 0.01	4	29
23.33 ± 0.01	3	19

Each HRT was maintained for at least a period equivalent to three HRTs to ensure stabilization and, thus, approximate as closely as possible to the steady-state condition.

2.3. Analytical methods

The analytical determinations of total and soluble chemical oxygen demand (tCOD and sCOD, respectively), dissolved organic carbon (DOC), total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), pH and alkalinity have been carried out according to APHA-AWWA-WPCF standardized methods (APHA-AWWA-WPCF, 2005).

For DOC determinations, a carbon/nitrogen analyzer (Analytic Jena® multi N/C 3100) with a chemiluminescence detector (CLD) was used. Volatile fatty acids (VFAs) determination was carried out by using a gas chromatograph (Shimadzu® GC-2010) with flame ionization detector (FID) and a capillary column (0.25 μm of diameter and 30 m length) filled with Nukol®, according to Aboudi et al. (2015b). The VFA concentrations from C2 (acetic acid) to C7 (heptanoic acid) were determined. The total volatile fatty acidity (TVFA) was calculated as the weighted sum (through molecular weights) of the concentrations of the different individual volatile fatty acids and expressed as acetic acid concentration.

Previously to CODs and DOC analysis, the samples were centrifuged for 15 min at 4500 rpm and, then, filtered through a 0.47 μm glass microfiber filter (ref. GF52047, Hahnemühle®, Dassel, Germany). For VFAs determination, the procedure was the same described previously but a second filtration was performed through a PTFE filter of 0.22 μm (Álvarez-Gallego, 2005).

Produced biogas was collected in 25 L and 50 L gas bags (Tedlar®, SKC). The volume of biogas produced was measured with a wet rotary drum gas flowmeter (Ritter TG5®) connected to a gas suction pump Laboport® KNF. The biogas composition was determined by using a gas chromatograph (Shimadzu®, GC-2014) with a thermal conductivity detector (TCD) and a packed column (3.2 mm of diameter and 2 m of length) Carbosieve® S-II.

3. Results and discussion

3.1. Analysis of the process stability

The analysis of the process stability can be performed through the different parameters related to acidification: pH, VFAs concentrations and distribution, alkalinity and their relationships (Gómez-Quiroga et al., 2020).

Fig. 1 shows the evolutions of pH, Total volatile fatty acidity (TVFA), and acidity/alkalinity ratio for the different HRTs tested. It should be pointed out that pH was daily measured and if necessary (<6.8) corrected by K_2CO_3 addition supplied with fed.

In a stabilized anaerobic digestion process, the VFAs production in the hydrolytic and acidogenic stages is balanced with their subsequent utilization by acetogenic and methanogenic microorganisms, resulting

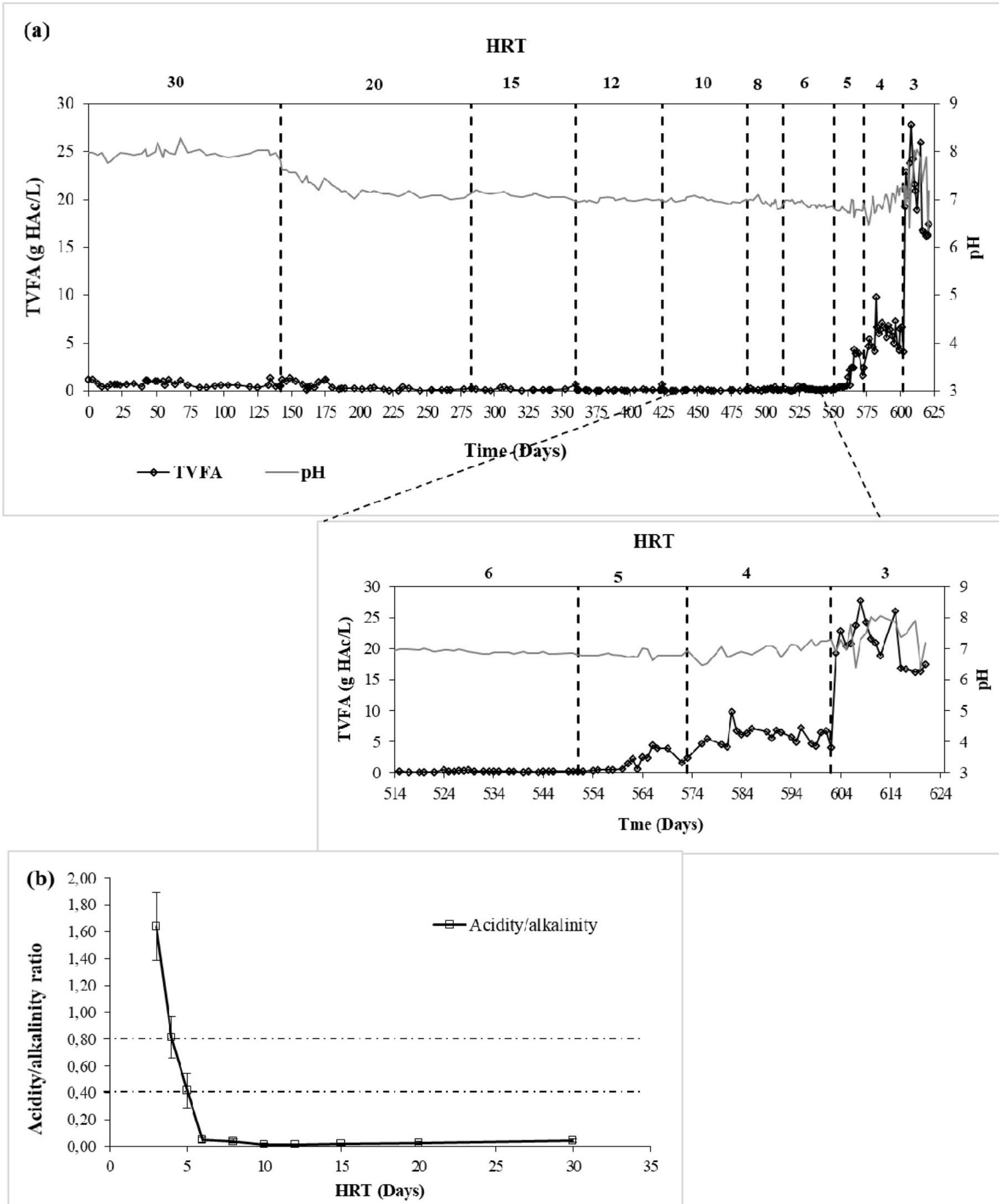

Fig. 1. (a) Evolution of TVFA and pH throughout the experiment (b) Final values of the acidity/alkalinity ratio for the different HRTs tested.

in relatively small levels of TVFA in the medium. In this study, the TVFA concentration at HRTs of 30, 20, 15, 12, 10, 8 and 6 days was under 2 g HAc/L and pH values were greater than 7, indicating a large performance of the co-digestion process in this range of HRTs.

Although pH values at HRTs of 8 and 6 days were slightly below 7, the process remained stable without any irreversible disturbance problems. It is noticeable that CM provides the required alkalinity to stabilize the process in spite of shortening HRTs since the anaerobic digestion of ESBP tends to spontaneous acidification when the HRT is shorter than 20 days (Aboudi et al., 2016a; Alkaya and Demirer, 2011). In the same line, Acosta et al. (2021) reported that the buffering provided by CM anaerobic co-digestion with cocoa waste was the key to to the process stability avoiding the system acidification.

On the other hand, it has been observed that the increase in OLRs (9.35, 12.47 and 17.19 gVS/$L_{reactor}$•d) and the shortening of the HRTs (6, 5 and 4-days), produced a gradual increase of TVFA values, along with the decrease in pH values despite the alkali addition. Although 4-day HRT required 7 times larger addition of the alkaline reagent than in 5-day HRT due to increased acidification, the microorganisms involved in the process have withstood since large biogas productions were observed in all HTRs, except for 3-day HRT. At 3-day HRT a dosage of 6 g K_2CO_3/L d was done. This was also corroborated by the ratios of some stability-indicating parameters such as the acidity/alkalinity ratio (Fig. 1 b). At the HRT 5-day, this ratio has a value of 0.4, considered as the lower tolerable limit of the AD system disturbance, while at the HRT of 4-day the ratio was at the upper admissible limit of 0.8 (Callaghan et al., 2002; Li et al., 2018; Switzenbaum et al., 1990; Zickefoose et al., 1976).

Nevertheless, the subsequent decrease of HRT to 3 days has triggered an important disturbance of the process, as can be seen in Fig. 1a. In this case, the increase of TVFA and the pH drop could not be controlled despite the addition of the alkaline reagent. The 3-day HRT was maintained for 18 days and the average TVFA rose to values of 21 g HAc/L, which is four times bigger than the TVFA level observed in the previous HRTs for a balanced process. At this HRT, the acidity/alkalinity ratio was bigger than 1.6, indicating that the AD reactor was critically affected by decreasing HRT (Fig. 1b). Furthermore, decreasing HRT to 3 days implies a sharp drop in pH (as can be seen in Fig. 1a). The amount of K_2CO_3 required at the end of this HRT to maintain pH close to neutrality was around 6 g/$L_{reactor}$·d and the methane production decreased significantly.

At mesophilic temperature, Aboudi et al. (2016a) observed that for the co-digestion of ESBP (a mixture of mechanically dried beet pulp and molasses) with CM, TVFA of 6 g/L was reached at a HRT of 12 days (OLR of 6.2 gVS/$L_{reactor}$•d), which induced system acidification and process inhibition. Therefore, 12-d HRT was found to be the limit admissible HRT at mesophilic temperature conditions. In the present study, working at thermophilic conditions allowed increasing the system's capacity to assimilate a bigger acidity. In the same way, on the effect of thermophilic temperature on increasing the system tolerance to VFAs increase, Suhartini et al. (2014) reported that for mesophilic AD of fresh sugar beet pulp (24% of TS), the system disturbance occurred at OLR of 5gVS/$L_{reactor}$•d (corresponding to the longer HRT of 54.8 days) at mesophilic temperature, while for thermophilic reactors, this OLR was tolerable and despite the TVFA increase, no disturbance was observed.

The main VFAs produced in all the HRTs tested were acetic, propionic and butyric acids, with acetic acid being the major one, Fig. 2 shows the maximum and ending values of VFAs for all the HRTs studied. As can be seen, the decrease in HRT below 6 days (9.35 gVS/$L_{reactor}$•d) leads to an increase in the maximum value of VFAs. A further decrease in HRT up to 3 days (equivalent to OLRs up to 23.33 gVS/$L_{reactor}$•d) caused an abrupt accumulation of VFAs. The final data for this HRT are 16, 1.4, and 2.2 g HAc/L of acetic, propionic and butyric acids, respectively. It can be pointed out that in 3-day HRT the maximum concentration reached for acetic acid was 21.8 g HAc/L which has been considered as inhibitory for the methanogenic microorganisms in literature (Pagés-Díaz et al., 2015; Rahman et al., 2019). It should be noted that the inhibitory level of individual VFAs in AD may vary according to substrates characteristics and system operation conditions. Aboudi et al. (2016a) found that in mesophilic semicontinuous anaerobic co-digestion of ESBP and CM, the maximum concentrations of acetic and propionic acids in the time considered as critical (HRT of 12 days) were 3.5 and 1.7 g HAc/L, respectively. However, in batch co-digestion studies, HPr concentrations up to 5.5 g/L have been observed and the system has not been inhibited (Aboudi et al., 2016b). In another study of Meng et al. (2020), authors reported that great concentrations of acetic acid could inhibit acetate-consuming methanogenic microorganisms and limit the degradation of propionic acid at concentrations above 2.2 gHAc/L. Nevertheless, previous studies of thermophilic anaerobic co-digestion of ESBP and animal manures (CM and PM), operating in batch, indicated that acetic acid concentrations reached values as great as 10–12 g HAc/L without system disturbing and acetic acid could be subsequently degraded without any inhibition of the process (Gómez-Quiroga et al., 2019, 2020).

In the present research, the shorter HRT of 3 days has been considered critical for the thermophilic anaerobic co-digestion of SD-ESBP and CM. Hence, the produced VFAs were not degraded and the acidification of the process occurred. Therefore, the washing-out of the acetoclastic methanogens likely produces decoupling of the rates of the microorganisms and impedes the normal performance of the anaerobic digestion process. It should be taken into account that the different groups of microorganisms involved in the anaerobic digestion process have different duplication times (Fdez.-Güelfo et al., 2013). Zhang et al. (2017) reported that the duplication time for acidogenic microorganisms is about 30 min while for acetogenic microorganisms is around 1.5–4 days. Finally, for methanogenic microorganisms, duplication times are 1 h for hydrogen utilizing archaea and 2–3 days for acetoclastic methanogens. According to above mentioned duplication rates, acetoclastic methanogenesis had resulted clearly affected by the shorter HRT.

Romero et al. (1988) working with wine vinasses, have reported that the maximum specific growth rate of thermophilic acetoclastic methanogens is 0.6 $days^{-1}$ and, therefore, the corresponding duplication time is 1.7 days. The ESBP and CM are substrates more difficult to degrade than wine vinasses. The lignocellulosic characteristic of ESBP can make it difficult for microorganisms to access the organic content of this agri-food by-product. The lignocellulose content of ESBP has been found to be as 22.5%, 21.2%, and 3.5% of hemicellulose, cellulose, and lignin,

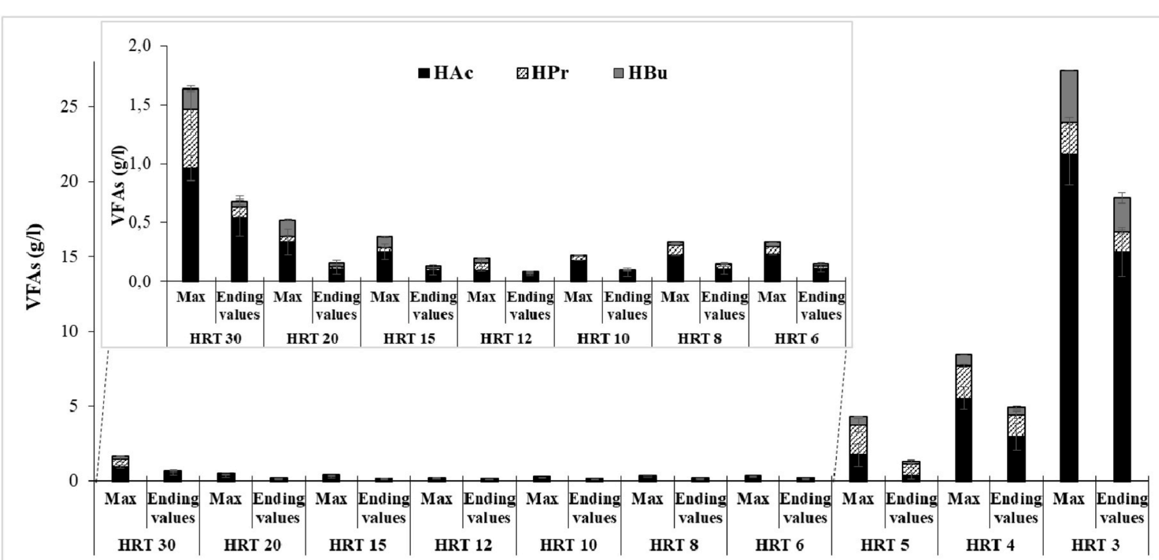

Fig. 2. Maximum and ending average values of the main volatile fatty acids (acetic, propionic and butyric acids) for the different HRTs tested.

respectively (Gómez-Quiroga et al., 2019). Therefore, this would cause an increase in the minimum HRT required by the system in the AD process (Cavinato et al., 2017; Şenol et al., 2020; Vats et al., 2019; Wang et al., 2020). The observed recovery in batch tests would point in the direction of a washing-out episode behind the failure of the semi-continuous reactor in this research.

The ratio between the concentrations of propionic and acetic acids is also considered as an early indicator of the process disturbance. According to literature, the maximum admissible value of this parameter for a suitable process performance is 1.4 (Franke-Whittle et al., 2014; Marchaim and Krause, 1993; T. Hill et al., 1987).

Fig. 3 shows the evolution of the propionic to acetic ratio for the different HRTs tested. As can be seen, for 5-day and 4-day HRTs an increase in the HPr/HAc ratio was observed, reaching the maximum value of 13.7 at the end of HRT of 5-day. It should be noted that prior to the HRT 5-day there had been no accumulation of propionic acid. At this time, the HPr/HAc ratio increased, despite the presence of methane, indicating the approach of the system to its limit. In these three HTRs, propionic remained below 2.2 g/L, but acetic continued to increase when moving to a shorter HTR, between the 5 and 4-day HTRs, the acetic was 9.7 times larger and between the 4 HTR and 3-days 6.5 times longer, for the final days of each HRT. Small values of the HPr/HAc ratio in 3-day HRT are a consequence of the very large values of the acetic acid concentrations reached in this period. Therefore, no inhibition by propionic acid has been detected in this study. According to the literature, the accumulation of propionic acid is, usually, related to an increase in the partial pressure of hydrogen which causes the inhibition of propionic-acetogenic bacteria (Mawson et al., 1991; Ruzicka, 1996). Therefore, for 4-day and 3-day HRTs, the syntrophic association between acetogenic bacteria and hydrogenotrophic methanogenic archaea was not affected and the interspecies hydrogen transfer appeared to be adequate. As can be seen in section 3.2, the presence of H_2 was never detected in the biogas. However, the large acetate concentrations for the HRT of 3-day were a consequence of the incapacity of the population of acetoclastic methanogenic archaea to use the acetate produced by acidogenic and acetogenic microorganisms, because the growth rate of acetoclastic methanogens is very small, requiring several days or even more for their duplication (Romero et al., 1988; Van Lier et al., 2008).

In summary, the shortest HRT tested for which all the stability parameters (including total acidity) were kept at optimal levels for the AD process was 6 days. Thus, even though the operation at 5-day HRT leads to the larger SMP value of all the HRTs studied (as can be seen in section 3.2), the first symptoms that the system is close to its operational limit are already beginning to be detected. Thus, the values of the acidity/alkalinity ratio begin to rise, although maintaining into the range considered adequate for the AD process. This destabilization is more evident for the 4-day HRT, despite the large SMP value, and leads to a total disturbance of the process when operating at 3-day HRT.

3.2. Evolution of the biogas and methane production for the different HRTs tested

Fig. 4 depicts different data related to biogas production: the methane production rate (MPR) (expressed as $L/L_{reactor}$•d) and the biogas composition (% CO_2 and % CH_4). H_2 was never detected in the biogas during the entire study.

As can be seen in Fig. 4 (a) the methane production rate increases continuously as HRT decreases between 30 and 4 days. However, this trend is not maintained in the last HRT tested. Thus, for 3-day HRT a significant decrease in MPR was observed compared to the previous HRT, obtaining an MPR 42.2% smaller (2.81 $L/L_{reactor}$•d) than that corresponding to 4-day HRT (5.04 $L/L_{reactor}$•d).

The biogas composition was quite stable in the range of HRTs between 20 and 5 days. Thus, except in specific and punctual episodes, due to changes in HRT or operational conditions, the methane content remained around 56%. For 4-day HRT, a change in the trend is observed and the percentage of methane starts to decrease. However, it can be observed that the methanogenic stage stabilizes leading to an increase in

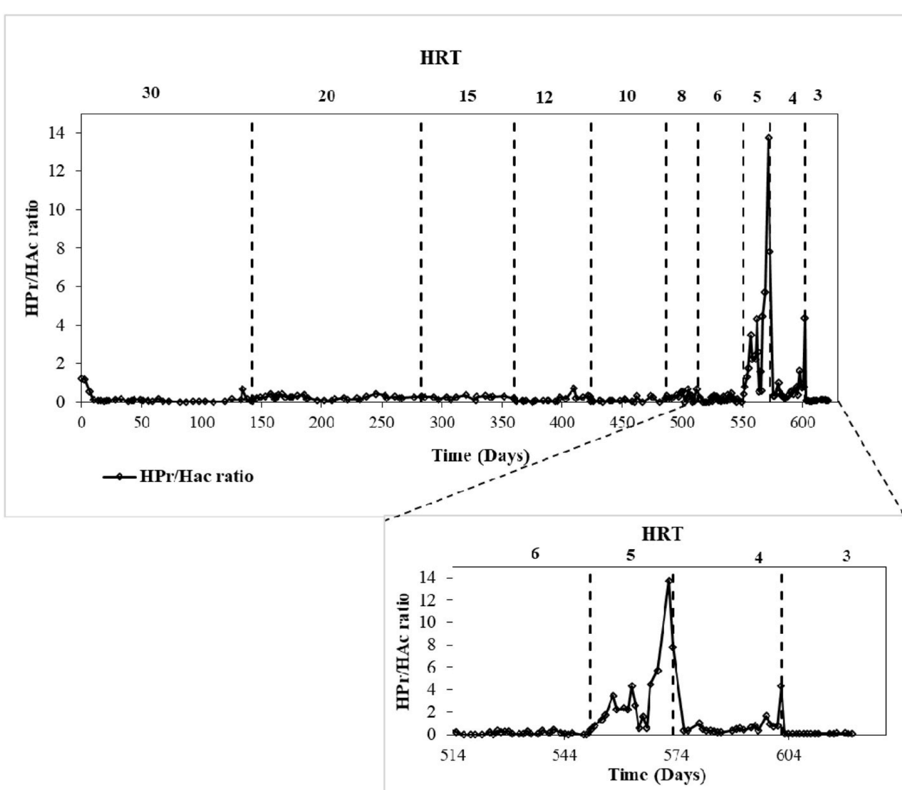

Fig. 3. Evolution of propionic to acetic acid ratio (HPr/HAc ratio).

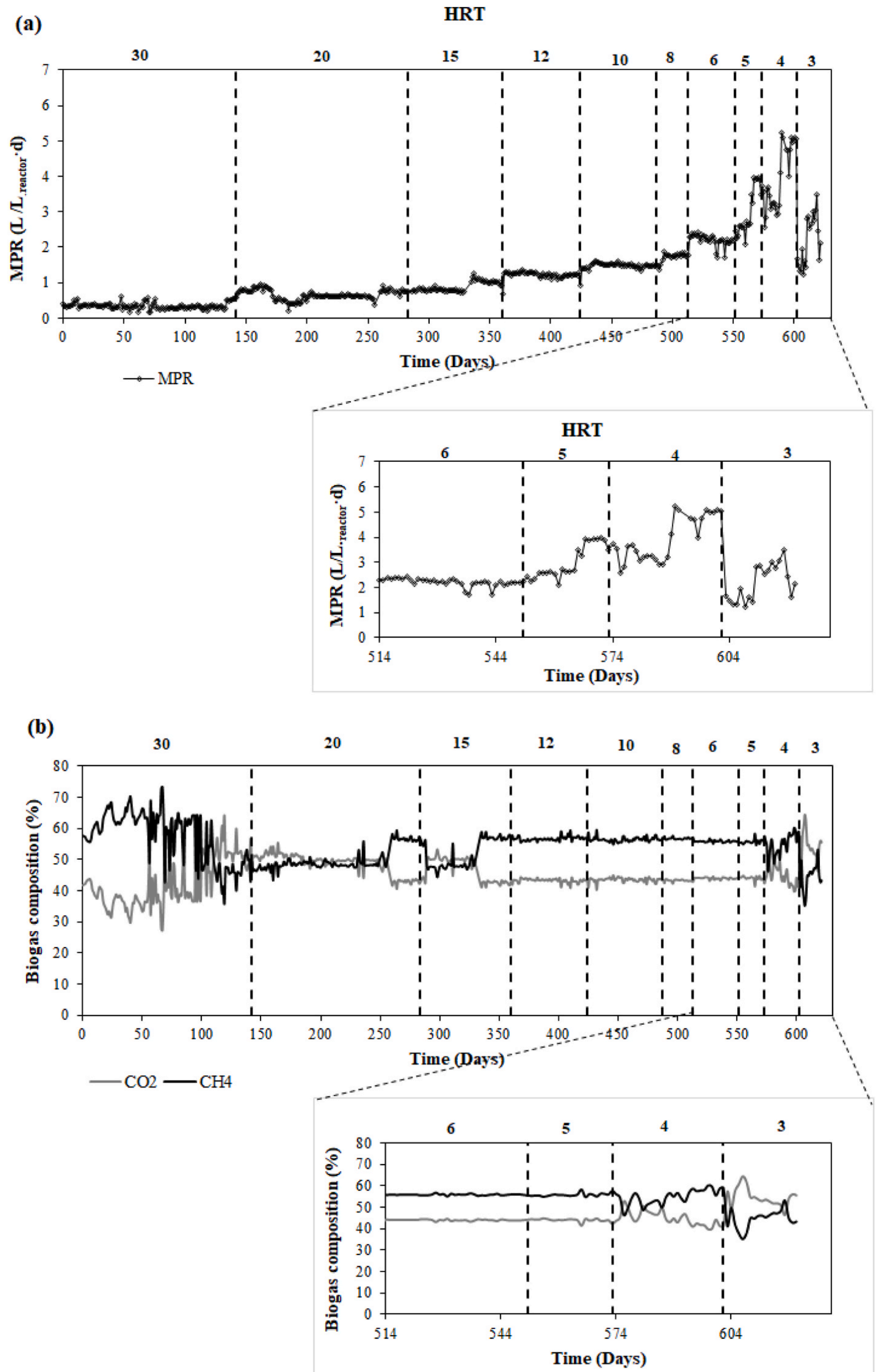

Fig. 4. Evolution of: (a) Methane production rate ($LCH_4/L_{reactor}.d$); (b) Biogas composition.

methane composition, reaching an average methane percentage similar to previous HRTs. For 3-day HRT, methane content decreased until stabilizing at 45.5% and CO_2 becoming the major component of biogas. Overall, the observed percentage of methane in the system was a consequence of pH control. In this study, the pH control was done by adding K_2CO_3, consequently this produces an increase in the concentration of CO_2 in the biogas.

Fig. 5 shows the correlation of the average methane production rate (MPR) and the specific methane production (SMP) ($mLCH_4/gVS_{added}$) versus the OLRs applied at each HRT. In addition, in the same graph, the evolution of the ratio between TVFA and the VS added to the system has been included for further discussion.

In Fig. 5, it can be observed that the MPR increased linearly with the OLR if the data of 3-day HRT is omitted. A linear regression fit has been applied with an acceptable value of the linear regression coefficient ($R^2 = 0.980$) and slope have showed a value of 0.286 L CH_4/gVS_{added} which is in accordance to the SMP values in the range of 2.71–17.19 gVS/$L_{reactor}$·d before the failure in 3-day HRT occurs.

From this figure, it is found that:

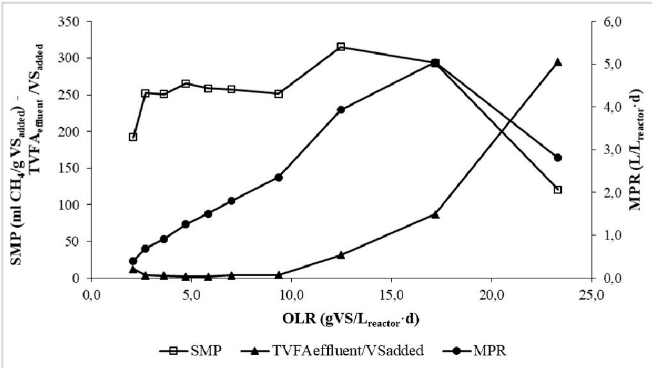

Fig. 5. Evolution of the specific methane production, methane productivity and the TVFA/VS_{added} ratio versus the OLR.

- At the first HRT of 30 days, the microbial population is in adaptation phase because the reactor was previously fed only with SD-ESBP and the system was not fully stabilized.
- For the range of HRTs between 20 and 4 days (OLRs ranges from 2.71 to 17.19 gVS/$L_{reactor}$·d), the system is working in stable conditions and the increase in the OLR supplied to the reactor leads to a proportional increase in methane production. Only could be detected a progressive accumulation of VFAs in the last two HRTs in this range (5-day and 4-day HRTs).
- At the HRT of 3 days (corresponding to OLR 23.33 gVS/$L_{reactor}$•d), the disturbance of the system occurs and the increase in the added OLR does not lead to an equivalent increase in methane production, while a substantial accumulation of VFAs is observed (mainly acetic acid).

According to the results obtained in the stable operating range (HRTs from 20 to 4 days), the experimental data of the methane yield for the anaerobic thermophilic co-digestion of SD-ESBP and CM were in the range 251–315 $mLCH_4$/gVS_{added}. Indeed, as can be seen in Fig. 5, for OLRs minor than 23.33 gVS/$L_{reactor}$·d (HRTs longer than 3 days) there is a linear relationship between MPR (LCH_4/$L_{reactor}$·d) and OLR (gVS/$L_{reactor}$·d). Thus, from the showed linear regression fitting of experimental data, a slope of 286 $mLCH_4$/gVS_{added} was obtained. This value is representative of the average methane yield for the considered range of HRTs if the process is well balanced and free of any significant disturbance.

The evolution of the ratio between total acidity (TVFA) and the volatile solids added to the system (VS_{added}) corroborates that system destabilization clearly occurs for HRTs shorter than 4 days or OLRs major than 17.19 gVS/$L_{reactor}$·d. However, an incipient accumulation of VFAs was also observed in the HRT of 4 days. Thus, the increasing fraction of the VS fed to the reactor at HRTs shorter than 4 days, was transformed into VFAs but not converted into methane due to the system overloading and methanogens washout, suggesting that the optimal organic matter removal is above this HRT. VS degradation efficiencies will be further discussed in section 3.3.

Considering the slope of the linear regression of MPR versus OLR (0.286 LCH_4/gVS_{added}), if there had been no alteration of the process in the HRT 3 days, the expected MPR would be 6.66 LCH_4/$L_{reactor}$·d. However, the real value obtained was 2.81 LCH_4/$L_{reactor}$·d, which supposes a 42.2% with respect to the potential of methane productivity for 3-day HRT.

This percentage referred to the theoretical one, obtained for 3-day HRT, is only slightly major than that generally accepted as corresponding to methane production derived from the activity of hydrogen-using archaea (27–30%) in the anaerobic digestion process (Awhangbo et al., 2020; Batstone et al., 2002; Romero García, 1991). This data, together with the large concentrations of acetic acid present in the process effluent and not used in the generation of methane for the 3-day HRT, constitutes an evidence that the activity of acetoclastic archaea is close to negligible. Thus, 3-day HRT is probably very close to the minimum useable HRT in semi-continuous thermophilic anaerobic co-digestion of SD-ESBP and CM, due to the washing-out of the acetoclastic methanogenic population.

Even when the optimum value for SMP was achieved in 5-day period, it could be very interesting the operation at 4-day HRT (17.19 g VS/$L_{reactor}$•d) because the specific methane production is only 7% minor than the maximum (5–day HRT) while the OLR added was 28% bigger. In addition, the accumulation of VFAs and the value of the acidity/alkalinity ratio in 4-day HRT were admissible, showing a good balance between acidogenic and methanogenic activities, and the percentage of volatile solid removal was (around 55%) (See section 3.3).

A maximum methane yield of 315 $mLCH_4$/gVS_{added} has been obtained in this study for the thermophilic co-digestion of SD-ESBP and CM, operating at 5-day HRT and an OLR of 12.47 gVS/$L_{reactor}$·d. This value of the methane yield is larger than obtained by Fang et al. (2011) operating in the thermophilic range, they obtained methane yields of 260 and 280 $mLCH_4$/gVS_{added} for co-digestion of fresh beet pulp and CM operating at OLRs of 2.95 and 6.75 gVS/$L_{reactor}$·d and mixing ratio 15:85 and 50:50 (%w/w), respectively. In a study of Li et al. (2014), mesophilic anaerobic co-digestion of chicken manure and corn stover was carried out in stirred tank reactors operating with a concentration of 12% in TS. Authors reported the need of operation at a long HRT (22.5-day) for an OLR of 4 gVS/$L_{reactor}$•d, to obtain a methane yield of 223 ± 7 mL/gVS_{added}. This SMP is 30% smaller and the OLR 3 times shorter than the obtained in the present research at a shorter HRT of 5 days and an OLR of 12.47 gVS/$L_{reactor}$·d, which is also 3-fold greater than that reported by Li at al. In another study, Lehtomäki et al. (2007) found that operation at HRT of 3-day and OLR of 2 gVS/L•d in a semi-continuous mesophilic digester fed with CM and 30% of sugar beet tops led to methane yield of 229 mL CH_4/gVS_{added}, which is 27.3% smaller than the results obtained in this study.

However, the maximum methane yields reported by Aboudi et al. (2015b), in the mesophilic anaerobic co-digestion of ESBP with PM was larger. These authors have obtained a maximum SMP of 362.2 $mLCH_4$/gVS_{added}, operating at 12-day HRT and an OLR of 7.4 gVS/$L_{reactor}$·d. Their study was performed using a different manure type (PM), which also may play a different role in improving AD in comparison to CM. In the present study, the maximum SMP occurred at 5-day HRT and an OLR of 12.47 gVS/$L_{reactor}$·d. It should be noted that the ESBP in the studies carried out by these authors was mechanically dried by extrusion. In addition, that sugar beet pulp contained molasses, which provides a greater amount of available and biodegradable organic matter for the process.

In another study of the same authors (Aboudi et al., 2016a), a maximum SMP of 314.0 $mLCH_4$/gVS_{added} was obtained in the mesophilic anaerobic co-digestion process of ESBP and CM operating at 15-day HRT and an OLR of 5.0 gVS/$L_{reactor}$·d. These results are according to the literature, in which it is generally considered that thermophilic range is capable of accepting bigger OLRs (Gou et al., 2014; Montañés et al., 2015; Neshat et al., 2017; Panichnumsin et al., 2010).

Nonetheless, the operation in the thermophilic range for the anaerobic co-digestion of SD-ESBP:CM carried out in this study has allowed a successful reduction of the operating HRT up to 4 days (which corresponds to an OLR of 17.19 gVS/$L_{reactor}$·d), much further than is possible in the mesophilic range. Moreover, it should be noted that the ESBP of both studies are not exactly the same. One of the differences between these two substrates is that the beet pulp used in this study, when dried in the sun, may have lost a part of its methanogenic potential.

In a recent study, Acosta et al. (2021) have studied the anaerobic co-digestion of cocoa wastes and CM at HRTs of 80, 56, and 28 days (OLRs of 1.4, 2, and 4 gVS/$L_{reactor}$.d, respectively). The authors found that the largest methane yield of 181 $mlCH_4$/gVS_{added} was obtained from co-digestion with CM. Furthermore, they concluded that CM has a key role in the stabilization of AD of cocoa waste. A similar conclusion was reported in previous studies, although without corroborating this finding with a microbial analysis (Aboudi et al., 2016a; Fang et al., 2011; Gómez-Quiroga et al., 2020).

3.3. Evolution of the organic matter concentration

The evolution of the organic matter concentration in the effluent of the reactor, expressed as soluble chemical oxygen demand (sCOD), is shown in Fig. 6 (a) for the different HRTs tested.

The sCOD reached their minimum values (3.06–3.10 g COD/L) for the HRTs between 20 and 6 days. In HRTs shorter than 6, a sharp initial increase in sCOD is observed when the OLR is increased as a consequence of the initial decoupling of the activities of the microbial groups. While in the last HRT 4 and 3, there is a clear and evident increase in sCOD, indicating that the system was in an extreme situation, prone to destabilization. Acosta et al. (2021) has confirmed the positive effect about the methanogenesis due to the usual presence of *Methanosaetaceae* in CM. However, the probably reduction of the size of the acetoclastic methanogenic population of microorganisms in 3-day HRT are behind the excessive accumulation of acetic acid (Aboudi et al., 2015a), As can be seen in Fig. 4 (a), the methane production decreases while sCOD increases (mean value of 29.50 g/L). This behavior is very similar to that described previously for both the TVFA concentration and the acidity/alkalinity ratio.

As previously stated, Aboudi et al. (2016a) have studied the anaerobic co-digestion of ESBP and CM in the mesophilic range. The authors indicated that the maximum organic matter removal (expressed as volatile solids) was obtained operating at 18-day HRT. In another research of (Aboudi et al., 2015a), the results of anaerobic mesophilic co-digestion of ESBP with PM the maximum organic matter removal was observed in 12-day HRT.

Data of volatile solids removal percentage, for the different HRTs tested in this study, are shown in Fig. 6 (b). The VS removal percentage was larger than 50% for all the HRTs tested, except for the HRT of 3 days. As previously commented for this HRT, this was likely due to that the size of the microbial populations was not enough to support the OLR added to the system (Neshat et al., 2017).

In semi-continuous mesophilic anaerobic co-digestion of sewage sludge and dry bagasse pellets from the brewing industry (Szaja et al., 2020), the removal percentages of VS were 44 and 36% for OLRs of 1.73 (20-day HRT) and 1.98 kgVS/m^3·d (18-day HRT), respectively. The authors consider that HRT should not be decreased due to the presence of hardly-biodegradable compounds related to the lignocellulosic character of the substrate. However, in the present study, the maximal OLR tested was as great as 23.33 kgVS/m^3·d (3-day HRT), which could be related to the small lignin content in SD-ESBP and CM mixtures.

4. Conclusions

According to the previous discussion, the main conclusions are the following:

- Exhausted sugar beet pulp dried by exposure at sun and cow manure are two complementary co-substrates, allowing to perform the thermophilic anaerobic co-digestion at HRT as short as 4 and 5 days before a drastic reduction in the specific methane production even when some punctual accumulation of total acidity have been observed.
- At the optimum operational conditions, based on the maximum of the specific methane production, (5-day HRT and 12.47 gVS/$L_{reactor}$·d) the system performance was: 3.93 $LCH_4/L_{reactor}$·d; 315 $mLCH_4/gVS_{added}$ and 53.7% VS removal.
- The methane production is proportional to the OLR for the range from 2.7 gVS/$L_{reactor}$·d (HRT of 20-day) to 17.19 gVS/$L_{reactor}$·d (HRT of 4-day) with an average methane yield of 286 $mLCH_4$/gVS.

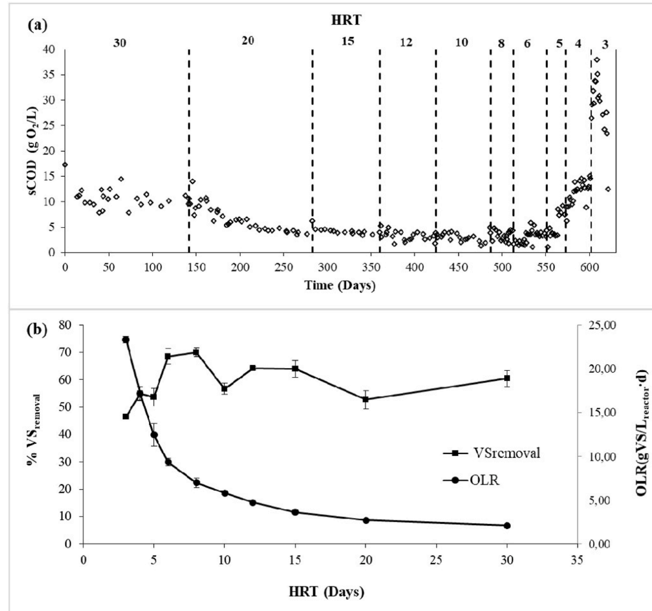

Fig. 6. Evolution of (a) sCOD and (b) VS removal for the different HRTs tested.

- Partial washing-out of acetoclastic methanogenic archaea would have occurred at 3-day HRT when the system failure occurred.

Author contributions

Author Contributions: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, writing—original draft preparation, writing—review and editing, Visualization, Supervision, Project administration, Funding acquisition were developed through equal contributions of all authors. All authors have read and agreed to the published version of the manuscript.

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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