Bioresource Technology Reports 19 (2022) 101168

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

From laboratory to farm-scale psychrophilic anaerobic co-digestion of cheese whey and cattle manure

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ARTICLE INFO

Keywords:
Co-digestion feasibility
Low-cost tubular digester
Biogas yields
Rural communities

ABSTRACT

This paper aims to prove the feasibility of cheese whey (CW) and cattle manure (CM) anaerobic co-digestion, from biomethane potential (BMP) laboratory assays to monitoring a farm-scale digester under psychrophilic conditions, and the impact on surrounding communities. The results show that while CW mono-digestion at 15 °C suffers inhibition, the CW:CM blend (70:30 volatile solids -VS- basis) is favorable at a similar temperature generating 0.24 m^3 CH_4/kg VS (35 °C yield: 0.60 m^3 CH_4/kg VS). A farm-scale digester (8 m^3) installed in a rural school operated at 17.7 °C reached 0.42 m^3CH_4/kg VS, 0.31 m^3 $CH_4/m^3_{digester}$ d with an organic loading rate of 0.61 kg VS/$m^3_{digester}$ d (CW:DM 54:46). Even with CW's high volatile fatty acids (VFA) load, the digester did not show metabolic activities inhibition: VFA consumption was around 96.45 ± 2.25 %. In the rural school, biogas generation replaced the wood utilization and reduced propane consumption by 33 %. Despite these results, there are issues around psychrophilic BMP test to be reviewed, and user's misperceptions of biogas technology to overcome.

1. Introduction

In rural areas of developing countries, it is common to find that economic activities focus on raising livestock and dairy production, which are developed by small enterprises or farmers. Dairy production improves local food security, generates local jobs, and represents an income for many smallholder families (Escalante et al., 2018). Dairy products demand in developing countries is expected to increase in the next years (FAO and GDP, 2018).

The main residues generated in this activity are cattle manure (CM) and cheese whey (CW) (Asas et al., 2021). CW has a high nutritional value represented by soluble organic matter content such as carbohydrates (5 %), lipids (0.5 %), and proteins (0.8 %) (Guimarães et al., 2010). Despite its nutritional value, this by-product disposal becomes a relevant environmental problem. Most of it is discarded directly to hydric sources due to a lack of systematization and technology transfer (Saddoud et al., 2007). From the perspectives of energy recovery, biofertilizer generation, and greenhouse gas mitigation, anaerobic digestion (AD) offers a long-term option for CW management.

AD of CW has been shown to be feasible since decades ago at a laboratory scale using an up-flow anaerobic sludge blanket digester (UASB; Rico et al., 1991). Chatzipaschali and Stamatis (2012) and Dereli

Abbreviations: AD, anaerobic digestion; ACoD, anaerobic co-digestion; BPR, biogas production rate; CM, cattle manure; CW, cheese whey; COD, chemical oxygen demand; HhD, household digesters; HRT, hydraulic retention time; ITAC, Cáchira Technical Agricultural Institute; m.a.s.l., meters above sea level; OLR, organic loading rate; SBP, specific biogas production; SMA, specific methanogenic activity; UASB, up-flow anaerobic sludge blanket digester; VFA, volatile fatty acids; VS, volatile solids.

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https://doi.org/10.1016/j.biteb.2022.101168
Received 21 April 2022; Received in revised form 16 July 2022; Accepted 19 July 2022
Available online 25 July 2022
2589-014X/

et al. (2019) show that CW AD research has been realized mainly at laboratory scale and mesophilic conditions, while there are a few literature about the performance of farm-scale digesters fed with CW. The digesters treating CW reported are industrial scale, usually UASB or modifications of this model (Chatzipaschali and Stamatis, 2012), that do not apply to small scale dairy farmers from developing countries. However, because of its low pH and high volatile fatty acids (VFA) content, AD of CW as the primary substrate has proven difficult resulting in process inhibition (Rico et al., 2015).

A low-cost alternative to treat the CW is to co-digest it with other dairy waste as cattle manure. The synergy of the mixture in anaerobic co-digestion (ACoD) allows the yields augmentation over the obtained by digestion of independent substrates (Bella and Rao, 2021). CW has a biodegradability of around 99 % (Comino et al., 2012), represented in an important macronutrient content such as proteins (9 %), lactose (70–80 %), organic matter (up to 80 g/L chemical oxygen demand COD), 8–20 % of minerals, and a low concentration of hydrolysed peptides and lipids. Those characteristics make the CW an attractive substrate for consortia to generate methane (Rico et al., 2015; Carvalho et al., 2013). For its part, CM characteristic apport high alkalinity (1.85 ± 0.17 g/L), refresh microorganisms' content and supplement the system with nutrients and trace elements that are important for the growth of bacteria and archaea. Additionally, CM has a low cost and easy availability and can replace the use of chemicals in pH regulation (Jaimes-Estévez et al., 2020).

Bertin et al. (2013) established that the best methane yield (0.320 m^3/kg VS), obtained from Biomethane Potential (BMP) test, was reached when the CW:CM ratio was 50:50. At mesophilic conditions and with a continuous feeding laboratory-scale digester, Comino et al. (2012) present that the best ratio CW:CM in volatile solids (VS) is 50:50 to achieve the best methane yield of 0.343 m^3/kg VS at 35 °C, an hydraulic retention time (HRT) of 42 d with an organic loading rate of 2.65 kg VS/m^3·d.

But currently in Latin America, the most widely used systems to carry out the anaerobic process are household digesters (HhD) that operate under psychrophilic conditions (Martí-Herrero et al., 2014a). The psychrophilic ACoD of CW and CM has been studied at laboratory scale. In a tubular digester at 25 °C, with a CW:CM ACoD (70:30), Jaimes-Estévez et al. (2021) obtained biomethane potentials ranging from 0.33 to 0.44 m^3_{biogas}/kg COD_{fed}, for an OLR of 0.5 to 1.0 Kg VS/m^3·d, and 129 and 69 d days of HRT, respectively. When tubular digester was operated at OLR 1.5 kg VS/m^3 d, biogas production was reduced to 0.09 m^3_{biogas}/kg COD_{fed}. Kavacik and Topaloglu (2010) realized ACoD CW:CM with a ratio 66:33 at 24 °C obtaining and methane yield equal to 0.0497 m^3/kg VS for OLR = 12.5 kg VS/m^3·d (HRT = 5 d), 0.090 m^3/kg VS for OLR = 6.26 kg VS/m^3·d (HRT = 10 d) and 0.0905 m^3/kg VS for OLR = 3.13 kg VS/m^3·d (HRT = 20 d). These results of psychrophilic ACoD of CW and CM at laboratory scale have not been transferred to farm-scale.

In the context of small and medium dairy farmers of developing countries, the HhD installed in rural areas are fed mainly with manures from livestock activities such as cattle, swine, and sheep breeding, providing a residues treatment and an energetic supplement to users (Garfí et al., 2016; Kinyua et al., 2016; Castro et al., 2017; Jaimes-Estévez et al., 2021). Nevertheless, in farm or household scale, the studies are applied to the mono-digestion process, and there are few successful reports about ACoD of CW and CM at this scale. So, while the dairy sector will grow in developing countries (FAO and GDP, 2018), there is a lack of experience reported in the ACoD of CW and CM in farm-scale psychrophilic simple digesters. The present paper examines the ACoD of CW and CM in a 8 m^3 HhD digester. The main objective was to establish the effect of substrate changes under psychrophilic conditions (15 °C) on the digester performance and quality and the bioprocess stability. Additionally, this study seeks to identify the social impact of digesters installation in areas without domestic gas service.

2. Materials and methods

2.1. Study case description

This study was carried out at a Colombian rural Institution located in the Cáchira Municipality (North of Santander Department; 7°44′10.6"N 73°03′03.0"W a 2025 m.a.s.l. average temperature: 15 ± 3 °C). A large area of the municipality (>9 thousand hectares) is part of the Santurbán moor, the main water source for the Santander and Norte de Santander departments. Given its geographic location, the region has a single access road limiting the natural gas supply. The Institution divides its activities into academic and agricultural areas. Nowadays, >200 students between 5 and 19 years old participate in presential classes focused on agribusiness education. Through its activities, the institution mainly generates organic residues from cattle raising and dairying. The key residues generated are acid cheese whey (CW; 30–50 L/d) and cattle manure (60–80 kg/d, where around 30 % are easily collectible). Some characteristics of used CW and CM, respectively are: carbohydrates 55.33 % and 47.7 %; lipids 2.24 % and 1.92 %; proteins 23 % and 9 %; C/N ratio of 22.1 and 27.14; pH 3.78 and 7.8; total alkalinity (TA) 1800 mg $CaCO_3$/L and 16,400 mg $CaCO_3$/L and VFA 4800 mg eq Acetic acid/L and 72 mg eq Acetic acid/L.

2.1.1. Biochemical methane potential experiments

The viability of CW biogas production under psychrophilic conditions was initially measured by determining biochemical methane potential (BMP). BMP tests were conducted by sets of triplicates in 500 mL glass flasks at 15 ± 2 °C, with an inoculum/substrate ratio of 2 (VS basis). The proportions of the substrate (CW) and co-substrate (CM) for each assay were 100:0 and 70:30 (on a volatile solids basis) in order to evaluate the mono-digestion process and a favorable mixing ratio for those substrates (Jaimes-Estévez et al., 2020). The inoculum was an anaerobic sludge from a tubular reactor (9.5 m^3 total volume) that digests cattle manure and has been operating for five years (local average temperature of 25 ± 5 °C). Inoculum VS concentration was around 24 ± 3 g VS/L. Additionally, sets of triplicate control assays (CW and CM mono-digestion at 35 ± 2 °C) and blank assays (without substrate) were performed to compare the AD at optimum temperature conditions and for endogenous methane production determination (Holliger et al., 2016). The methane production was measured daily by the volume displacement of an alkaline solution (NaOH 5 N). BMP assays were finalized when methane quantity was undetectable or <1 % of the total produced (in this case, 35 and 45 days for assays at 35 °C and 15 °C, respectively). Total VFA and pH were measured by titration (Jobling Purser et al., 2014) and a pH meter (691, Metrohm) to determine possible acidification at the end of the BMP assays. The statistical significance of the experimental results was assessed using a one-way ANOVA with a confidence level of 95 %, with *p*-values <0.05 considered significant.

2.1.2. Rural anaerobic digestion unit

The anaerobic digestion unit consisted of a digester coupled with an inlet mixing tank and an 0.5 m^3 outlet digestate container. The digester was a black colour, high-density, and heat-sealed geomembrane bag (density > 0.94 g/cm^3; thickness 1.5 mm; Breaking strength 400 kN/m). Its dimensions were 2.2 m in width, 3 m in length, and 8 m^3 total volume. The operational volume was 5.2 m^3. The digester is semi-buried in the ground with a 1 cm thickness geotextile as protection in the trench. On top of reactor, a pipeline was located as biogas outlet. During bioprocess, biogas passed through pipeline to a H_2S filter, and later it was stored in a 2 m^3 reservoir to be combusted posteriorly in a stove. Before the H_2S filter, a gasometer was installed to quantify biogas production and a safety valve fixed to a maximum biogas pressure of 15 cm water column. The total pipeline length (from the digester to the stove) was around 200 m with 2.5 cm diameter (Fig S1). Additionally, two datalogger sensors were installed to monitor environmental and slurry (internal) temperature.

2.2. Anaerobic co-digestion feeding

The HhD start-up consisted of a three-month discontinuous phase (batch) with a single feed of 1.3 m^3 of fresh manure and 3.9 m^3 of water. The preceding was done to stimulate the growth of microbial communities' content in CM and adapt them to the system conditions (Castro et al., 2017). After batch period, the AD semi-continuous process was carried out in two feedings stages: stage A) mono-digestion of the mixture CM: water (1: 3 v/v) and stage B) ACoD of the mixture CW:CM: water. To reduce hindrances and shocks due to microorganism no adaptation to CW characteristics (as low pH and high acid content), a feeding strategy was proposed during stage B: the water in the mixture was periodically replaced by CW until reaching a blend composition of 70 % CW and 30 % CM on a volume basis (0.54–0.67 g VS_{cw}/g $VS_{total\ loaded}$;0.60–0.72 g COD $_{CW}$/g $COD_{total\ loaded}$). Due to the COVID-19 pandemic, the monitoring of final blend just lasted 20 days. As the total volume fed was fixed, the hydraulic retention time (HRT) was constant (75 days). Substrates used in the HhD feeding have no pretreatment and were attempted to be loaded immediately after its recollection.

2.2.1. AD process evolution during monitoring

The biochemical and microbiological behaviour in the digester inlet and outlet flows were studied to evaluate the progression of AD process during each stage. The biochemical behaviour consisted of the determination of the content of total volatile fatty acids (tVFA) and the individual concentration of VFA (C2-C6) via chromatography using a BP21 GC capillary column (packing material: treated polyethylene glycol) coupled to a flame ionization detector (Raposo et al., 2013). The microbiological behaviour was based on determining the Specific Methanogenic Activity (SMA) in fed and effluent, following the guidelines proposed by Astals et al. (2015). The SMA consisted of the indirect measurement of microorganism's capacity to generate methane from acetate as substrate in 500 mL batch reactors with an inoculum/substrate ratio of 5 (VS basis). The methane produced was measured similarly to BMP assay and expressed as g COD CH_4/g VS d. Once per week, the fed and effluent samples were taken from the digester mixture zone and the outlet pipeline, respectively. Samples were immediately refrigerated and transported in plastic bottles before analysis. Analyses of volatile solids and COD were performed according to the standard methods for examining wastewater (APHA, 2005).

2.2.2. Variation in biogas production and quality

The biogas production rate (BPR) and the specific biogas production (SBP) were determined daily as the relation between biogas produced over the digester volume as an indicator of the technology efficiency, and the biogas produced over organic matter fed as an indicator of the anaerobic digestion process efficiency, respectively (Martí-Herrero et al., 2015). The volume of biogas produced was measured with a gasometer (Fig S1) and normalized to standard conditions (0 °C, 1 atm). The biogas quality (CH_4 and CO_2 content) was determined by gas chromatography at the end of each period.

2.3. Social impact

The ITAC educational community comprises teachers, administrators, students, and parents. 95 % of ITAC students live in rural areas, and agriculture is the only economic activity of their families. The digester was used as a demonstrative pedagogical tool. The social appropriation of knowledge about the implementation and use of the ITAC digester was developed in two steps:

2.3.1. Training of students in anaerobic technology

There are three education levels at ITAC: Kindergarten, elementary and middle school, and high school. Topics related to the anaerobic digestion process as digester, biogas, and digestate uses were implemented in the theoretical-practical subjects “small farmers” and “productive projects” (provided at the kindergarten, elementary school, and middle school, with an intensity of two hours per week). In high school, the subject “ecological agricultural system” was imparted with an intensity of seven hours per week. This subject included installation, management, and maintenance of the biogas plant, uses of biogas, and digestate, among others.

2.3.2. Diagnosis and training on knowledge about digesters addressed to administrators, teachers, and parents

First, the focus group technique was used as a qualitative research alternative to generate information about the beliefs of anaerobic technology. It was formed into five focus groups of ten people each. A representative from each group presented the identified beliefs, and a rapporteur consolidated the information from the five focus groups. Afterward, the Universidad Industrial de Santander (Colombia) organized three theoretical-practical workshops of 2 h each.

3. Results and discussion

3.1. Effect of cheese whey adding on process performance and biogas production

3.1.1. BMP assays as an indicator for AD viability

The BMP results for the mono-digestion and co-digestion process of CW and CM are presented in Table 1. The obtained values indicate that CW mesophilic mono-digestion is feasible and out of inhibition risk: the VFA content was lower than 1500 mg/L, values reported as stable for farm-scale or household biogas plants (Angelidaki et al., 2005). On the other hand, there is inhibition for CW psychrophilic mono-digestion, which can be assured due to the VFA remaining over 4500 mg eq Acetic Acid /L (pH = 5.4 ± 0.5). The addition of cattle manure in psychrophilic conditions allows the system to support the acid charge, to reduce the VFA content (final concentration of 1150 ± 212 mg eq Acetic Acid /L). This behaviour is similar to other studies that mention CW co-digested with cattle manure is more robust, and high concentrations of whey can acidify the medium (Bertin et al., 2013; Jaimes-Estévez et al., 2020). The mesophilic ACoD of CW and CM and a ratio of 70:30 results in 0.6 m^3 CH_4/kg VS, almost double than the 0.32 m^3 CH_4/kg VS reported by Bertin et al. (2013) at a 50:50 ratio.

The methane yield of CM at psychrophilic conditions is 45 % concerning mesophilic ones, 29 % for the CW, and 40 % for the co-digestion CW:CM (70:30). These low values for psychrophilic conditions with respect to mesophilic can be due to: the inocula used for these BMPs came from a digester working at 25 °C, so it is possible that the change to 15 °C without previous acclimatization has affected the methane potential; and the criteria to stop measuring the BMP test three days after the daily methane production is 1 % respect the accumulated methane during the assay, underestimated the methane yield for the

Table 1
BMP, VFA content, and pH for cheese whey and cheese whey co-digested with cattle manure.

Assay	Temperature (°C)	BMP (m^3 CH_4/kg VS)	Final VFA (mg eq Ac Acid/ L)	Final pH
AD CM	15	0.14	200 ± 0.0	7.4 ± 0.2
AD CW		0.16	4750 ± 300	5.8 ± 0.5
ACoD CW: CM		0.24	1150 ± 212	7.36 ± 0.2
AD CM	35	0.32	300 ± 0.0	7.8 ± 0.0
AD CW		0.55	700 ± 70.1	6.8 ± 0.4
ACoD CW: CM		0.6	510 ± 120	7.21 ± 0.5

psychrophilic conditions, which could produce methane in very low rates (lower than 1 % respect to accumulated methane production) for long periods of time.

The co-digestion CW:CM (70:30) at psychrophilic conditions has more synergic results than mesophilic co-digestion. If the synergy of the codigestion is measured as the ratio between the methane produced during the co-digestion with respect to the sum of the methane produced by the mono-digestion of the substrate (VS based), it can be shown that the mesophilic co-digestion has a ratio 1.25, while psychrophilic has 1.56. This result indicates that the codigestion has more positive effects in psychrophilic conditions than mesophilic ones.

This behaviour indicates the presence of viable microbial communities to hydrolyze macromolecules and consume soluble compounds such as VFA to produce methane. Those results show the viability of ACoD in a psychrophilic regimen (Temperatures around 15 °C).

3.1.2. Effect of cheese whey addition on HhD biogas production behaviour

Once the viability of CW:CM ACoD through BMP was studied, the next step was to start the digestion process in a farm-scale 8 m^3 HhD. Forty days after installation and initial load, the HhD was fed daily with 0.07 m^3/d cow manure mixed with water (30 % CM and 70 % water) with 75 d for the HRT (Stage A). Then, stage B began, characterized by the gradual change in the diet of the digester, replacing water with CW, and keeping a daily inflow equal to 0.07 m^3/d, so 75 d for the HRT. The HhD total monitoring period lasted 300 days for stages A and B. Fig. 1 illustrates the net biogas production behaviour accompanied by environmental and slurry temperature fluctuations. The unmeasured area (School break) corresponds to the period of scholar vacations. Accumulated biogas production showed a linear tendency, stabilized through the whole monitored period.

Concerning temperature, the maximum and minimum environmental values were 18.6 and 16.0 °C, respectively, while the slurry temperature remained constant with a mean value of 21.3 °C ± 0.2. These 3.6 °C of temperature gained by the slurry with respect to the mean ambient temperature can be explained by the solar radiation gain of the system due to the black colour of the geomembrane exposed, that acts as a passive solar heating system (Martí-Herrero et al., 2018).

During stage A (mono-digestion of CM), biogas output was 1.2 Nm^3/d ($R^2 = 0.99$). When Stage B began (after 145 d for stage A), replacing water with CW and keeping the CM daily load, it can be seen in Fig. 1 the augmentation in the slope of the cumulative biogas production vs. time. The biogas production is increased according to the increase of CW in the inflow, from 1.68 Nm^3/d for 0.2 g COD_{CW}/g $COD_{total\ loaded}$, to 2.7 Nm^3/d for 0.6 g COD_{CW}/g $COD_{total\ loaded}$. An interesting behaviour is observed in 0.5 g COD_{CW}/g $COD_{total\ loaded}$ area; at the beginning of this zone, biogas production was diminished by irregularities in feeding during the school break. However, the system responded positively, showing an increasing trend in biogas production.

In Table 2, the HhD's operational conditions, parameters measured, and performance characterization are summarized. Aimed to evaluate the bioprocess and digester yields, the BPR and the SBP were determined for stage A, obtaining 0.24 $Nm^3_{biogas}/m^3_{digester}$ d and 0.66 Nm^3_{biogas}/kg VS d, respectively, for an OLR equal to 0.36 kg VS/ $m^3_{digester}$·d. The results found in Stage A are consistent with those reached in other studies. Digesters fed with cattle manure operated at 26 °C reported values around 0.35 m^3_{biogas}/kg VS and 0.37 $m^3_{biogas}/m^3_{digester}$d (Lansing et al., 2008), while others at 16.6 °C stated 0.17 to 0.23 $m^3\ _{biogas}$/kg VS and 0.07 to 0.06 $m^3_{biogas}/m^3_{digester}$d (Martí-Herrero et al., 2014b; Martí-Herrero et al., 2015). In Stage B, the highest yields attained were 0.41 $m^3_{biogas}/m^3_{digester}$·d and 0.72 $m^3\ _{biogas}$/kg VS when OLR was between 0.69 and 0.72 kg VS/ $m^3_{digester}$·d (CW fraction of 0.5 to 0.6 g COD_{CW}/g $COD_{total\ loaded}$). With 58.96 % of CH_4 in the biogas, the digester at 21.3 °C (internal temperature) reached 0.42 m^3 CH_4/kg VS, which is in the range of 0.24 to 0.6 m^3 CH_4/kg VS of The BMP test run at 15 and 35 °C, respectively. Interestingly, keeping HRT fixed to 75 d along the whole monitoring period, despite the organic load increasing from 0.36 to 0.61 kg VS/ $m^3_{digester}$·d, the SBP keeps almost stable in a range of 0.60 to 0.72 $m^3\ _{biogas}$/kg VS.

The 0.42 m^3 CH_4/kg VS obtained from the current farm-scale psychrophilic digester is higher than the 0.34 m^3 CH_4/kg VS at 35 °C obtained by (Comino et al., 2012). The ratio CW:CM in volatile solids was 54:46 for current research, while Comino et al. (2012) was 50:50. The main difference is between OLR and HRT: Comino et al. (2012) operate with an OLR (2.65 kg VS/m^3·d, HRT = 42 d) four-folds higher than current research (0.72 kg VS/m^3·d, HRT 75 d). So, CW proportion, dilution, and higher HRT are the operational parameters that could explain that psychrophilic ACoD has a better SBP to mesophilic one. Respect psychrophilic continuous fed digesters at laboratory scale, Jaimes-Estévez et al. (2020) reported, for a similar range of OLR and 25 °C (4 °C more than current farm-scale digester) with a CW:CM ACoD

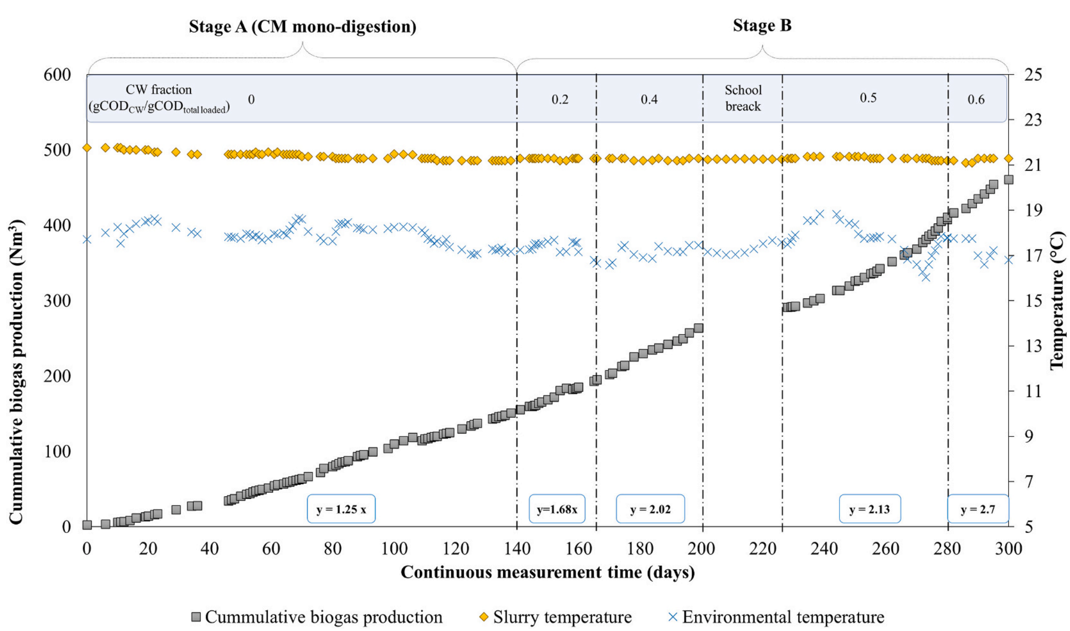

Fig. 1. Variation in biogas production by cheese whey augmentation (grey squares) and slurry (yellow rhombus) and environmental temperature (blue cruxes) throughout measurement time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The HhD's operational conditions and performance characterization.

Operational conditions		
Parameter	Units	Value
Installation	Year	2019
Total volume (operational volume)	m^3	8 (5.2)
Daily mean load	m^3/d	0.07
HRT	Days	75
Mean slurry temperature	°C	21.3 ± 0.2
Mean environmental temperature	°C	17.7 ± 0.5

Performance characterization											
Parameter	Units	Value									
Sample point		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
CW fraction	g CODCW/g COD total loaded	0		0.2		0.4		0.5		0.6	
CW:CM ratio	VS basis	0:100		13:87		31:69		43:57		54:46	
OLR (experimental average)	kg COD/$m^3_{digester}$·d	0.36 ± 0.07		0.45 ± 0.03		0.53 ± 0.12		0.77 ± 0.19		1.34 ± 0.17	
	Kg VS/$m^3_{digester}$.d	0.36 ± 0.03		0.46 ± 0.01		0.56 ± 0.13		0.69 ± 0.10		0.72 ± 0.09	
COD	g COD/L	26.76 ± 3.89	4.29 ± 0.33	28.29 ± 1.70	6.32 ± 0.01	32.27 ± 0.66	13.63 ± 4.58	50.69 ± 2.74	15.76 ± 2.27	91.81 ± 5.95	24.04 ± 0.79
	% Removal	83.59 ± 5.86		79.75 ± 27.05		85.36 ± 29.28		68.84 ± 1.75		72.72 ± 4.39	
pH	–	6.63 ± 0.09	6.30 ± 0.02	4.64 ± 0.02	6.67 ± 0.14	3.74 ± 0.03	6.98 ± 0.02	3.73 ± 0.02	6.74 ± 0.03	4.05 ± 0.04	6.96 ± 0.01
tVFA	mg/L	892.99 ± 22.11	31.50 ± 2.25	1018.65 ± 25.28	74.75 ± 2.37	1295.15 ± 170.28	24.16 ± 4.48	1661.95 ± 211.04	52.00 ± 8.39	2428.26 ± 69.28	44.89 ± 9.81
SMA	g COD CH_4 /g VS·d	0.025 ± 0.01	0.037 ± 0.01	0.018 ± 0.01	0.056 ± 0.002	0.015 ± 0.01	0.069 ± 0.02	0.003 ± 0.001	0.090 ± 0.004	0.006 ± 0.002	0.092 ± 0.006
CH_4 (CO_2 balance)	%	50.55 ± 2.38		56.99 ± 0.50		55.37 ± 0.18		50.50 ± 0.07		58.96 ± 0.05	
Process yield (SBP)	Nm^3_{biogas}/kgVS d	0.67		0.7		0.69		0.6		0.72	
Reactor yield (BPR)	Nm^3 $_{biogas}$/m^3 $_{digester}$ d	0.24		0.32		0.39		0.41		0.52	
Specific methane production (SMP)	Nm^3 CH_4/kg VS	0.34		0.4		0.38		0.3		0.42	

(70:30), biomethane potentials ranged from 0.33 to 0.44 m^3 $_{biogas}$/kgCOD$_{fed,}$ (0.28 to 0.37 m^3 $_{biogas}$/kg VS$_{fed,}$) which is comparable with the present research. Faster digesters reported by Kavacik and Topaloglu (2010) at 24 °C with 5, 10 and 20 days of HRT (12.5, 6.26 and 3.13 kg VS/m^3·d) and ACoD CW:CM with a ratio 66:33, obtained 0.0497, 0.090 and 0.0905 m^3 $_{CH4}$/kg VS respectively. These results from fast digesters permit to reach better biogas production rate (0.62, 0.56, and 0.28 m^3_{CH4}/$m^3_{digester}$·d) but much lower organic matter reduction, related to

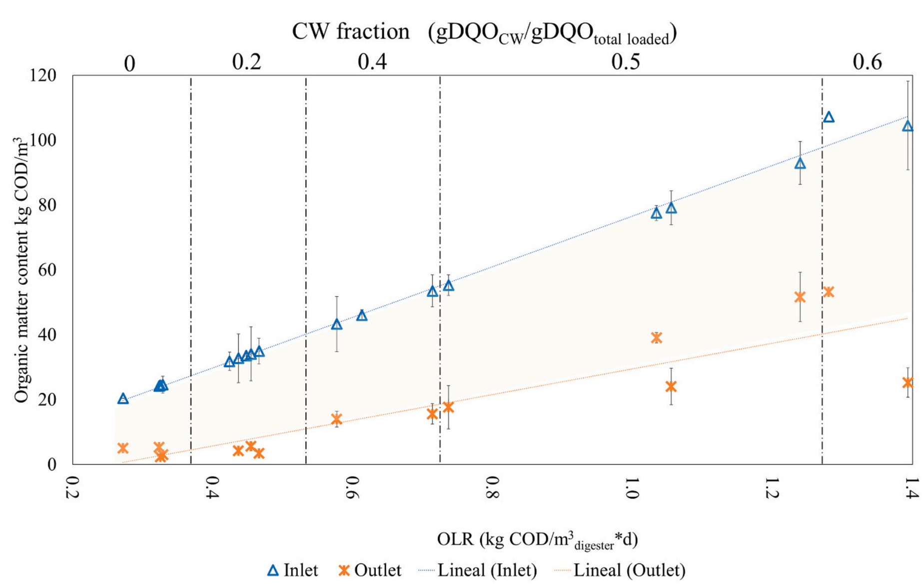

Fig. 2. Organic matter variation by cheese whey augmentation for influent (blue triangles) and outlet (orange cruxes). The shadowed area represents the organic matter removed during the bioprocess. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the low specific methane production.

Regarding biogas use in the ITAC, this fuel has been used for: i) cooking in the school restaurant, ii) to heat water in the sterilization process of the milking materials, and iii) during poultry processing as an agricultural practice of the school (Section 3.2).

3.1.3. Organic matter reduction

Fig. 2 presents the variation in organic matter content as a function of the OLR. During monitoring time, OLR ranged from 0.27 kg COD/$m^3_{digester}$ ·d (minimum value) to 1.39 kg COD/$m^3_{digester}$ ·d (highest value) with an average HRT of 75 d. Those OLR changes are because the water that dilutes CM was replaced progressively by CW. Generally, in the AD process, an increase in OLR causes a decrease in COD removal efficiency (Jaimes-Estévez et al., 2021). Despite the augmentation of organic matter loaded, its removal was not affected significantly. Organic matter removal went from 83.59 ± 5.86 % to 72.72 ± 4.39 %, with an OLR increase of around 450 %. These removal values indicate the possibility of increasing the treated CW volume under psychrophilic conditions. The removal reached in this study is comparable with a mesophilic CM mono-digestion system (fed with CM and rainwater in a 1:3 volumetric ratio; average temperature upper than 25 °C) that reached an organic matter removal near 76 % with an OLR around 1 kg COD/$m^3_{digester}$ d (Castro et al., 2017). Studies treating CW, as presented by Kavacik and Topaloglu (2010) and Comino et al. (2012), reported removals of around 49.4 % and 82 % at 34 °C and 35 °C, respectively. This comparison allows inferring those nutrients contributed by CW do not affect the organic matter removal. So, the CW addition could balance nutritional content, leaving available easy-biodegradable compounds to be treated by microorganisms.

3.1.4. Anaerobic digester stability

The biochemical performance of the AD process was assessed in this study using a complete VFA profile for each stage (Fig. 3). Under the operating conditions of farm-scale biogas plants or HhD, the VFA concentration indicates a healthy process. In that sense, VFA concentrations of <1500 mg/L indicate a low risk of inhibition (Angelidaki et al., 2005). The VFA concentration loaded to the process increased significantly with the CW addition, starting at an average of 892.99 ± 22.12 mg/L (Stage A: 0 g COD_{CW}/g $COD_{total\ loaded}$), reaching 2428 ± 23.88 mg/L for CW fraction of 0.6 g COD_{CW}/g $COD_{total\ loaded}$. In those cases, the most representative acid in the inlet was acetic acid which went from 63 % in stage A to 80.50 % for all stage B.

The above represents a high availability of easily degradable organic matter. Fatty acid levels are important in anaerobic digestion for two reasons: (a) short-chain organic acids are the immediate precursors in the metabolic chain leading to methane formation, where, particularly acetic acid is the ancestor of approximately 70 % of all CH_4 formed during digestion (Poh and Chong, 2009) and (b) a high concentration of acids causes stress in the microbial population, leading to process inhibition. When there is an accumulation of VFA (due to variations in temperature or organic load), there is a decline in methanogenic activity. This activity decrement generates a reduction in methane production and deficits in the consumption of organic matter. Despite the OLR supplied for the stage B (that experimentally increased from 0.36 to 1.34 kg COD/$m^3_{operation}$·d on average), the high concentration of VFA loaded and the reduced local temperature of 17.3 °C (21.3 °C ± 0.2 in the slurry), the HhD did not show inhibitions of metabolic activities. That is justified by the VFA outlet content that remained below 80 mg/L. So, during anaerobic process, the global consumption of VFA was around 96.45 ± 2.25 %. The pH value of the AD system is a significant element for stability because it affects the microbial community structure, metabolic pathways, and enzyme activities, so VFA consumption (Tang et al., 2017). Inlet VFA concentrations represented pH values that decreased from 7.03 to 3.73 by the CW increase. Analyzing outlet pH values, it is possible to deduce that AD system had the capacity to settle it to 6.68 ± 0.52. This result emphasizes there was no risk of inhibition by acidification due to the CW:CM mixture and volume loaded daily. So, it can be understood that the addition of the new substrate improved the capacity of the microbial consortium to transform organic matter, allowing to maintain pH constant throughout the process. The synergism between used substrates can justify this due to the balance in nutrient availability and the alkalinity contributed by CW and CM, respectively. Some authors observed that using co-substrates optimizes operational parameters such as the C/N ratio while enhancing microorganisms syntrophism, giving balance to the AD pathway (Kibler et al., 2018; Chuenchart et al., 2020).

According to the generalized metabolic pathway of the methane formation process, the propionic acid/acetic acid ratio is an indicator of the proper functioning of the bioprocess (Hill et al., 1987). An increase in the P/A ratio may indicate process inhibition: values >1.4 indicate

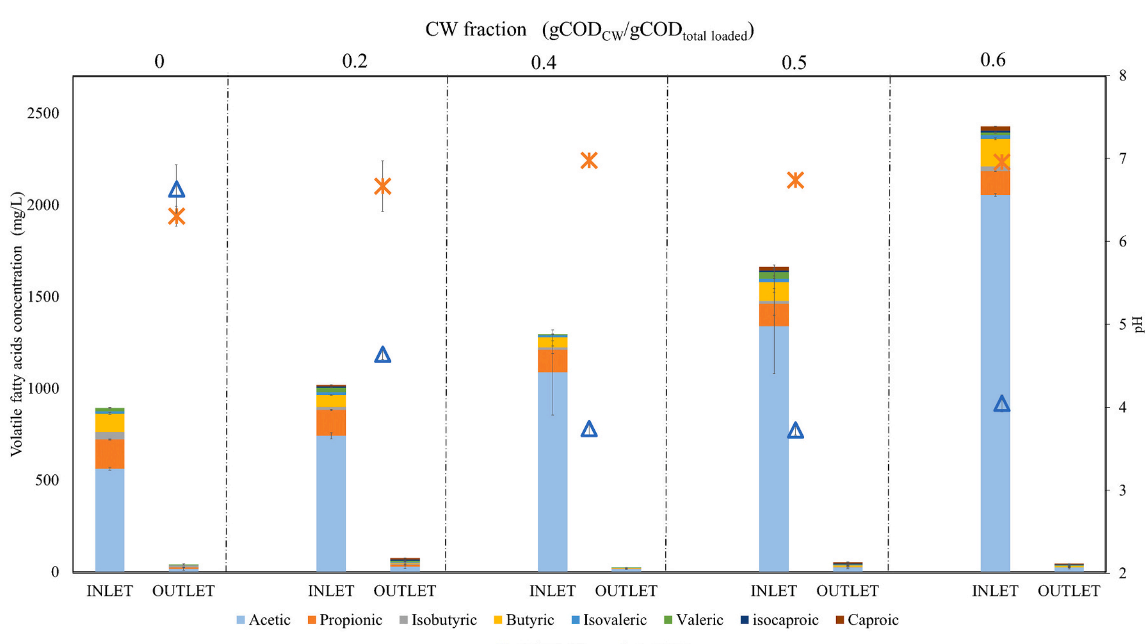

Fig. 3. Individual VFAs (C2–C6) concentration changes in inlet and outlet by cheese whey augmentation and pH behaviour for inlet (triangles) and outlet (cruxes).

imminent digester failure. In that sense, an increase in the acetate level suggests a problem with the methanogenic population, represented in the inefficiency of acetate conversion to CH_4 (Palatsi et al., 2009). For the stages studied, the P/A ratio was below 0.52 (Fig. S2), which indicates that microorganisms could produce methane from available acetic acid. That reinforces the reduction of inhibition risk due to VFA, showing that the population could adapt to the consumption of organic matter with a high content of VFA. Those results are similar to P/A ratio in a tubular digester treating swine manure under psychrophilic conditions where 0.11 mg/mg was reached (Jaimes-Estévez et al., 2021).

3.1.5. Biomethane potential assays contrasted to real scale digester

The BMP assays allowed the determination of the viability of ACoD implementation. However, the results obtained on a real scale were higher. In this study, methane production values as a function of organic matter were higher for the farm-scale continuous process (SMP = 0.42 m^3 CH_4/kg VS·d) than for the batch assay (SMP = 0.24 m^3 CH_4/kg VS·d). Those differences can be explained in three inferences:

i) *Higher treatment time.* The BMP assay time at 15 °C was 45 days, while for the farm-scale semi-continuous process HRT was 75 days A longer treatment time for the fed substrates increases organic matter consumption, which carries higher biogas generation values. The daily biogas production at psychrophilic conditions is produced at very low rates after the first 30 or 40 d of retention time, inferior to the 1 % of the accumulated biogas produced. So, when psychrophilic BMP tests are stopped (when daily biogas is lower than 1 % of the accrued biogas), 30 or 40 % of potential biogas are still generated. In farm-scale psychrophilic digesters, as HRT is higher than in the BMP test and does not depend on "1 %"criteria, although the low rate of biogas production, more accumulated biogas is produced per kg VS.

ii) *Controlled temperature.* Temperature is a variable that directly and significantly affects the bioprocess. In this study, the average temperature value for the batch tests was controlled (average temperature 15 ± 2 °C). For its part, the mean ambient temperature during the monitored period of the farm-scale HhD was 17.7 °C, but through the passive solar heating, the slurry temperature of the farm-scale digester was 21.3 °C. The lower temperature for BMP affects the speed of chemical and biological reactions, causing them to occur more slowly (Lettinga et al., 2001), reducing methane production.

iii) *Inoculum adaptation.* According to Feller (2017), only microorganisms adapted to psychrophilic conditions can cope with the limitations that occur with temperatures below 20 °C. For the BMP test, the inoculum used was one from a digester operated at a mean temperature of 25 ± 5 °C. On the other hand, the HhD start-up consisted of adapting the inoculum for at least 90 days under temperatures around 17 °C. This longer adaptation time could improve the inoculum characteristics to support lower temperatures. Even with the differences in results between BMP assay and HhD monitoring, the BMP test could indicate the feasibility of the anaerobic digestion process of one or more substrates. Otherwise, the variations obtained show the necessity to establish a methodology for psychrophilic BMP test that can be representative of full scale psychrophilic digesters.

3.1.6. Specific methanogenic activity dynamics

The SMA is an indicator of the ability of microbial biomass to transform a specific substrate such as acetate into methane (Astals et al., 2015). Fig. 4 shows the SMA behaviour at the input and output during operation in stages A and B. During CM mono-digestion (CW fraction equal to 0), inlet SMA is higher than in all ACoD cases studied. The feed of fresh manure can renovate microbial communities present in the system; therefore, the reduction in CM fed diminishes the SMA that could be contributed. Outlet SMA values are significatively above average for stabilized dairy manure (around 0.04 g COD/g VS d; (Quintero et al., 2012). In all cases, the outlet had at least 47 % higher methanogenic activity of the inlet, suggesting that the microbial consortia had adapted successfully to the anaerobic codigestión process with a reduced microorganisms contribution by CM (with a maximum ORL around 1.24 kg COD/$m^3_{digester}$ ·d). The key reasons for the stability of digestion were traditionally thought to be the nutrient balance and relatively high buffering capacity contributed by CM (Xing et al., 2020). The additional nutrients given by the CW used as the main substrate and the synergism achieved during the treatment may explain these improvements in methanogenic activity. When microbial activity is compared to catalytic activity in a chemical process, these results are profitable. In that sense, it is possible to mention that SMA could be an easy assay to monitor the digester performance and stability.

3.2. Community experiences through anaerobic digestion

In kindergarten and elementary, and middle school, the theoretical-practical subjects allowed the student to identify and understand the

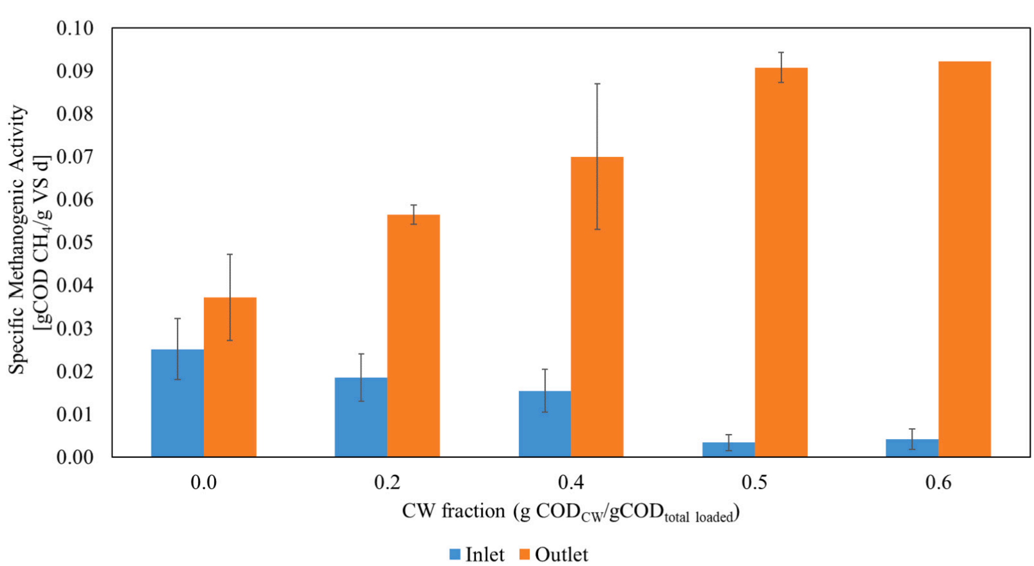

Fig. 4. Specific methanogenic activities as a function of CW fraction.

components of the digester and its relationship with agricultural activities. One hundred forty-five students have been trained in basic knowledge of anaerobic technology. In the subject ecological agricultural systems (high school level), the students had the opportunity to feed the digester and use the biogas to heat water and clean the milking equipment. Further, the digestate was applied to the ITAC crops. Besides, students at this level contributed to the training of partners at lower levels through collaborative learning. At this level, fifty-five students have been trained in digesters installation, management, and maintenance. Moreover, biogas was used for cooking the food consumed by the students in the school restaurant and for poultry processing. This experience led the students to verify the usefulness of biogas as renewable energy.

Before the digester installation, the ITAC used a 100 lbs./month propane cylinder (45.36 kg/month) and 600 kg/month of dry wood (two bundles of 15 kg, five days per week). The generated energy was employed for cooking proposes (for 40 people per day), heating water, cleaning milking devices, and heating for chicks. After introducing the digester, which produces 2.7 Nm^3/d of biogas (59 % CH_4) per day during its last stage, the wood was completely replaced, and the propane consumption was reduced to 0.67 propane cylinders per month (33.75 kg/month). So, biogas has avoided consuming 11.61 kg/month of fossil propane and 600 kg/month of dry wood. An energetic analysis shows that before the digester, the primary energy composition for cooking and heating was 14,124.51 MJ/month. In comparison, after the digester, it is 3110.59 MJ/month (energy considerations based on the ITAC's consumption of traditional fuel are shown y Table S1). That implies that the heating efficiency of dry wood combustion had been very low, so biogas offers a better energetic performance reducing the exposure to harmful gases produced by wood and the environmental impact that this entails.

3.2.1. Diagnosis and training on digesters for administrators, teachers, and parents

From the analysis of the preconceived beliefs of the focus groups, it is inferred that knowledge of anaerobic technology generates positive and negative beliefs in the educational community (the beliefs identified -knowledge/preconception- in the focus groups, which are related to anaerobic technology, from the implementation of the digester in ITAC, are shown in Table S2). Those beliefs were statements, thoughts, or ideas that they assumed to be true about the functioning of the digester. However, most of these assumptions lack scientific foundations representing a barrier to the acceptance and appropriation of anaerobic technology. For example, waste treatment or nutrient recycling and the fertilizer potential of digestate are not mentioned in focus groups; neither the reduction of using fossil fuels or wood for cooking to avoid greenhouse emissions and deforestation. The lack of information on previous experiences with digesters in the community generates preconceived and unconfirmed information that represents a barrier to the diffusion and implementation of the technology, in concordance with Astals et al. (2015). The availability of digesters in educational institutions focused on agricultural training allows the integration of AD in the academic content. Also, counting with an operational bioreactor allows to carry out workshops on digesters with the rest of the neighbors. In the rural context, “seeing is believing,” so demonstrative digesters overcome barriers to installation and AD acceptation (Martí-Herrero et al., 2014b). As an example, based on the workshops carried out, a family accepted and appropriated anaerobic technology by installing a digester on their farm.

4. Conclusions

The synergism in an appropriate CW:CM blend provides stability to the system and improves yields with a strong increase in the process SMA. This work highlights the importance of investigating new waste farm-scale treatment in rural areas, and the necessity to establish a clear procedure to determine the viability of BMP under psychrophilic conditions as the link between laboratory assays and real-scale monitoring. This first monitoring experience opens up new possibilities for rural users to handle acids subproducts as CW, promoting renewable energy production and waste treatment, improving the economic condition and environmental sustainability in areas with no favorable temperature.

CRedit authorship contribution statement

Jaimes-Estévez, J.: Conceptualization, methodology, investigation, visualization, writing - review & editing. **Vera E.**: social analysis – writing. **Jaramillo, J**.: Formal analysis. **Rodríguez, P**.: Formal analysis. **Martí-Herrero, J.**: Conceptualization, writing - review & editing. **Escalante H.**: Resources, Conceptualization, Writing – review, supervision & Editing. **Castro L.**: Funding acquisition, conceptualization, writing & editing - original draft.

Funding sources

This research was funded by Newton Fund Institutional Links (Project code: 527677146: Integrated anaerobic digestion and gasification systems for sustainable farming in Colombia), the Ministerio de Ciencia Tecnología e Innovación (Minciencias-Colombia), Programa de Becas de Excelencia Doctoral del Bicentenario Cohorte I (project: *Mejoramiento de la Biometanización Psicrofílica de Lactosuero mediante el uso de Biochar como soporte orgánico*), and the Universidad Industrial de Santander, Colombia (Project: *Desarrollo de un plan de transferencia tecnológico para valorizar residuos pecuarios del Instituto Técnico de Cáchira mediante digestión anaeróbica*).

Declaration of competing interest

J. Jaimes-Estevez reports financial support was provided by Colombia Ministry of Science Technology and Innovation. Liliana Castro reports financial support was provided by Newton Fund. Liliana Castro reports financial support was provided by Industrial University of Santander.

Data availability

Data will be made available on request.

Acknowledgments

Authors are grateful to the Instituto Técnico Agrícola de Cáchira and the Universidad Industrial de Santander for the development of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2022.101168.

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