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Original Article

Sustainable biogas production via anaerobic co-digestion of cheese whey and cattle manure

Producción sostenible de biogás mediante co-digestión anaerobia de lactosuero y estiércol bovino

Marco Arturo Velasco Morales[1], José Apolonio Venegas-Venegas[2*], Mariela Beatriz Reyes Sosa[2], Raúl Hernández-Altemirano[3*], Violeta Y. Mena-Cervantes[3], Rene Pinto Ruíz[1], Pascual López de Paz[4], Carlos Alonso Meza Avendaño[4], Fidel Alejandro Aguilar-Aguilar[3*]

[1] Doctorado en Ciencias Agropecuarias y Sustentabilidad-Facultad de Ciencias Agronómicas, Universidad Autónoma de Chiapas (UNACH), Carretera Ocozocoautla Villaflores, Chiapas #230, CP 30470., México.

[2] Consejo Nacional de Ciencia y Tecnología- Universidad Autónoma de Chiapas, Carretera Ocozocoautla Villaflores, Chiapas # 230, CP 30470., México.

[3] Centro Mexicano para la Producción más Limpia y Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de Biocombustibles (LaNDACBio) del Instituto Politécnico Nacional, Av. Acueducto s/n, Col. La Laguna Ticomán, 07340, Ciudad de México, México.

[4] Instituto de Investigación e Innovación en Energías Renovables, Universidad de Ciencias y Artes de Chiapas. Libramiento norte poniente 1150 Lajas Maciel, 29039 Tuxtla Gutiérrez, Chiapas, México.

ABSTRACT

In Mexico, specifically in Chiapas, the dairy cattle industry plays a significant role in cheese and milk production. However, the large quantities of cattle manure (CM) and cheese whey (CW) generated as byproducts pose environmental challenges if not managed properly. To address this issue, anaerobic digestion (AD) technology offers a sustainable solution for organic waste treatment, and biogas production. This research study focuses on assessing the potential of CW and CM, both individually and in co-digestion, in an anaerobic environment, as a potential treatment for such wastes. The study also evaluated biogas yield and composition using an up-flow anaerobic sludge blanket (UASB) reactor with different CW and CM mixtures. The findings indicate that the 30CM:70CW ratio exhibited the highest methane yield, surpassing other assays in co-digestion and mono-digestion. Furthermore, the UASB reactor showed that a 90CW:10CM mixture produced 25.73 L of biogas per gram of volatile solids (VS) daily, comprising 60 % methane (CH_4) and 40 % carbon dioxide (CO_2). This research demonstrates the potential for efficient and environmentally friendly treatment of CM and CW through optimized co-digestion and UASB technology, highlighting the opportunity to generate biogas while reducing waste.

Keywords: Biogas, anaerobic digestion, biochemical methane potential, substrates, methane yield

RESUMEN

En México, específicamente en el estado de Chiapas, la industria del ganado lechero juega un papel importante en la producción de queso y leche. Sin embargo, las grandes cantidades de estiércol de ganado (EB) y Lactosuero (LS) generados como subproductos generan desafíos ambientales si no se gestionan adecuadamente. Para abordar este problema, la tecnología de digestión anaerobia (DA) ofrece una solución sostenible mediante el tratamiento de residuos orgánicos y la producción de biogás. Este estudio se centra en evaluar el potencial de EB y LS, tanto individualmente como en co-digestión, en un entorno anaerobio, para el manejo de tales residuos. El estudio también evalúa el rendimiento y la composición del biogás utilizando un reactor anaerobio de flujo ascendente (RAFA) con diferentes mezclas EB y LS. Los resultados indican que la relación 30EB:70LS exhibió el mayor rendimiento de metano (CH_4), superando a otros ensayos realizados tanto en codigestión como en monodigestión. Además, el reactor RAFA mostró que una mezcla de 90EB:10LS produjo 25,73 L de biogás por gramo de sólidos volátiles por día, con una composición de 60 % de metano (CH_4) y 40 % de dióxido de carbono (CO_2). Esta investigación demuestra el potencial para el tratamiento eficiente y respetuoso con el medio ambiente de EB y LS a través de la codigestión optimizada y la tecnología RAFA, destacando la oportunidad de generar biogás mientras se reducen los desechos.

Palabras clave: Biogás, digestión anaeróbica, potencial bioquímico de metano, sustratos, rendimiento de metano.

INTRODUCTION

Anaerobic digestion (AD) is a biotechnological process involving a series of metabolic reactions in the absence of oxygen. It effectively converts organic matter, including food waste, industrial and sewage effluents, and animal organic waste (Molino *et al*., 2013; Piñas *et al*., 2016; Mainardis *et al*., 2017). AD is a highly effective technology for renewable energy (biogas) generation, while simultaneously reducing greenhouse gas emissions (GHGs) according to previous studies (Mainardis *et al*., 2017, Ohimail and Izah, 2017). Biogas is predominantly composed of CH_4 ranging from 45 % to 70 %, CO_2 ranging from 30 % to 45 %, and impurities (Molino *et al*., 2013; Venegas *et al*., 2017). Before implementing large-scale biodigesters for controlled biogas production, it is crucial to assess the CH_4 yield and biodegradability of substrates at the

*Author for correspondence: José Apolonio Venegas-Venegas; Raúl Hernández- Altamirano; Fidel Alejandro Aguilar-Aguilar
e-mail: falexaguilar@gmail.com; polo_tex1@hotmail.com; rhaltamirano@gmail.com
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laboratory scale (Labatut *et al*., 2011; Da Silva *et al*., 2018), emphasizing that laboratory-scale AD tests conducted in batch reactors provide valuable insights into substrate biodegradability. Biochemical Methane Potential (BMP) tests are specifically designed to determine the methane yield and biodegradability of individual substrates or substrate mixtures. Furthermore, anaerobic co-digestion has emerged as an alternative for the utilization of multiple organic wastes. In a previous study, it was reported that combining two or more substrates in co-digestion satisfies the nutritional requirements of the microbial community and enhances stability during the organic matter decomposition process, compared to conventional AD (Náthia-Neves *et al*., 2018).

CW represents a significant organic by-product in the agro-industry, which has been reported to originates from cheese production and contain a high concentration of organic matter, constituting 55 % of the total nutrients found in milk (Mazorra-Manzano and Moreno-Hernández, 2019). Specifically, CW is composed of approximately 96 % lactose (ranging from 46 g L^{-1} to 52 g L^{-1}), 25 % protein (ranging from 6 g L^{-1} to 10 g L^{-1}), and 8 % lipids (5 g L^{-1}). Small and medium-sized companies in the dairy industry face challenges in valorizing CW. It has been reported that in Mexico, 30 % of CW is primarily utilized as animal feed or for the production of ricotta cheese or cottage cheese (Sebastián-Nicolás *et al*., 2020). Cheese production is considered a vital economic activity in La Frailesca, Chiapas, with an estimated daily CW production of 106 m^3. However, in the region, approximately 56 % of CW is discarded, leading to its improper disposal in rivers, streams, lagoons, or directly into sewers (Esnoval *et al*., 2017). According to official data from SIAP (Sistema de Información Agroalimentaria y Pesquera), the Frailesca region produced 72.44 million L of milk in 2021. Approximately 60 % of milk production in Chiapas is used for cheese production, indicating that 43.46 million liters were dedicated to this purpose (Esnoval *et al*., 2017). It is stated that for every kg of cheese produced, 8 to 9 L of CW are generated (Mazorra-Manzano and Moreno-Hernández, 2019). It is estimated that 39.12 million L of CW were produced, of which 19.56 million L were discarded without any treatment. Furthermore, cattle production plays a crucial role in the global supply of meat and milk, and has experienced an exponential growth in recent years. It is projected that cattle consumption will reach 76 Mt (megatonnes) over the next decade, contributing to 16 % of the total increase in meat consumption compared to the base period of 2020 (OECD, 2023).

According to SIAP (2021), cattle production in Chiapas reached 2,627,827 heads in 2021, with a predominance of 12.5 % in the Frailesca region. However, this livestock activity generates significant amounts of CM. It has been estimated that an adult cow excretes 15 kg of manure per day, resulting in a daily manure total of 4,927,175 t. Unfortunately, there is no treatment in place for CM, leading to infections, water contamination, and environmental pollution in surrounding areas (Vera-Romero *et al*., 2017). CM consists of 45 % readily biodegradable organic matter, including carbohydrates, proteins, and lipids, which can be utilized for biogas production. However, CW has a high fibrous material content (> 30 %), making it difficult to biodegrade resulting in low biogas production potential (Fagbohungbe *et al*., 2019). Neshat *et al.* (2017) proposed the use of complementary substrates, rich in readily biodegradable components in combination with CM, to compensate for the carbohydrate deficiency, enhance anaerobic process stability, improve treatment efficiency, and increase biogas yield. When it comes to the implementation of anaerobic digestion (AD), there is a wide range of reactor options available, such as the up-flow anaerobic sludge blanket (UASB) reactor. UASB reactors offer advantages such as the ability to handle higher organic loads, achieve greater organic matter reduction, and generate less sludge compared to conventional reactors (Hublin *et al*., 2014; Neshat *et al*., 2017; Magdalena *et al*., 2020). The use of biofilms within UASB-type reactors has gained considerable attention as they facilitate the attachment of bacterial consortia, increase organic matter consumption, and improve biogas production (Chatterjee *et al*., 2018).

This study aimed to evaluate the potential of CW and CM for bioenergy production through anaerobic co-digestion. Specifically, the research aimed to determine the maximum amount of biogas and CH_4 that can be obtained from various CW and CM mixtures, and assess the performance of the anaerobic digestion process. The study also highlighted the advantages of anaerobic co-digestion in enhancing biogas production and its potential role in promoting sustainable energy solutions. This study hypothesized that the anaerobic co-digestion of CW and CM results in a higher yield of biogas and methane, compared to the mono-digestion of each substrate individually. Furthermore, it was expected that biomethane derived from this process exhibits qualities that make it a competitive alternative to conventional fuels, with the additional benefit of being a more durable and reliable energy source.

MATERIALS AND METHODS

Organic residues and inoculum

The CW and CM residues used in this study were sourced from "La Quesería", a dairy processing unit located in the Frailesca region, in the municipality of Villaflores, Chiapas, at 16º 35′ 45″ North Latitude and 93º 31′ 49″ West longitude. The CW was obtained from the by-product of quesillo manufacturing, while the CM was collected from adult cows in the milking area. Both residues were stored in an industrial freezer at -15 °C at the Facultad de Ciencias Agronómicas plant physiology laboratory, Universidad Autónoma de Chiapas.

The inoculum used in the BMP assays and the UASB reactor in this study, was obtained from a functioning anaerobic tubular biodigester. Before the start of the experiment, the inoculum, which contains the bacteria responsible for methane production, was stored in a hermetically sealed anaerobic reactor, ensuring the absence of oxygen, to maintain the activity of the microorganisms responsible for the degradation of organic matter. Additionally, a daily feed of

2 g of reactive-grade glucose ($C_6H_{12}O_6$) was provided to keep the inoculum active until its use in the assays and the UASB reactor.

Batch Reactor Set-up

The main objective of the BMP assays, was to determine the maximum amount of biogas that could be obtained through anaerobic co-digestion while optimizing its performance. For these assays, two key variables were used: the concentration of CM (X1) and the concentration of CW (X2) (Table 1). An experimental design based on mixtures was implemented, resulting in a total of eight assays. Assays 1 to 5 focused on anaerobic co-digestion, where the concentrations of CM and CW were varied. Subsequently, assays 6 and 7 evaluated the anaerobic digestion of each substrate individually (Table 1). Finally, assay number 8 was established as a control, using only inoculum. All experimental assays were conducted in triplicate, to reduce experimental errors and increase the reliability of the obtained results. Therefore, in this study, a total of 24 experiments were performed to evaluate the BMP of the waste mixtures (CM/CW). This rigorous approach to replication ensured the robustness of the collected data and the precision of the conclusions derived from this study.

The experimental assays were conducted using 100 mL glass bottles equipped with rubber stoppers and aluminum seals to maintain optimal anaerobic conditions. The selection of these bottles was based on their ability to maintain the desired experimental conditions, and allow for the adaptation of an outlet port for the quantification and collection of biogas. In each of the 24 reactors, an inoculum of 45.69 mL, equivalent to 5 g of VS, was added along with the respective substrates in the proportions described in Table 1. Additionally, a solution of micro and macro nutrients were added to each reactor to ensure the availability of essential nutrients for the microbial consortia. The macronutrients added included NH_4Cl (1112.0 mg L^{-1}), $(NH_4)H_2PO_4$ (132.5 mg L^{-1}), $(NH_4)_2HPO_4$ (44.50 mg L^{-1}), $MgCl_2·6H_2O$ (250.00 mg L^{-1}), $CaCl_2·2H_2O$ (189.00 mg L^{-1}), and $NaHCO_3$ (2500.00 mg L^{-1}. The micronutrients included $FeCl_3·6H_2O$ (5.00 mg L^{-1}), $ZnCl_2$ (0.13 mg L^{-1}), $MnCl_2·4H_2O$ (1.25 mg L^{-1}), $(NH_4)6Mo_7O_{24}·4H_2O$ (1.60 mg L^{-1}), $AlCl_3·6H_2O$ (0.13 mg L^{-1}), $CoCl_2·6H_2O$ (5.00 mg L^{-1}), $NiCl_2·6H_2O$ (13.00 mg L^{-1}), H_3BO_3 (3.00 mg L^{-1}), $CuCl_2·2H_2O$ (8.00 mg L^{-1}), and HCl (1.00 mg L^{-1}) (Aguilar-Aguilar *et al*., 2027). To establish a completely anaerobic environment, the batch reactors for each experimental assay were purged of any excess air, as methanogenic bacteria thrive without oxygen. This was achieved by using a 60 mL syringe to remove air from the headspace of the reactors. Subsequently, the reactors were maintained at a constant temperature of 30 ± 3 °C using a Memmert brand oven, model D91126, for an incubation period of 50 d or until biogas production ceased. Before the commencement of the assays, the initial pH of the reactors was adjusted to 7.5 using a 2 N Na_2CO_3 buffer solution. This step ensured the maintenance of an optimal pH range between 6.5 and 7.5 for the anaerobic digestion process.

Biogas Measurement

The gas volume gauging system was adapted from the work of Aguilar-Aguilar *et al*. (2017) and was composed of a 500 mL inverted glass vial containing a 3 mol L^{-1} NaOH solution, whose function was to capture CO_2. The vial was adapted with a cover with an opening for the entrance of the gas produced in the anaerobic reactor, and another opening for the exit of the liquid displaced by the gas (Figure 1). Measurements were performed every 24 h after the first day of incubation by the liquid displacement method, in which the liquid displaced by the gas (Figure 1) was collected in a graduated cylinder, and the volume was then converted to the standard biogas volume (NmL) under normal conditions of pressure and temperature (1 atm, 0 °C), according to the Ideal Gas Law.

Table 1. Experimental design for the evaluation of the biochemical methane potential of cattle manure (CM) and cheese whey (CW).
Tabla 1. Diseño de experimento para la evaluación del potencial bioquímico de metano del estiércol de ganado EB y lactosuero LS.

Assays	X1: CM	X2: CW	Nomenclature	Raw matter (mL)	*VS (initial)
	(%)	(%)			(g L^{-1})
1	80	20	CoAD 80CM:20CW	6.47	11.50±0.17
2	70	30	CoAD 70CM:30CW	7.55	11.50±0.62
3	50	50	CoAD 50CM:50CW	9.72	10.87±0.14
4	30	70	CoAD 30CM:70CW	11.88	12.60±0.65
5	20	80	CoAD 20CM:80CW	12.96	14.30±0.20
6	100	0	MoAD CM	4.30	10.70±0.28
7	0	100	MoAD CW	15.13	14.40±0.00
8	-	-	MoAD Inoculum	45.69	5.63±0.15

VS=Volatile solids.
VS= Solidos volátiles.

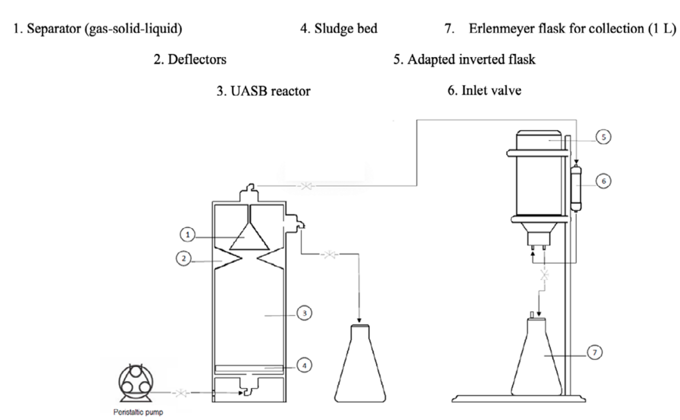

Figure 1. Diagram of biogas production and quantification in an anaerobic UASB reactor.
Figura 1. Diagrama de producción y cuantificación de biogás en un reactor anaerobio UASB.

Start-up of Up-flow Anaerobic Sludge Blanket

The reactor used in the study was a custom-designed five-liter capacity unit. It was built using 6" PVC pipe and end caps made of the same material (PVC). The reactor included 1" plastic B3 flanges at the inlet and outlet ports, as well as 1/2" stopcocks and 1/2" elbows for fluid control. Flexible silicone hose, 8 mm x 100 latex rubber hose, 3-way valves, and 4 mm silicone hose were also incorporated into the design (Figure 1). To facilitate the anaerobic digestion process, ceramic cylinders were added to create a fixed sludge bed within the reactor. These cylinders provided a surface area for the attachment and concentration of microbial biomass. Feeding of the reactor was carried out discontinuously using a Cole-Parmer masterflex® L/S® peristaltic pump. The feeding schedule involved three times per week, specifically on Mondays, Wednesdays, and Fridays. This feeding regimen was applied to each mixture in the co-digestion and mono-digestion assays involving the CW. Throughout the evaluation period, which lasted five months, the ambient temperature surrounding the reactor was monitored. This temperature recording was performed using a Fluke 28 II digital multimeter, providing valuable information regarding the temperature conditions during the experiment. Figure 1 describes the production, quantification, and characterization of the biogas produced from the experimental tests with the CM and CW mixture.

To optimize the CW concentration in the anaerobic co-digestion process, the results from the BMP tests were taken into consideration. Based on these results, the CW concentration was adjusted and evaluated in a UASB reactor as shown in Figure 1. To ensure an anaerobic condition, the reactor was fed using a masterflex® L/S® Cole-Parmer peristaltic pump. The inlet flow rate was adjusted according to the Hydraulic Retention Time (HRT), which was calculated to maintain the stability of the AD process throughout the evaluation period of the treatments. The treatments were evaluated for 15 days in each phase. Feedings were carried out every Monday, Wednesday, and Friday by adding 100 mL of the respective treatment according to Table 2. At the start-up of the reactor, 450 mL of inoculum were added using a peristaltic pump for 10 days to allow for adaptation. After the adaptation phase, the evaluation of biogas production commenced by adding only undiluted CW for a 15 days feeding period in the first phase**.** In the second phase (Table 2), the 95CM:05CW treatment was added while maintaining the volume of 100 mL of a substrate. In the third phase, the 90CM:10CW treatment was added with the same volume, and in the fourth phase, the 85CM:15CW treatment was added with 100 mL of substrate, as indicated in Table 2.

Table 2. Physicochemical characterization and dilutions of the treatments evaluated in the UASB reactor.
Tabla 2. Caracterización fisicoquímica y diluciones de los tratamientos evaluados en el reactor UASB.

Treatment	Cheese whey (%)	Cattle manure (%)	COD (g L^{-1})	pH	Nomenclature
1	100	-	59.0±0.70	3.8	MoAD CW
2	95	5	59.9±0.84	3.9	CoAD CW95:05CM
3	90	10	63.2±1.76	3.9	CoAD CW90:10CM
4	85	15	65.0±1.41	4	CoAD CW85:15CM

COD= Chemical Oxygen Demand.
DQD= Demanda química de oxígeno.

Analytical Methods

Before conducting the experimental assays, the CW, CM, and inoculum were subjected to physicochemical characterization. The following parameters were determined, and all measurements were performed in triplicate to ensure greater reliability:

Total Solids and Volatile Solids

Total Solids (TS) and Volatile Solids (VS) are critical parameters for assessing the organic matter available for anaerobic digestion. The determination of TS and VS was performed according to the Mexican Official Standard NMX-AA-034-SCFI-2015. Approximately 10 g of a representative sample were weighed and placed in a pre-weighed, heat-resistant crucible. The sample was then dried at 105 °C in a drying oven until a constant weight was achieved, ensuring all the water content had evaporated. After drying, the crucible was removed from the oven and allowed to cool in a desiccator to prevent moisture absorption from the air. The weight of the dried sample and crucible were recorded, and the TS calculated using the formula:

$$TS\left(\frac{mg}{g}\right)=\frac{\text{Final weight of the crucible with residue }(g)-\text{initial weight of the empty crucible}(g)}{\text{Sample }(g)}x\ 1000$$

Following the TS determination, the dried sample was combusted in a muffle furnace at 550 °C for 2 hours. After combustion, the crucible was removed from the furnace and allowed to cool in a desiccator. The weight of the ash remaining in the crucible was recorded. The VS were then calculated using the formula:

$$VS\left(\frac{mg}{g}\right)=\frac{\text{Crucible weight with TS }(g)-\text{Crucible weight after calcination}(g)}{\text{Sample }(g)}x\ 1000$$

pH

The pH of the samples is an essential parameter for maintaining optimal conditions for AD. The pH measurement was conducted using an OAKTON® WD-35619-series potentiometer, following the Mexican Official Standard NMX-AA-008-SCFI-2016. The potentiometer was calibrated with standard buffer solutions at pH 4.0, 7.0 and 10. The electrode was then immersed in the sample, and the pH value was recorded once the reading stabilized.

Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) indicates the amount of organic matter present in the samples. The COD was determined by closed reflux with a Spectroquant® Prove 100 spectrophotometer, using the photometric method 5000-90000 mg L^{-1} Spectroquant® to DIN ISO 15705. A known volume of the sample was added to a digestion vial containing a premeasured amount of potassium dichromate in sulfuric acid. The vial was sealed and heated in a reactor for 2 h to allow complete oxidation of the organic matter. After cooling, the absorbance was measured at 600 nm using the spectrophotometer, and the COD value was calculated based on the calibration curve prepared with standard solutions.

Biogas Characterization

The biogas generated during the experimental assays was stored in 1.5 L medical-grade plastic enteral feeding bags, equipped with stop valves (three-way). To quantify the captured biogas, the liquid displacement method was employed. An inverted flask, adapted with valves and filled with distilled water, was used to displace the biogas. The displaced volume was then measured using a 100 mL graduated cylinder (Aguilar-Aguilar *et al*., 2017). The Multitec® 540 equipment has proven to be an indispensable tool in our research, as it is used to measure and analyze the composition of gases in biological processes, detecting up to five different gases, including methane CH_4, CO_2, carbon monoxide CO, hydrogen sulfide H_2S, and oxygen O_2 (Figure 2). It is worth noting that the presence of CH_4 in a range of 50 to 70 % in the samples indicates the presence of methanogenic bacteria in the inoculum used in this study. During the measurement of biogas, the Multitec® 540 equipment played a pivotal role in allowing us to accurately quantify the concentration of these gases in each of the experimental assays, as illustrated in Figure 2.

From Figure 3, it is evident how the biogas generated in each assay was stored in the bag identified as (4), and subsequently, we used the Multitec® 540 equipment to conduct a detailed characterization of its composition (Figure 3). This device provided essential data that proved crucial in understanding and evaluating CH_4 production in our experiments, significantly contributing to the results and conclusions of our study.

Figure 2. Multitec® 540 equipment used for the characterization of biogas from each of the experimental trials with the mixture of cattle manure and cheese whey.
Figura 2. Equipo Multitec® 540 utilizado para la caracterización del biogás de cada uno de los ensayos experimentales con la mezcla de estiércol de ganado y suero de queso.

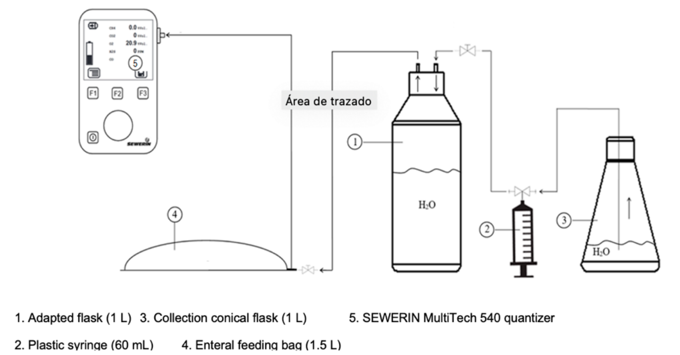

Figure 3. Diagram of the biogas characterization analysis.
Figura 3. Diagrama de análisis de caracterización del biogás.

Statistical analysis

To assess the CH_4 production potential, this study implemented a mixed experimental design, focusing on the added proportions of two specific wastes: cattle manure (X1) and cheese whey (X2). The mixing of these components was carried out according to a mixed experimental design (Table 1). This design aimed to achieve as homogeneous a mixture as possible to maintain consistency across experimental batches. The proportions of X1 and X2 were determined using a predefined matrix that allowed for an exhaustive analysis of the design space, covering a broad range of possible combinations.

Statistical analysis was used to interpret the data obtained. This included significance tests to determine if the differences in CH_4 production were the result of the variations in the proportions of the wastes and not due to random variation. In addition to biogas production, the homogeneity of the mixture was evaluated in each trial. This was done through physical and chemical tests to ensure that the waste mixture was consistent and uniform throughout all experiments.

The biogas production results obtained from the anaerobic digestion were analyzed using StatSoft software. An analysis of variance (ANOVA) was conducted to determine if there were significant differences between the experimental trials in co-digestion and mono-digestion. To further evaluate the differences, a Tukey test was performed at a significance level of 5 %. The purpose of this analysis was to identify the best BMP assay that demonstrated superior biogas production, which could then be selected for further evaluation in the UASB reactor at a laboratory scale. By comparing the results and conducting statistical tests, the study aimed to determine the most promising assay for subsequent testing and implementation.

RESULTS AND DISCUSSION

Characterization of cattle manure and cheese whey (CW)

The results indicate that the CM had a TS content of 425 g L^{-1}, but a low biodegradability (32.7 %) as determined by the VS to TS ratio. On the other hand, the CW had a TS content of 55.9 g L^{-1} and maintained a higher VS/TS ratio of 82.5 % (Table 3). These findings align with a study by Fagbohungbe *et al.* (2019) which also highlighted the low biodegradability and biogas production potential of CM, emphasizing the need for a readily biodegradable co-substrate like CW to enhance biogas production. The pH values of CM and CW were measured to be 6.8 and 3.9, respectively. These pH values can be complementary to stabilize the overall pH AD digestion or CoAD processes. According to Appels *et al.* (2008) maintaining a pH between 6.5 and 7.5 is crucial for process stability and optimal biogas production. Additionally, the COD values for CM and CW were determined to be 89 g L^{-1} and 59 g L^{-1}, respectively (Table 3). These results are in line with expectations, as CM contains a higher concentration of organic matter compared to CW, which has a more liquid composition with a lower organic matter content. Overall, the physicochemical characterization of CW, CM, and inoculum provided valuable insights into their biodegradability, pH, and organic matter content, which are crucial parameters for AD and CoAD processes.

From Table 4, it is evident that the final VS concentrations of the anaerobic CoAD trials, specifically the CoAD 50CM:50CW, CoAD 30CM:70CW, and CoAD 20CM:80CW, were 8.80 g L^{-1}, 9.63 g L^{-1}, and 9.47 g L^{-1}, respectively (Table 4). These values indicate that these trials had lower VS concentrations compared to the other assays, and lower than the initial VS concentrations at the beginning of the process. Furthermore, the pH values at the beginning of the anaerobic digestion were recorded as 7.5, which falls within the recommended range (6.5 to 7.5) for an adequate AD treatment. Some reactors maintained their pH within this suggested range until the end of the process (Table 4). These findings suggest that the anaerobic co-digestion trials with different proportions of CM and CW (CoAD 50CM:50CW, CoAD 30CM:70CW, and CoAD 20CM:80CW) exhibited effective biodegradation of volatile solids and maintained appropriate pH levels throughout the digestion process. According to the results, it was observed that the CoAD 20CM:80CW treatment did not remain stable throughout the anaerobic co-digestion. This instability may be attributed to an inhibition process or the spontaneous consumption of carbohydrates, leaving behind only the fibrous material in the reactors.

Table 3. Physicochemical characterization of CW and CM organic waste and inoculum.
Tabla 3. Caracterización fisicoquímica de los residuos orgánicos e inóculo de LS y EB.

Parameters	Cattle manure	Cheese whey	Inoculum
pH	6.8±0.0	3.9±0.0	6.9±0.0
TS (g L^{-1})	425±1.0	55.9±0.13	11.56±0.14
VS (g L^{-1})	139±0.28	46.2±0.15	7.66±0.15
VS/TS (%)	32.7	82.75	66.26
COD (g L^{-1})	89±1.41	59±0.70	14.8±0.84

TS=Total solids, VS=Volatile solids, COD= Chemical Oxygen Demand.
ST=Sólidos totales, SV=Sólidos volátiles, DQO= Demanda química de oxígeno.

Kinetics of biogas production

Figure 3 displays the methane production curves for each of the treatments, showing the three characteristic periods described by Guerrero-Toledo *et al.* (2020), lag phase, exponential phase, and stationary phase. The co-digestion treatments demonstrated the best methane production yields, with the highest observed in the 70CM:30CW treatment (223.54 mL CH_4 g^{-1} VS) and the 50CM:50CW treatment (215.82 mL CH_4 g^{-1} VS) (Figure 3). These treatments exhibited stable kinetics throughout the anaerobic digestion process. The results indicate that the co-digestion treatments resulted in higher biogas yields compared to the mono-digestion treatments. This suggests that the combination of CM and CW in different proportions improved the biodegradability and methane production efficiency of the substrates.

The research conducted by Álvarez *et al.* (2010) indicates that anaerobic co-digestion can lead to a significant increase in biogas production compared to mono-digestion, with potential improvements of up to 200 %. The specific increase in biogas production may vary depending on the operational conditions and the characteristics of the substrates used. In the current study, the 30CM:70CW co-digestion treatment generated 130 % more biogas compared to the CM mono-digestion treatment (Figure 3). This highlights the effectiveness of co-digestion and the high biodegradability of CW. The rapid and higher biogas production observed in the first five days of the co-digestion treatment, indicates the potential of CW as a substrate for methane production. However, it is important to note that successful and efficient degradation requires appropriate control and management of the co-digestion process. The results of this study demonstrate that all co-digestion treatments outperformed the mono-digestion treatment, confirming the potential of co-digestion for enhancing biogas production. The high biodegradability of CW makes it a promising waste substrate for achieving methane production in a shorter timeframe, provided that proper control and management strategies are implemented.

Biogas production

The yields of accumulated biogas production and biogas regarding volatile solids (VS) for each treatment are presented in Table 5. These values were subjected to a mean comparison test using Analysis of Variance (ANOVA) with a significance level of 5 % ($p < 0.05$). These indicate a significant difference between the anaerobic mono-digestion and co-digestion of the CW/CM mixture. Furthermore, when comparing the CoAD 50CM:50CW and CoAD 30CM:70CW treatments, no significant differences in biogas production were observed. The biogas production values for these treatments were 215.82 mL CH_4 g^{-1} VS and 223.54 mL CH_4 g^{-1} VS, respectively,

Table 4. Initial and final values of total and volatile solids for batch tests in co-digestion (CoAD) and mono-digestion (MoDA).
Tabla 4. Valores iniciales y finales de sólidos totales y volátiles para pruebas discontinuas en codigestión (CoDA) y monodigestión (MoDA).

Assays	$pH_{Initial}$	$TS_{Initial}$ ($g\ L^{-1}$)	TS_{Final} ($g\ L^{-1}$)	$VS_{Initial}$ ($g\ L^{-1}$)	VS_{Final} ($g\ L^{-1}$)
CoAD 80CM:20CW	7.5	18.80 ± 0.36	18.60 ± 3.29	11.50 ± 0.17	10.60 ± 2.02
CoAD 70CM:30CW	7.5	18.20 ± 0.88	18.57 ± 1.70	11.50 ± 0.62	10.23 ± 1.05
CoAD 50CM:50CW	7.5	16.07 ± 1.50	15.63 ± 2.75	10.87 ± 0.14	8.80 ± 1.83
CoAD 30CM:70CW	7.5	17.57 ± 0.51	16.10 ± 2.77	12.60 ± 0.65	9.63 ± 2.14
CoAD 20CM:80CW	7.5	19.27 ± 0.41	14.83 ± 1.25	14.30 ± 0.20	9.47 ± 1.05
MoAD Cattle manure	7.5	17.55 ± 0.35	19.90 ± 0.65	10.70 ± 0.28	11.20 ± 0.26
MoAD Cheese whey	7.5	17.97 ± 0.11	17.03 ± 2.26	14.40 ± 0.00	10.17 ± 1.51
MoAD Inoculum	7.5	7.30 ± 0.20	8.17 ± 0.05	5.16 ± 0.11	5.63 ± 0.15

TS=Total solids, VS=Volatile solids.
ST=Sólidos totales, SV=Sólidos volátiles.

Table 5. Comparison of CH_4 production in the different essyas with different CW and CM mixtures in anaerobic digestion (AD) and anaerobic co-digestion (CoAD).
Tabla 5. Comparación de la produccion de CH_4 en los diferentes ensayos con diferentes mezclas de LS y EB en digestion anaerobia (DA) y co-digestion anaerobia (CoDA).

Assays	Cumulative biogas (mL)	Biogas (mL ton^{-1} VS)
CoDA 80EB:20L	138.62 ± 21.73	^{bc}172.20 ± 27.0
CoDA 70EB:30L	129.49 ± 11.61	b160.86 ± 14.42
CoDA 50EB:50L	164.20 ± 4.70	c215.82 ± 6.18
CoDA 30EB:70L	197.16 ± 12.55	^{a}223.54 ± 14.23
CoDA 20EB:80L	52.65 ± 3.88	^{d}52.60 ± 3.87
MoDA EB	72.80 ± 3.06	^{d}97.19 ± 4.09
MoDA LS	66.52 ± 6.38	^{d}65.99 ± 6.33

Different letters (a, b, c, d, and e) show significant differences between treatments (*p-value*> 0.05).
Letras diferentes (a, b, c, d, y e) muestran diferencias significativas entre tratamientos (*valor-p* > 0.05).

which are very close in value (Ergüder *et al*., 2001). This suggests that these two treatments resulted in similar levels of methane production efficiency. The ANOVA test allowed for a statistical comparison of the different treatments, revealing the significant differences in biogas production between mono-digestion and co-digestion, as well as the similarities between certain co-digestion treatments in terms of CH_4 yield.

CH_4 production varies significantly among the different mixtures studied, particularly highlighting the differences between those with high proportions of CM (CoDA 30CM:70CW) and those with high contents of CW (CoDA 20CM:80CW), as well as the more balanced mixtures (CoDA 50CM:50CW) (Figure 4 and Table 5). Interestingly, the monoculture mixtures of CM (MoDA CM) and CW (MoDA CW) did not show significant differences between them, but they did in comparison with most of the other mixtures, especially those with a higher proportion of CM. Notably, the CoDA 30CM:70CW mixture stands out significantly from all others, indicating that this specific proportion is the most effective for CH_4 production (Figure 4 and Table 5). This suggests a notable synergistic effect in biogas production when specific proportions of CM and CW are used. On the other hand, the mixture with the lowest CH_4 production was CoDA 20CM:80CW, underscoring how specific variations in mixture composition can significantly influence biogas production performance.

It is important to note that no significant differences were found between some specific mixtures, such as CoDA 70CM:30CW and CoDA 80CM:20CW (Figure 4 and Table 5). This finding suggests that minor adjustments in the proportion of components do not necessarily result in significant changes in CH_4 production, which could indicate a threshold in the benefit of adjusting the proportions of the substrates used.

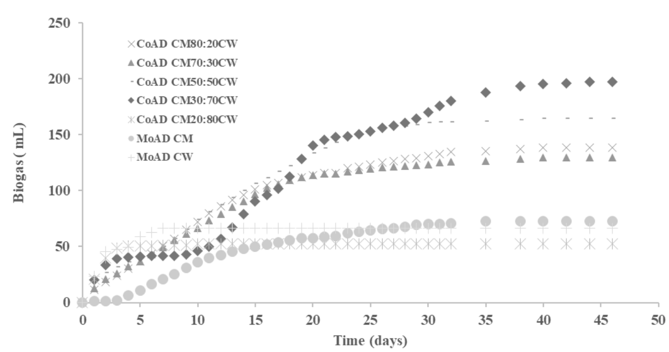

Figure 4. Kinetics biogas yield accumulated in the different treatments in mono-digestion (MoDA) and co-digestion (CoDA) in batch tests.
Figura 4. Cinética del rendimiento de biogás acumulado en los diferentes tratamientos en monodigestión (MoDA) y codigestión (CoDA) en pruebas discontinuas.

The visualization of these differences through the confidence interval graph, provides an intuitive graphical representation of the statistical significance between groups, with non-overlapping lines indicating significant differences. This graphical analysis highlights how the specific composition of the mixtures critically influences biogas production, offering valuable insights for optimizing biogas production. The CoDA 30CM:70CW mixture, in particular, emerges as the most promising for efficient biogas production, suggesting that the precise balance between CM and CW can play a key role in maximizing biogas production.

These findings have significant implications for the design and operation of biogas production processes, suggesting that careful selection of substrate proportions can substantially improve production efficiency. This detailed analysis and interpretation of the results provide a solid foundation for future research in the field of biogas production, guiding towards more effective strategies for the management of organic waste and the production of renewable energy.

Energy recovery from biogas and methane

The BMP assays yielded an average of 62.1 % CH_4, establishing a basis for estimating the energy generated in each trial shown in Table 6. According to the study by Castellanos-Sánchez *et al*. (2023) biogas with a 60 % CH_4 content can produce between 18 to 22 MJ per cubic meter, while pure CH_4(100 %) reaches between 30.67 to 36.68 MJ per cubic meter. Based on these averages, the energy generated in each trial was estimated.

As shown in Table 6, the CoAD 30CM:70CW mixture generated the highest amount of energy per ton of dry matter, both in biogas and CH_4, with 4,671.24 MJ/ton and 4,674.71 MJ/ton, respectively. This result underscores the potential of the combination of CW and CM for energy production through AD. Additionally, the biogas from the CoAD 50CM:50CW mixture and the methane from CoAD 80CM:20CW (4,509.92 MJ/ton and 4,513.27 MJ/ton respectively) showed superior energy per ton compared to conventional sources. Despite being lower than the 32,000 MJ/m^3 of gasoline, these results position biogas and methane competitively with natural gas and far exceed the energy content of wood. Despite the similarities in energy yield between biogas and CH_4 purified biomethane can be utilized as a high-quality fuel capable of competing with conventional fuels. Purified biomethane has the added advantage of not corroding pipes and machinery, making it a more durable and reliable option for energy applications (Noor *et al*., 2013).

These findings highlight the efficiency of biogas and CH_4 as sustainable alternatives to fossil fuels and traditional biomass. The adoption of technologies based on these renewable sources can transform the energy landscape by reducing dependence on non-renewable fuels, minimizing greenhouse gas emissions, and promoting a green energy economy. The large-scale implementation of these energy solutions opens a promising path toward a cleaner and renewable future, demonstrating the viability and potential of these sources to significantly contribute to energy sustainability (Lönnqvist *et al*., 2018).

Substrate Evaluation in a UASB reactor

The results of the biogas production assays in the UASB reactor showed that the highest yields were obtained when using a higher concentration of CW and a lower concentration of CM. This combination improved the carbon-to-nitrogen ratio, nutrient supply, and synergy between the substrates, resulting in enhanced biogas production. After the reactor start-up, where only inoculum and nutrients were added to allow microorganisms to establish a biofilm on the reactor surfaces, the addition of 100 mL of CW (MoAD CW) initiated biogas production. The CoAD CW95:05CM treatment achieved an average daily production of 687 mL of biogas, with a total production of 10,300 mL, and 50.77 % CH_4 content in the biogas (see Figure 5). For the CoAD CW90:10CM treatment, the average daily production was 1,240.78 mL of biogas, with a total production of 19,099 mL and 50.25 % methane content. The CoAD CW85:15CM treatment generated an average daily production of 1,597 mL of biogas, with a total production of 23,116 mL. It is noteworthy that as the concentration

Table 6. Energy potential of biogas and CH_4 production from the anaerobic digestion and co-digestion of CM with CW.
Tabla 6. Potencial energético de la producción de biogás y CH_4 proveniente de la digestión y codigestion anaerobia del EB con LS.

CM (%)	CW(%)	Assays	Biogas ($m^3\ ton^{-1}VS$)	Methane ($m^3\ ton^{-1}VS$)	Biogas (MJ/ton)	Methane (MJ/ton)
80	20	CoAD 80CM:20CW	172.20	106.94	3598.40	3601.08
70	30	CoAD 70CM:30CW	160.86	99.89	3361.44	3363.93
50	50	CoAD 50CM:50CW	215.82	134.02	4509.92	4513.27
30	70	CoAD 30CM:70CW	223.54	138.82	4671.24	4674.71
20	80	CoAD 20CM:80CW	52.60	32.66	1099.16	1099.98
100	0	MoAD CM	97.19	60.35	2030.95	2032.45
0	100	MoAD CW	65.99	40.98	1378.97	1379.99

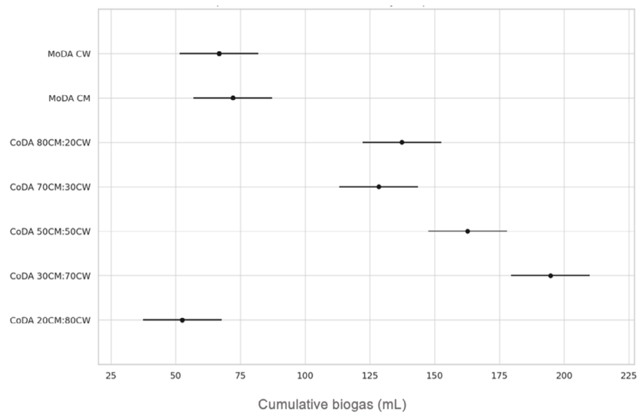

Figure 4 . Comparison of means using the Tukey test to produce biogas from the different mixtures of CM and CW in AD and CoAD.
Figura 4 . Comparación de medias mediante la prueba de Tukey para la produccion de biogás de las diferentes mezclas entre el CM y CW en DA y CoDA.

of CW decreases below 90 %, biogas production increases (Figure 5). The pH of the system remained stable within the range of 6.5 - 7.2, which is favorable for anaerobic digestion. However, once a 50 % CW concentration is reached, the accumulated biogas production starts to decrease, possibly due to the increase in fibrous material contributed by the CM. The use of a biofilm with porous material in the reactor had a positive effect on biogas production, since by keeping the microbial consortium attached to the biofilm, the waste mixture could be more efficiently and rapidly consumed, leading to improved biogas production. These findings suggest that the combination of a higher concentration of CW and a lower concentration of CM, along with the use of a biofilm, can optimize biogas production in anaerobic digestion processes.

The kinetics of biogas production varied among the different assays, with notable differences in the amount of biogas generated. The CoAD CW90:10CM assay exhibited the highest biogas production, reaching 2,964 mL in 24 h on the sixth day of the evaluation. In comparison, the CW85:15CM assay produced 2,604 mL of biogas, and the CoAD CW90:05CM mixture produced 1,036 mL (Figure 5). The use of a biofilm with a porous material had a positive effect on biogas production, as it facilitated the attachment and activity of the microbial consortium. This led to greater and faster consumption of the waste mixture, resulting in increased biogas production. The pH inside the reactor is an important indicator of process stability in anaerobic digestion. A pH range of 6.5 to 7.5 is considered favorable for maintaining biogas production, while values outside this range can indicate an inhibition process (Appels *et al*., 2011). In this study, the pH fluctuated in all treatments, as shown in Figure 6, but remained within the favorable range. This indicates that there was stability in the anaerobic digestion process inside the reactor, despite the variation in the concentration of CW and CM. Although the pH of the mixtures was initially acidic, ranging from 3.8 to 4.0, which could potentially lead to medium acidification, the observed biogas production process remained stable (Figure 6). This suggests that the microbial consortium adapted and maintained its activity, resulting in sustained biogas production throughout the evaluation period.

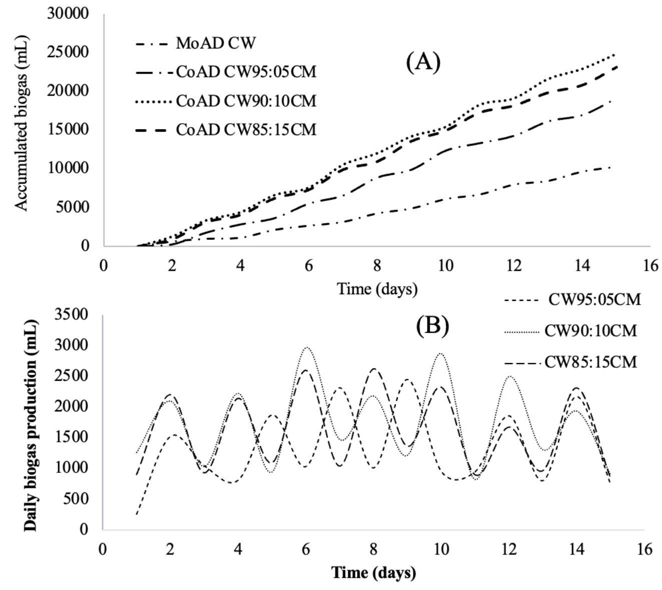

Figure 6. Accumulated (A) and daily biogas (B) production from CM and CW mixtures in CoAD within the UASB reactor.
Figura 6. Producción acumulada (A) y diaria (B) de biogás de mezclas de CM y LG en CoDA dentro del reactor UASB.

Our results show that both, co-digestion and anaerobic mono-digestion, of CW led to biogas production within the first 24 h of operation in the UASB reactor under mesophilic conditions (28 °C). In all treatment trials, a CH_4 concentration greater than 50 % was obtained, which is consistent with the findings of Neshat *et al.* (2017) who also reported methane concentrations of approximately 50 %. Specifically, the CoAD CW90:10CM mixture had an average CH_4 concentration of 62.25 %, indicating a high CH_4 content in the biogas produced (Figure 7). The CoAD CW85:15CM mixture exhibited a slightly lower methane concentration (56.43 %), and the

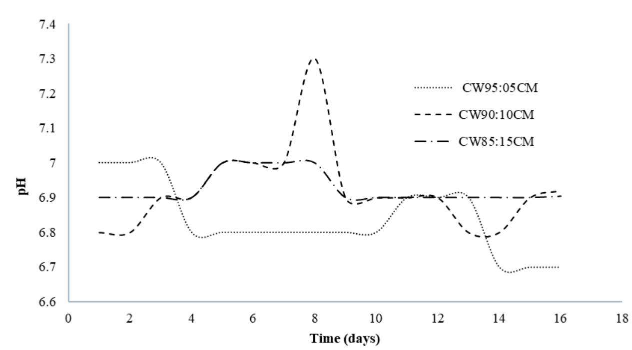

Figure 7. Monitoring of the pH value in anaerobic co-digestion treatments in the UASB reactor.
Figura 7. Monitorización del valor de pH en los tratamientos en codigestión anaerobia en el ractor UASB.

CoAD CW95:05CM mixture had a methane concentration of 54.55 %. It is important to note that the CoAD CW85:15CM treatment has a higher CM concentration and a lower CW concentration. Therefore, based on these results, it is considered that the use of a mixture consisting of 90 % CW and 10 % CM in an up-flow reactor is an appropriate configuration for achieving optimal CH_4 production.

CONCLUSION AND PERSPECTIVES

The study confirms that co-digesting CW with CM significantly boosts biogas yield, especially with a dominant CW proportion. Employing a UASB reactor and porous materials for biofilm support emerges as a potent method for this process, resulting in robust biogas production. High CW mixtures excel in both biogas yield and methane richness, presenting a promising avenue for energy conversion into heat or electricity. The practicality and scalability of this approach suggest its potential for extensive adoption, promoting the sustainable management of agricultural waste streams. Furthermore, the digestate, a nutrient-rich byproduct, could be repurposed as a biofertilizer, with future research needed to devise safe and efficient application strategies that consider nutrient balance, environmental impact, and compliance with regulations.

Future research can focus on optimizing the design and operation of UASB reactors for enhanced biogas production, including the integration of advanced materials and technologies to improve microbial activity and substrate utilization. Scaling up the process to pilot-scale trials will be crucial to assess the feasibility and efficiency of large-scale biogas production from CW and CM, addressing practical challenges and refining the process parameters. Exploring the integration of biogas production with other renewable energy systems, such as solar or wind power, can result in hybrid energy solutions that maximize resource utilization and energy output. Comprehensive economic and environmental assessments will be essential to evaluate the cost-effectiveness, sustainability, and overall impact of implementing anaerobic co-digestion of CW and CM on a larger scale. Engaging with policymakers to develop supportive regulations and incentives for the adoption of biogas technologies, can drive the transition toward a green energy economy, ensuring that the benefits of this sustainable energy source are realized. These perspectives highlight the potential advancements and areas of focus that can further enhance the viability and impact of biogas production from CW and CM, contributing to a sustainable and renewable energy future.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aguilar-Aguilar, F.A., Nelson, D.L., Pantoja, L. de A. and Santos, A. 2017. Study of anaerobic co-digestion of crude glycerol and swine manure for the production of biogas. Rev Virtual Quim, (9): 2383-2403.

Álvarez, J., Otero, L. and Lema, J. 2010. A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresour. Technol, (101): 1153-1158.

Appels, L., Baeyens, J., Degrève, J. and Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci, (34): 755-781.

Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Willems, K., Van Impe, J. and Dewil, R. 2011. Anaerobic digestion in global bio-energy production: potential and research challenges. Renew. Sustain. Energy Rev, (15): 4295-4301.

Castellanos-Sánchez, J.E., Aguilar-Aguilar, F.A., Hernández-Altamirano, R., Venegas Venegas, J.A. and Raj Aryal, D. 2023. Biogas purification processes: review and prospects. Biofuels, pp.1-13.

Chatterjee, P., Ghangrekar, M. and Rao, S. 2018. Sludge granulation in an UASB–moving bed biofilm hybrid reactor for efficient organic matter removal and nitrogen removal in biofilm reactor. Environ. Technol, (39): 298-307.

Da Silva, C., Astals, S., Peces, M., Campos, J. and Guerrero, L. 2018. Biochemical methane potential (BMP) tests: Reducing test time by early parameter estimation. Waste Manag, (71): 19-24.

Ergüder, T., Tezel, U., Güven, E. and Demirer, G. 2001. Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors. Waste Manag, (21): 643-650.

Esnoval, C.O.V., Ruiz, R.P., Hernández, R.R., de la Torre, J.C. and de Jesús, A.G. 2017. Uso, produc-ción y calidad nutricional del lactosuero en la región central de Chiapas. Av. En Investig. Agropecu, (21): 65-77.

Fagbohungbe, M.O., Onyeri, C.A. and Semple, K.T. 2019. Co-fermentation of whey permeates and cattle slurry using a partitioned up-flow anaerobic digestion tank. Energy, (185): 567-572.

Guerrero-Toledo, F. de M., Espinosa-Solares, T., Balagurusamy, N., Guerra-Ramírez, D., Huber, D. and Hernández-Eugenio, G. 2019. Biochemical methane potential of chicken litter added with propionate in mesophilic conditions. Sci. Agropecu, (10): 307-311.

Hublin, A., Schneider, D.R. and Džodan, J. 2014. Utilization of biogas produced by anaerobic digestion of agro-industrial waste: energy, economic and environmental effects. Waste Manag. Res, (32): 626-633.

Labatut, R.A., Angenent, L.T. and Scott, N.R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol, (102): 2255-2264.

Magdalena, J.A., Greses, S. and González-Fernández, C. 2020. Anaerobic degradation of protein-rich biomass in an UASB reactor: organic loading rate effect on product output and microbial communities dynamics. J. Environ. Manage, (274) : 111201.

Mainardis, M., Cabbai, V., Zannier, G., Visintini, D. and Goi, D. 2017. Characterization and BMP tests of liquid substrates for high-rate anaerobic digestion. Chem. Biochem. Eng. Q. 31, 509-518.

Mazorra-Manzano, M.Á. and Moreno-Hernández, J.M. 2019. Properties and options for the valorization of whey from the artisanal cheese industry. CienciaUAT 14, 133-144.

Molino, A., Nanna, F., Ding, Y., Bikson, B. and Braccio, G. 2013. Biomethane production by anaerobic digestion of organic waste. Fuel 103, 1003-1009.

Náthia-Neves, G., Berni, M., Dragone, G., Mussatto, S. and Forster-Carneiro, T. 2018. Anaerobic digestion process: technological aspects and recent developments. Int. J. Environ. Sci. Technol. 15, 2033-2046.

Neshat, S.A., Mohammadi, M., Najafpour, G.D. and Lahijani, P. 2017. Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. Renew. Sustain. Energy Rev. 79, 308-322.

Noor, M.M., Wandel, A.P. and Yusaf, T. 2013, January. The development of MILD combustion open burner experimental setup. In Proceedings of the 2nd International Conference of Mechanical Engineering Research (ICMER 2013). University of Southern Queensland.

Lönnqvist, T., Sandberg, T., Birbuet, J.C., Olsson, J., Espinosa, C., Thorin, E., Grönkvist, S. and Gómez, M.F. 2018. Large-scale biogas generation in Bolivia stepwise reconfiguration. Journal of cleaner production, 180, pp.494-504.

OECD, Food, Nations, A.O. of the U. 2021. OCDE-FAO Perspectivas Agrícolas 2021-2030. https://doi.org/10.1787/47a9fa44-es

Ohimain, E.I. and Izah, S.C. 2017. A review of biogas production from palm oil mill effluents using different configurations of bioreactors. Renew. Sustain. Energy Rev. 70, 242-253.

Piñas, J.A.V., Venturini, O.J., Lora, E.E.S., Oliveira, M.A. de and Roalcaba, O.D.C. 2016. Aterros sanitários para geração de energia elétrica a partir da produção de biogás no Brasil: comparação dos modelos LandGEM (EPA) e Biogás (Cetesb). Rev. Bras. Estud. Popul. 33, 175-188.

Sebastián-Nicolás, J.L., González-Olivares, L.G., Vázquez-Rodríguez, G.A., Lucho-Constatino, C.A., Castañeda-Ovando, A. and Cruz-Guerrero, A.E. 2020. Valorization of whey using a biorefinery. Biofuels Bioprod. Biorefining 14, 1010-1027.

Servicio de Información Agroalimentaria y Pesquera (SIAP). 2021. Población de Ganado de carne y leche 2012-2021. [Consultado: Febrero 20, 2023] Disponible en: http:/ www.gob.mx/siap/documentos/poblacion-ganadera-136762.

Venegas Venegas, J.A., Espejel García, A., Pérez Fernández, A., Castellanos Suárez, J.A. and Sedano Castro, G. 2017. Potencial de energía eléctrica y factibilidad financiera para biodigestores motogenerador en granjas porcinas de Puebla. Rev. Mex. Cienc. Agríc, (8): 735-740.

Vera-Romero, I., Estrada-Jaramillo, M., González-Vera, C., Tejeda-Jiménez, M., López-Andrade, X. and Ortiz-Soriano, A. 2017. Biogás como una fuente alternativa de energía primaria para el Estado de Jalisco, México. Ing. Investig. Tecnol, 18, 307-320.