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Use of mezcal vinasses to produce methane by co-digestion with bovine manure

Uso de vinazas de mezcal para producir metano por co-digestión con estiércol de bovino

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RESUMEN

Se investigó la mejor proporción de co-digestión entre vinazas de mezcal y estiércol de bovino para producir metano. Se compararon cinco mezclas de vinazas con estiércol en diferentes proporciones (v/v): 25-75, 50-50, 75-25, 100-0 y 0-100, con seis repeticiones. Como reactores se utilizaron envases de vidrio de 496 mL con sello hermético, con 300 mL de volumen activo en condiciones mesofílicas ($36 \pm 1^\circ\text{C}$) durante 10 días. El pH, los sólidos totales y los sólidos volátiles de vinazas, estiércol de bovino y sus mezclas se determinaron por triplicado. El volumen acumulado de metano fue mayor con 0-100 y 75-25, seguido por el 100-0; con 286.23, 286.40 y 225.48 NmL de CH_4 , respectivamente. El rendimiento de metano fue mayor con 100-0 y 75-25 con 28.27 y 22.77 NmL de $\text{CH}_4 \text{ g}^{-1}$ sólidos volátiles, respectivamente. El modelo de crecimiento bacteriano de Gompertz mostró que el período de adaptación de los microorganismos en las vinazas fue mayor que en el estiércol de bovino. La co-digestión de vinazas y estiércol de bovino en una proporción de 75-25 % mejoró la producción de metano 26.7 % con respecto a la digestión de vinazas solas y redujo el tiempo de adaptación de las bacterias a éstas en 4.12 días.

Palabras clave: agave, residuos agroindustriales, biogás, biometano.

ABSTRACT

The best co-digestion ratio between mezcal vinasses and bovine manure was investigated to produce methane. Five vinasses and bovine manure mixtures in different proportions were compared (v/v): 25-75, 50-50, 75-25, 100-0, and 0-100, with six replications. As reactors, 496 mL glass containers with hermetic seal were used, with 300 mL of active volume under mesophilic conditions ($36 \pm 1^\circ\text{C}$) for 10 days. The pH, total solids, and volatile solids of vinasses, bovine manure, and their mixtures were determined in triplicate. The methane accumulated volume was greater with the 0-100 and 75-25, followed by the 100-0, with 286.23, 286.40, and 225.48 NmL CH_4 , respectively. Methane yield was higher with 100-0 and 75-25 with 28.27 and 22.77 NmL $\text{CH}_4 \text{ g}^{-1}$ volatile solids, respectively. The Gompertz bacterial growth model showed that the micro-organisms adaptation period in vinasses was longer than in bovine manure. Co-digestion of vinasses and bovine manure in proportion 75-25 % improved the methane production by 26.7 % with respect to the

digestion of vinasses alone, and reduced the adaptation time of bacteria to vinasses by 4.12 days.

Key words: agave, agro-industrial waste, biogas, biomethane.

INTRODUCTION

In Mexico, the growing of *Agave* crops such as *A. angustifolia* Haw. and *A. tequilana* Weber to produce mezcal and tequila, respectively, has socioeconomic and environmental relevance. The state of Oaxaca produces 92.3 % of mezcal in Mexico; in 2018, the agave-mezcal production chain generated 19,000 direct jobs and 85,000 indirect jobs, making it the greatest economic activity in the state (CRM, 2019). However, the by-products that this agro-industry generates, bagasse and vinasses, have a strong environmental impact when discharged without treatment. For every liter of mezcal produced in a factory with intermediate technology, 9 to 15 L of vinasses are generated (Beltrán *et al.*, 2001; Jiménez *et al.*, 2006). In 2018, the Mexican states that manufactured spirits from *Agave* species with a protected designation of origin, produced 5,914,200 L of mezcal (CRM, 2019), generating at the same time between 53,227,800 and 88,713,000 L of vinasses. It is estimated that each year 80 % of vinasses is discharged directly into rivers, lakes, and water reserves or municipal drainage systems, as well as agricultural land, without treatment (Rodríguez and De La Cerna, 2017). Thus, in Mexico, annually between 42,582,240 and 70,979,400 L of vinasses are spilled into the environment with harmful effects due to its recalcitrance, resistance to biological or chemical degradation, and corrosive and contaminating properties (Cervantes-Carrillo, 2008; Heredia-Solís *et al.*, 2014).

Therefore, the treatment and use of this residue are urgently needed. Energy recovery as methane through anaerobic digestion (AD) is one option (Robles-González *et al.*, 2012; Arreola-Vargas *et al.*, 2016). However, the acidic pH and high content of phenolic and ferulic compounds derived from the thermal hydrolysis of lignin, which is present in vinasses, might inhibit methanogenesis (Chávez and Domine, 2010; Paul *et al.*, 2018). Recent research has shown that due to its sugar content, mezcal vinasses inoculated with bovine manure is an efficient substrate for AD (López-Velarde *et al.*, 2020).

Given this, co-digestion or simultaneous digestion of two or more substrates, improves AD and methane yield,

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utilizing nutrient balance and improvement of the medium buffer capacity (Akyol *et al.*, 2016); i.e., the co-digestion of cow dung and maize waste in batch reactors improved the biogas yield by 92 % when a 10:5 cow dung:maize waste ratio was used compared with a 10:1 ratio (Abdoli *et al.*, 2014); the co-digestion of llama (*Lama glama*), sheep (*Ovis orientalis aries*), and cattle (*Bos primigenius taurus*) manures in a semi-continuous system increased methane yield by more than 50 % compared to only llama manure (Álvarez and Lidén, 2009). López-Velarde *et al.* (2019) improved the buffer capacity of the mixture by using activated sludge at a pH of 7 to evaluate vinasses adaptation to inoculation in a semi-continuous flow reactor; they obtained up to 85.5 mL CH₄ g⁻¹ volatile solids (VS) of vinasses. However, some doubts remain, especially when the vinasses content in mixtures increases. In contrast, there is an inhibitory effect by increasing the amount of mezcal vinasses in the co-digestion with substrates like agave bagasse. In this regard, Gómez-Guerrero *et al.* (2019) evaluated the co-digestion of mezcal vinasses and two particle sizes of agave bagasse (1 and 100 mm), inoculated with pig manure and granular sludge in batch flow reactors. Methane production with 100 mm of bagasse decreased 76 % by substituting 12 % of the reactor working volume for vinasses and stopped after 24 % substitution.

Therefore, it is important to study other options for the co-digestion of mezcal vinasses with local substrates abundantly available in mezcal production areas, such as bovine manure (BM) in different proportions, to determine its optimal level of use in biodigesters. The aim of the present work was to investigate the best proportion of co-digestion between the vinasses and BM to produce methane in a batch system.

MATERIALS AND METHODS

Substrates and inoculum

The study was carried out from January to May 2019 in the Soils Laboratory of CIIDIR Oaxaca, in Santa Cruz Xoxocotlan, Oaxaca, Mexico (17° 01' 30.3'' N and 96° 43' 12.5'' W, at 1530 meters above sea level). The BM was donated by the municipal slaughterhouse of Santa Cruz Xoxocotlan, Oaxaca. The vinasses were obtained at the end of the distillation process at the "Don Agave" mezcal factory, located in the municipality of Tlacolula de Matamoros, Oaxaca. Both substrates were stored at 4 °C until their use. The inoculum was prepared from the effluent of an active biodigester from a pig farm in Santa Ana Zegache, Oaxaca. A mixture of the effluent, water, vinasses, and BM in 4:2:1:1 volume/volume ratio (v/v), respectively, was prepared for its adaptation (Angelidaki *et al.*, 2009) and incubated under mesophilic conditions (36 ± 1 °C) in a water bath, in two airtight plastic containers of 10.1 L each, with 9 L of liquid volume per container, for 25 days until gas production stopped. The pH, total solids (TS), and VS of the substrates and inoculum were determined in triplicate according to APHA (APHA, 2012). The pH was determined with a Hanna brand digital potentiometer (Hanna Instruments[®]), TS and VS were determined using an oven at 105 and 550 °C

until constant weight with a digital analytical balance (model MSL, Brand Mettler Toledo[®]), respectively.

Experimental design and data collection

A completely randomized design with six repetitions per treatment was used. Five vinasses-BM (V-BM) mixtures at different percentage (v/v) proportions (25-75, 50-50, 75-25, 100-0, and 0-100) were used as treatments, with no pH adjustment nor TS balance to reproduce as normal conditions as possible. The experimental unit consisted of a 496 mL glass bottle with a metal lid and hermetic seal, with 300 mL of active volume, consisting of 75 mL of V-BM mixture, according to the proportions described above, 75 mL of water to avoid overload and keeping TS content below 10 % which is optimal for AD performance (Lorenzo-Acosta and Obaya-Abreu, 2005; Angelidaki *et al.*, 2009), and 150 mL of previously prepared inoculum, and immersed in water in mesophilic conditions (36 ± 1 °C) for 10 days to promote methanogenic activity (Espinoza-Escalante *et al.*, 2009; Abdoli *et al.*, 2014). The experiment was stopped when the volume of biogas produced per day during the last three days was <1 % of the total accumulated biogas (Holliger *et al.*, 2016).

The biogas produced per day and accumulated during the evaluation period, normalized (NmL) to standard temperature and pressure (273.15 °K, 101.33 kPa), was obtained using the ideal gas law equation (Holliger *et al.*, 2016). Biogas methane content was determined by the volumetric displacement method of 5 % NaOH solution (Viquez, 2017). The methane yield was obtained with equation 1:

$$Y_{CH_4} = \left(\frac{A_v \cdot \%_{CH_4}}{VS_R} \right) \quad (\text{Equation 1})$$

where Y_{CH_4} is the methane yield (NmL CH₄ g⁻¹ VS), A_v is the accumulated volume of biogas during the period of the experiment (NmL CH₄), and VS_R is the mass of volatile solids in grams added to the reactor. The daily methane yield was calculated dividing the accumulated yield by 10, which corresponds to the number of days of the experiment.

Statistical analysis

An analysis of variance was used to determine significant differences between treatments in the accumulated volume of methane, a means separation Tukey test was used to determine the best V-BM proportion according to data. To identify the critical stages of AD, such as the start of methane production and the microbial adaptation phase (Zwietering *et al.*, 1990) a nonlinear regression ($p < 0.05$) was performed with the collected data, using the SPSS v. 22 software (Statistical Package for the Social Sciences) to adjust the methane yield to the Gompertz bacterial growth model (Equation 2).

$$P = A \exp^{-\exp\left(\frac{\mu_{me}}{A}(\lambda - t) + 1\right)} \quad (\text{Equation 2})$$

In this model, methane production is proportional to microbial activity. P is the prediction of methane production in a certain period (t), A is the accumulated volume of meth-

ane in a given time ($\text{NmL CH}_4 \text{ g}^{-1} \text{ VS}$), μ_m is the maximum rate of methane yield per day ($\text{NmL CH}_4 \text{ g}^{-1} \text{ VS d}^{-1}$) defined by the tangent of the slope in the exponential growth phase of bacteria, and λ is the adaptation period (d) defined by the intercept of the line of the slope with the x-axis (Zwietering *et al.*, 1990).

RESULTS AND DISCUSSION

Characterization of the inoculum, substrates, and mixtures

Fresh vinasses presented acidic pH compared to bovine manure. The pH of the inoculum adapted to the vinasses was slightly alkaline, higher than vinasses. The highest vinasses content presented a more acidic pH than those with the highest manure content. The pH in 100-0 treatment was higher than the original substrates because of the 75 mL of water added to avoid overload of the system. The content of total solids and volatile solids of vinasses was lower than that of the inoculum, and bovine manure. The vinasses-bovine manure mixtures showed different total solids content. The 100-0 mix had the lowest total solids and volatile solids content, while the 0-100 mix had the highest content (Table 1).

The acidity of vinasses is determined by the content of organic acids, such as acetate, which is synthesized during the fermentation of the agave sugars to obtain alcohol; therefore, as the vinasse in the mixture increases, the pH becomes acidic. On the other hand, bovine manure addition to the mixture increased pH to a methanogenic level. The vinasses pH was comparable to that reported by other researchers for mezcal vinasses with values between 3.5 and 4.77 (Gómez-Guerrero *et al.*, 2019; López-Velarde *et al.*, 2019). The initial pH of the mixtures studied was at optimal levels for anaerobic digestion (6.8 and 7.4), except for the 100-0 mixture with 5.49, which is closest to the optimal pH level of acidogenic bacteria, between 5.5 and 6.5 (Khanal, 2009). When inhibitions to vinasses anaerobic digestion occur with pH values lower than 5.0 (Gómez-Guerrero *et al.*, 2019), pH adjustments are needed to carry out the anaerobic digestion (Espinoza-Escalante *et al.*, 2009).

The total solids content of vinasses studied was similar to those obtained from industrial-type mezcal factories in Oaxaca (43.4 g L^{-1}) (Villalobos *et al.*, 2009) and San Luis Potosí (49.17 g L^{-1}) (López-Velarde *et al.*, 2019), but lower than artisan-type factories ($91\text{--}94.7 \text{ g L}^{-1}$) (Gómez-Guerrero *et al.*, 2019). In contrast, the volatile solids content of vinasses was lower than those from artisan-type mezcal factories (72 g L^{-1}) (Gómez-Guerrero *et al.*, 2019) and Comiteco liquor vinasses (62.90 g L^{-1}) obtained from agave juices (Cruz-Salomón *et al.*, 2017). Regarding total solids content, it was less than 10 % in all vinasses-bovine manure mixtures, values below which the anaerobic digestion “usually operates in better conditions” (Lorenzo-Acosta and Obaya-Abreu, 2005). It was found that food waste anaerobic digestion performance was improved by increasing total solids content from 5 to 20 % (Yi *et al.*, 2014). In these studied mixtures, the volatile solids content was recorded in the 20 to 60 g L^{-1} range, recommended for anaerobic digestion tests (Holliger *et al.*, 2016), except for treatment 0-100, which had lesser values.

pH changes in mixtures

In the V-BM mixtures the pH increased as a function of the bovine manure content, decreasing significantly ($p < 0.0001$) in every mixture at the end of the experiment (5.64 ± 0.87). The lowest pH value was obtained in the mixtures with the highest vinasses content (Figure 1).

Archaea bacteria synthesize volatile fatty acids during the acidogenesis stage for methane synthesis, thus the pH values of the mixtures during anaerobic digestion decreases (Khanal, 2009; Pibul and Towprayoon, 2015). The pH variation at the end of the anaerobic digestion process is expected when no pH conditioner is added (Gómez-Guerrero *et al.*, 2019), as in the present study. The highest and most significant pH variation in the mixtures with the highest bovine manure content could be due to a higher volatile fatty acids production, associated with higher total solids content used for its synthesis (Parra-Orobio *et al.*, 2015).

Table 1. Physicochemical characterization of the substrate, inoculum and vinasses-bovine manure (V-BM) mixtures used in the experiment of anaerobic co-digestion.

Tabla 1. Caracterización fisicoquímica del sustrato, inóculo y mezclas de vinazas-estiércol bovino (V-EB) utilizadas en el experimento de co-digestión anaerobia.

Parameters	Inoculum / substrates								V-BM mixtures (%)							
	I		V		BM		100-0		75-25		50-50		25-75		0-100	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.75	0.05	3.52	0.04	7.98	0.11	5.49	0.03	6.39	0.03	6.77	0.03	7.19	0.04	7.74	0.03
TS (%)	6.55	0.14	4.93	0.17	21.8	0.59	3.8	0.11	5.38	0.23	6.2	0.18	7.51	0.18	8.79	0.13
TS (g L^{-1})	64.7	0.72	46.2	2.01	243	8.23	37	0.57	54.6	3.04	62.4	1.57	72.1	3.94	90.6	3.99
VS (g L^{-1})	54.4	0.68	42.3	1.60	193	6.02	26.7	0.60	42	2.07	48.2	1.23	56.8	3.16	66.2	5.00

I, inoculum; V, vinasse; BM, bovine manure; SD, standard deviation; TS, total solids; VS, volatile solids.

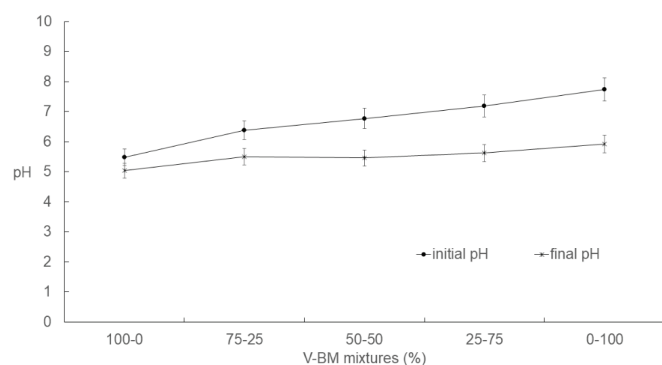


Figure 1. pH changes of the vinasses-bovine manure (V-BM) mixtures at the beginning and at the end of the experiment.

Figura 1. Cambios de pH de las mezclas de vinazas-estiércol bovino (V-EB) al inicio y al final del experimento.

Methane production

The accumulated volume of methane was significantly different ($p < 0.05$) between vinasses-bovine manure mixtures. The highest volume was obtained with 0-100 and 75-25 mixtures, with no significant difference between them, and the lowest volume with 25-75 and 50-50 mixtures. The 100-0 mix produced an intermediate methane volume (Figure 2).

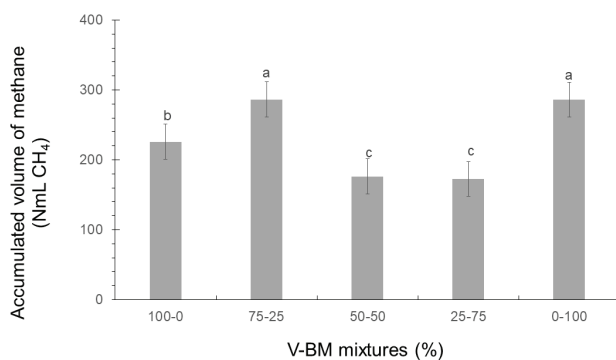


Figure 2. Methane accumulated volume from the vinasses-bovine manure (V-BM) mixtures. Different letters indicate significant differences between treatments ($p < 0.05$).

Figura 2. Volumen acumulado de metano de las mezclas de vinazas-estiércol bovino (V-EB). Letras diferentes indican diferencias significativas entre tratamientos ($p < 0.05$).

Substrates such as vinasses, with dissolved organic matter and highly degradable sugars, allow higher methane yields (Yi *et al.*, 2014) due to fructans present in the agave “piñas” (Vera-Guzmán *et al.*, 2009; Chávez-Parga *et al.*, 2016). During their cooking in the mezcal process, fructans are hydrolyzed into fermentable sugars, mainly fructose, glucose, xylose, and maltose, at different concentrations according to the degree of plant ripeness. These sugars are used in microbial fermentation for alcohol synthesis (Michel-Cuello *et al.*, 2008). However, a portion of the sugars cannot be fermented and go to vinasses; for example, 77.1 mg g⁻¹ of total sugars were found in the wort after fermentation, i.e., 14 % of the

initial total present in the juice of the cooked agave “piñas” (Vera-Guzmán *et al.*, 2009), and 51 mg g⁻¹ of total sugars in vinasses after distillation (López-Velarde *et al.*, 2019). These sugars present in vinasses, along with other suspended organic solids (Espinoza-Escalante *et al.*, 2009), are used by bacteria during anaerobic digestion to obtain energy and forward substrates for methanogenesis. In the case of the 75-25 mix, the bacterial community present in bovine manure uses these sugars and the optimized pH conditions to produce methane. The 75-25 and 0-100 mixtures had the highest cumulative methane production with 286.40 and 286.23 NmL CH₄, respectively. The 100-0 mix produced 225.98 NmL CH₄, ranking third; the 75-25 mix produced 26.7 % more methane than the 100-0 mix, indicating that vinasses-bovine manure co-digestion can be used for improving methane production from the mezcal vinasses.

Methane yield kinetics

The bacterial kinetic curve coincided with the Gompertz prediction model growth. The mixtures with higher vinasses content obtained a higher accumulated methane yield, while mixtures with higher bovine manure content presented lower yields; in addition, they produced 80 % of the methane produced in the first four days, while the 100-0 mix obtained that production after six days, indicating that co-digestion with bovine manure reduces the digestion time of vinasses by two days (Figure 3).

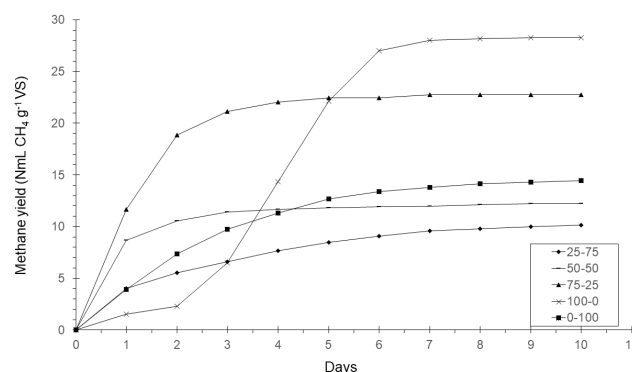


Figure 3. Methane yield kinetics from vinasses-bovine manure (V-BM) mixtures during the experimental period.

Figura 3. Cinética de los rendimientos de metano de las mezclas de vinazas-estiércol bovino (V-EB) durante el periodo experimental.

The methane yield expresses the volume of methane produced per gram of volatile solids invested in the system; this explains that despite having lower accumulated production (Figure 2), in the 100-0 mix methane yield was higher (28.27 NmL CH₄ g⁻¹ volatile solids) compared to the 0-100 mix (14.43 NmL CH₄ g⁻¹ volatile solids). Regarding the performance of the yield curve, the conversion rate of complex substrates such as vinasses with acidic pH presents a more prolonged hydrolysis stage at the start of anaerobic digestion, which was evidenced in the S-shaped methane yield curve. In contrast, simpler substrates with more balanced properties, such as neutral pH in mixtures with bovine manure, have shorter

incubation periods, as evidenced in the inverted L-shaped curve (Ware and Power, 2017).

The cumulative methane yield estimated by the Gompertz model was adjusted to the results obtained in the experiment with R^2 values close to 1 (Table 2). The bacterial kinetics for each of the vinasses-bovine manure mixtures described the expected bacterial growth behavior by the theoretical model. The 100-0 mix had the highest daily and cumulative return rate; nevertheless, it had the highest adaptation phase due to the lower pH value (Figure 2). This implies the need for a longer hydraulic retention time and larger reactors, or in the best case, pH adjustment. On the other hand, in the mixtures with bovine manure, the degradation began before its preparation, indicated by the λ negative values. This phenomenon is possibly caused by the microbiological activity present in the manure before the anaerobic digestion, which could start vinasses digestion in a shorter time, i.e., the 75-25 mix adaptation period was -1.83, which means that methane production started 4.12 days earlier than in the 100-0 mix, and had the best performance regarding the maximum rate of methane yield per day and cumulative methane yield, comparable to the 100-0 mix. This indicates that the vinasses-bovine manure co-digestion in a 75-25 proportion best favors the mezcal vinasses anaerobic digestion treatment process.

Table 2. Results of the Gompertz growth model for methane production from vinasses-bovine manure (V-BM) mixtures used in the experiment.

Tabla 2. Resultados del modelo de crecimiento de Gompertz para la producción de metano de las mezclas de vinazas-estiércol bovino (V-EB) utilizadas en el experimento.

V-BM mixtures	A (NmL CH ₄ g ⁻¹ VS)	μ_m (NmL CH ₄ g ⁻¹ VS d ⁻¹)	λ (d)	R ² (adjusted)
100-0	28.97	9.03	2.29	0.994
75-25	22.64	10.09	-1.83	0.998
50-50	12.08	3.68	-1.56	0.992
25-75	10.54	1.44	-0.17	0.999
0-100	14.44	3.02	-0.37	0.998

A, methane accumulated volume; μ_m , maximum rate of methane yield per day; λ , adaptation period; VS, volatile solids.

The accumulated methane yield was higher with a higher vinasses content because "vinasses are more efficient substrates for anaerobic digestion than other substrates due to the quantity of soluble sugars they contain", contrary to what happened with the cellulose present in the bovine manure (López-Velarde *et al.*, 2020). During the adaptation period, bacteria grow and prepare to hydrolyze organic compounds; it takes longer for complex substrates under acidic pH (Parra-Orobio *et al.*, 2014), such as the vinasses alone. Nevertheless, this period can be shortened by adding bovine manure, as shown by the 75-25 mix. The co-digestion of substrates demonstrates the shortening of the hydrolysis period, i.e., when maize waste was added to cow dung digestion, the incubation period was 4 days in contrast with 11 days for cow dung digestion alone (Abdoli *et al.*, 2014). Negative λ values

have been observed in anaerobic digestion bacterial growth curves of substrates with an initial bacterial load, such as bovine manure (Ware and Power, 2017; López-Velarde *et al.*, 2020). This implies that vinasses-bovine manure co-digestion favors the initial presence of a bacterial community for vinasses anaerobic digestion in a shorter time.

CONCLUSIONS

The vinasses-bovine manure co-digestion raised the pH and anticipated the start of anaerobic digestion by 2 days. The 75-25 vinasses-bovine manure mixture produced the highest accumulated methane production, with 286.40 NmL CH₄, 26.7 % more methane than vinasses alone. The highest methane yield was produced with the 100-0 mixture, with 28.27 NmL CH₄ g⁻¹ volatile solids, followed by the 75-25 mixture with 22.8 NmL CH₄ g⁻¹ volatile solids. The 100-0 mixture presented the most prolonged adaptation period of anaerobic digestion, 2.29 days. The vinasses-bovine manure co-digestion in proportion 75-25 was the best mixture to improve the mezcal vinasses anaerobic digestion process and shortened the bacterial adaptation period to 4.12 days.

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