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Anaerobic co-digestion of *Opuntia ficus-indica* (L.) Mill. cladode with cow manure: Effect of different cultivars on biochemical methane potential

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Abstract. Nopal (*Opuntia ficus-indica* (L.) Mill.) cladode has garnered great interest recently in the area of agro-energy as emerging biomass due to its sustainable production. The objective of this study was to compare the biochemical methane potential of different nopal cultivars in co-digestion with cow manure. For this purpose, two different nopal cladodes: cow manure proportions (75:25 and 82:18) and three different cultivars (Atlixco, Copena V1, and Milpa Alta) were evaluated. The results indicated that the treatments with higher biochemical methane potential (mL CH_4 $g^{-1}{}_{VSfed}$) were Milpa Alta 75:25 (71.4), Copena V1 75:25 (66.5), Milpa Alta 82:18 (64.6), and Copena V1 82:18 (59.0), which showed no statistical difference (P>0.05) between them, whereas the Atlixco treatments (75:25 and 82:18) had the lowest (P<0.05) values (52.8 and 41.5, respectively). The results suggest that the cow manure proportion and nopal cultivar used in a co-digestion system may influence its biochemical methane potential.
Keywords: *Nopal cultivars; co-digestion; energy crop; bioenergy, Gompertz model.*

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Introduction

Crassulacean acid metabolism (CAM) plants can be used as a source of food, fiber, bioenergy, and pharmaceutical products (Davis *et al*., 2019). Their high-water use efficiency, even under drought conditions, makes them a good candidate for biomass production. *Opuntia ficus-indica* (L.) Mill. (OF) has become of great interest in the agro-energy area as emerging biomass due to its sustainable production (Yang *et al*., 2015). It has been reported that OF is an excellent candidate for supplying the biomass needed for global energy production levels expected by 2050 (IEA Bioenergy, 2007). It has also been noted that the expected productivity levels of CAM plants may be underestimated, particularly in the Eastern hemisphere, so these plants could play an important role in biomass production for bioenergy in the coming years (Hartzell *et al*., 2021). Quiroz e*t al*. (2021) indicated that intensification of cultivar management would increase the methane potential. These authors reported that the theoretical methane potential for *Opuntia* could range from 681 to 17,433 m^3 ha^{-1}, depending on the dry biomass produced. In a five-year biomass production trial using three different *Opuntia* species (*O. cochelenillifera*, *O. ficus-indica*, and *O. streptacantha*), Neupane *et al*. (2021) reported a maximum of 15.52 Mg ha^{-1} $year^{-1}$ (in dry biomass units). These authors have shown that, although no statistical difference was found, OF is the most promising plant due to its positive response to water inputs. There are some proposals for using OF as a feedstock for bioethanol (Alencar *et al*. 2020; Perez-Cadena *et al*., 2018; Santos *et al*., 2016) and methane (Ramirez-Arpide *et al*., 2018; Ramirez-Arpide *et al*., 2019; Santos *et al*., 2016) production.

Our research group has reported that using the digestate obtained after co-digestion of OF cladodes and cow manure has the potential to support the reduction of global warming (Ramirez-Arpide *et al.,* 2018). The use of biofertilizer obtained with the anaerobic digestion of *Opuntia heliabroavana* has been reported (Quintanar-Orozco *et al*., 2018). OF digestate can also be used as an alternative biofertilizer since it conserves most of the nutrients provided by the plant (Krumpel *et al*., 2020). In addition to the amount of dry mass, biomass chemical composition plays an important role in methane production. A study conducted to evaluate the influence of co-digestion of agro-food biowastes with manure on methane production found that it could increase by 30 to 250 % (Duarte *et al*., 2021).

Regarding the influence of chemical composition on methane production, Yan *et al*. (2017), in a study of 20 different leafy vegetables, found that the volatile solids: total solids ratio and lignin and hemicellulose content influence methane production. The chemical composition of cultivars of the same crop may vary in quantity and diversity of some constituents such as carbohydrate, protein, ash, lipid, phenolic, pigment, and soluble/insoluble fiber contents (Ragaee *et al*. 2012; Xiao *et al*. 2015). In the area of biofuel production, for example, Sheetal *et al*. (2019) reported that different rice (*Oryza sativa* L.) cultivars (i.e. Pusa 44, IR36, Pusa basmati 1121, PRH 10, Taraori basmati) produced different ethanol yields. Among the cultivars, Taraori basmati had the highest ethanol yield. Similarly, Pazderů *et al*. (2014) reported different methane yields obtained from three different sorghum (*Sorghum bicolor* L.) cultivars: Botival (207.4 m^3 t^{-1}), Sucrosorgo (243.8 m^3 t^{-1}), and Goliath (246.4 m^3 t^{-1}). On the other hand, in Tunisian date seed (*Phoenix dactylifera* L.), Souli *et al*. (2020) found that the cultivar Deglet Nour had a higher cumulative methane yield (0.327 Nm^3 CH_4 kg^{-1} COD) than the other cultivars tested (Benjou, Ammari, Kentichi, Alig, Kenta), with values ranging from 0.267 to 0.318 Nm^3 CH_4 kg^{-1} COD.

Taking into consideration what has been stated above, different OF cultivars can be expected to have different methane production levels. However, no reports are evaluating the influence of different OF cultivars on their biochemical methane potential (BMP). Thus, the objective of this work was to compare the BMP of different OF cultivars in co-digestions with cow manure (CM). For this purpose, we evaluated two different OF: CM proportions (75:25 and 82:18) and three different OF cultivars (Atlixco, Copena V1, and Milpa Alta). Anaerobic digestion performance was evaluated using the modified Gompertz model.

Materials and Methods

Cladode samples from three cultivars (Atlixco, Copena V1 and Milpa Alta) of *Opuntia ficus-indica* (L.) Mill. and cow manure was provided by a dairy cattle farm in Chapingo, State of Mexico, Mexico. Both OF and CM were used as substrates for co-digestion systems. Table 1 summarizes the characteristics of the OF Atlixco, OF Copena V1, OF Milpa Alta, and CM substrates. For each OF cultivar, two different OF: CM proportions (75:25 and 82:18) were evaluated. These proportions were chosen based on C: N ratios close to 20 (considered as the best ratio for efficient anaerobic digestion) (Tufaner and Avşar, 2016).

The anaerobic digestion was performed in microcosms using 305-mL serum bottles with 250 mL of working volume. The inoculums were added at 10% (v/v) and were obtained from two 10-L mesophilic digesters fed with OF: CM ratios of 75:25 and 82:18, respectively, both at 4% of total solids. The treatments feed was prepared with two different feedstock ratios of OF cladode and cow manure in aqueous solution, in triplicate (three experimental replications). Parameters characterizing the initial and final chemical composition of feed treatments are reported in Table 2. In all treatments, the anaerobic condition was produced by purging microcosms with nitrogen gas and incubating at 37 ± 1 °C.

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Table 1. Chemical composition of substrates.

Parameters	Units	Substrate			
		OFA	**OFC**	**OFM**	**CM**
pH	-	4.52	4.05	4.15	8.61
TS	[%]	4.37	3.67	4.47	5.56
VS/TS	[%]	68.56	76.08	82.49	53.00
Protein	[%]	6.88	7.74	4.71	10.8
Lipids	[%]	2.07	1.99	5.4	2.0
C	[%]	37.36	35.93	36.06	24.55
H	[%]	4.92	4.54	4.93	3.19
N	[%]	1.52	1.52	1.03	2.28
S	[%]	0.42	0.29	0.08	0.29
C/N	-	24.58	23.72	35.01	10.78

TS: total solids; **VS:** volatile solids; **OFA, OFC,** and **OFM** codes are *Opuntia ficus-indica* (L.) Mill. cladode cultivar Atlixco, Copena V1, and Milpa Alta, respectively; **CM:** Cow manure.

Table 2. Initial and final chemical composition of treatments.

Treatments	Substrates			Parameters							
	OF	**CM**	**C/N**	**pH**		**TS [%]**		**VS/TS [%]**		**COD [mg L^{-1}]**	
				Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
OFA75	75	25	21.1	6.1	7.8	3.5	2.6	63.3	40.5	30,526	12,098
OFC75	75	25	20.5	5.4	7.8	3.5	2.3	66.5	37.9	31,243	15,640
OFM75	75	25	29.0	5.7	7.6	3.7	3.1	67.7	40.9	33,984	22,261
OFA82	82	18	22.1	5.9	7.8	3.2	2.4	71.3	32.4	29,640	14,924
OFC82	82	18	21.4	5.0	7.8	3.5	2.9	70.8	43.0	32,719	14,586
OFM82	82	18	30.7	6.6	6.7	4.2	2.7	61.6	29.9	33,098	6,532

TS: total solids; **VS**: volatile solids; **OFA**, **OFC**, and **OFM** codes are *Opuntia ficus-indica* (L.) Mill. cladode cultivar Atlixco, Copena V1, and Milpa Alta, respectively; **CM**: Cow manure; **COD**: chemical oxygen demand.

Analytical methods

Total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) were determined using procedures laid out in the American Public Health Association's Standard Methods for the Examination of Water and Wastewater manual (1998). The content of several volatile fatty acids and methane was determined according to Meneses-Reyes *et al*. (2017).

Biochemical Methane Potential

Experimental data from methane production were modeled using the Gompertz model as reported in detail by Meneses-Reyes *et al*. (2017) to obtain the kinetic parameters of methane production, including the Biochemical Methane Potential (BMP, mL CH_4 g^{-1} $_{VSfed}$), methane production rate (μ_m, mL CH_4 g^{-1} $_{VSfed}$ d^{-1}), and lag phase (λ, d) values according to Equation 1 below:

$$AMY = BMP \cdot exp\left\{-\,exp\left[\frac{\mu_m \cdot e}{BMP}(\lambda - t) + 1\right]\right\} \quad (1)$$

AMY is the accumulated methane yield (mL CH_4 g^{-1} $_{VSfed}$) at time t (d), *e* is the mathematical constant (2.718282), and ***t*** is the digestion time (d). Modeling was performed using SigmaPlot 14.0 software (Systat Software, Inc. USA).

Statistical analysis

The experimental data were statistically analyzed using ANOVA. To determine significant differences among treatments, Fisher analyses were performed, with P<0.05 being considered significant. ANOVA and Tukey's means comparison test was performed using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The accumulated methane yield and the volatile fatty acids profile for all treatments is shown in Figure 1 and Figure 2**,** respectively. The higher methane yield was obtained by treatment OFM75, followed by OFC75, OFM82, and OFC82, which showed no statistical difference (P>0.05) among them, whereas the two treatments derived from the cultivar Atlixco (OFA75 and OFA82) had the lowest (P<0.05) methane production. On the other hand, considering the OFM75 methane yield value, our data indicated that other treatments showed 3.7% (OFC75), 9.4% (OFM82), 16.0% (OFC82), 28.6% (OFA75), and 43.8% (OFA82) lower methane production values. The methane yield obtained in treatments OFM75 and OFC75 was slightly higher than that previously reported by Uribe *et al*. (1992) (70.5 mL CH_4 g^{-1} $_{VSfed}$) but lower than that reported by Jigar *et al*. (2011) (123.5 mL CH_4 g^{-1} $_{VSfed}$).

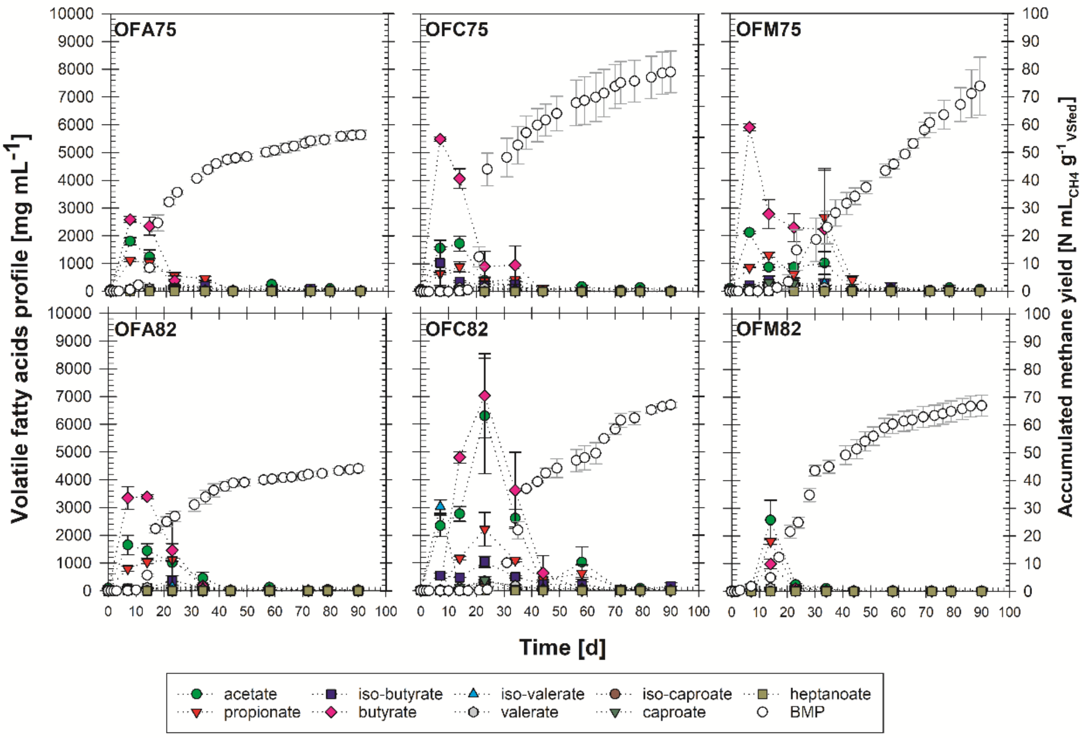

Figure 1. Accumulated methane and volatile fatty acids profile per treatment during the anaerobic digestion process. **OFA**, **OFC** and **OFM** codes are *Opuntia ficus-indica* (L.) Mill. (OF) cladode cultivar Atlixco, Copena V1 and Milpa Alta, respectively. The ratio between **OF** and cow manure for each treatment is defined in Table 2. Data are the average (± standard deviation) of three replications.

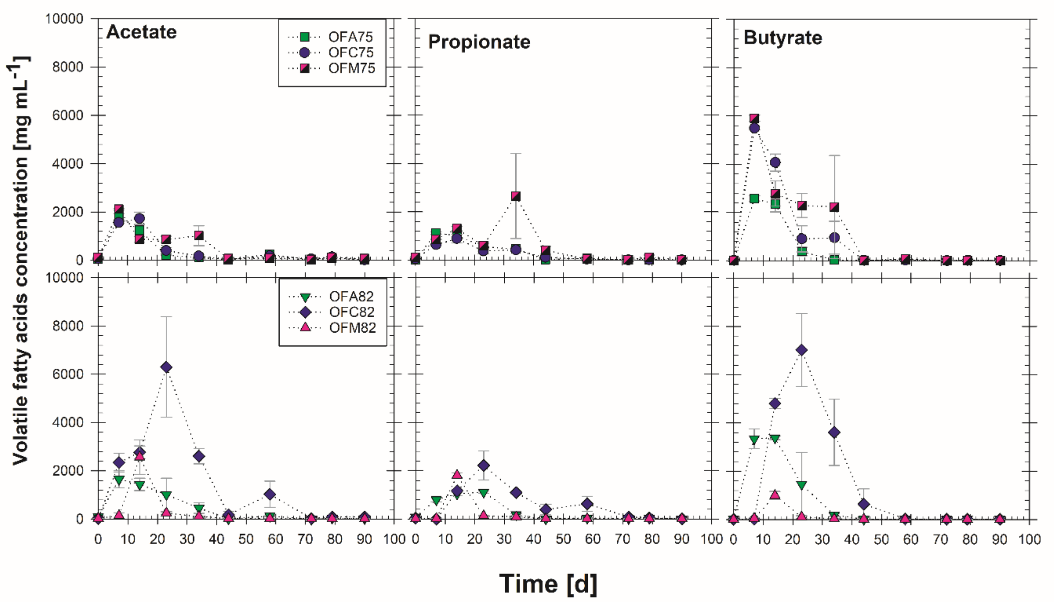

Figure 2. Changes in the acetate, propionate, and butyrate concentration in the anaerobic digestion. **OFA, OFC** and **OFM** codes are *Opuntia ficus-indica* (L.) Mill. (OF) cladode cultivar Atlixco, Copena V1 and Milpa Alta, respectively. The ratio between OF and cow manure for each treatment is defined in Table 2.

Methane production curves of all treatments showed three phases: 1) slow gas production period (lag phase), 2) rapid gas production period (exponential phase), and 3) period in which the rate of gas production slows and eventually trends to zero (asymptotic phase) (Figure 1). Based on the above-mentioned methane production results and to analyze the anaerobic digestion performance of the treatments, kinetic parameters of methane production were calculated (Table 3). Methane production of all treatments fitted accurately to the Gompertz model. Similarly, with the methane yield results, the higher BMP was obtained by OFM75, followed by OFC75, OFM82, and OFC82, whereas OFA75 and OFA82 had the lowest BMP.

The difference observed in methane production among different studies may be attributed to several factors such as the OF cultivar used and its chemical composition, which may affect the methane production, and the co-digestion conditions. Thus, the higher methane production of OFM75 may be attributed to the chemical composition of this cultivar, because of its high lipid content, as well as its low content of S and N (Table 1). In this regard, Edwiges *et al*. (2018) reported that chemical composition influenced the methane potential of fruit and vegetable waste under anaerobic digestion. The authors found that lipid content correlated positively with methane production. In a related study, Cu *et al*. (2015) found that lipid content explained most of the variation (59.9%) in a model to predict the methane production from combined animal and plant biomass.

In addition, S and N levels could be an indicator of minor methane production inhibition by potential sulfhydric acid and ammonia accumulation. In contrast, the high S and N content in the cultivars Copena and Atlixco (Table 1) can cause inhibitory effects on biogas and methane production. Accordingly, Yenigün and Demirel (2013) stated that even though ammonia is an essential nutrient for bacterial growth, it might inhibit methanogenesis during anaerobic digestion if high concentrations are reached. Similarly, potential inhibition of methane production by S conversion is due to competition for common organic and inorganic substrates from sulfate-reducing bacteria, which cause less methane production, or to the toxicity of these elements to bacterial groups (Chen *et al*. 2008). However, the presence of these inhibitors was not evaluated in this study and requires additional experimental confirmation. The data suggest that lipid content and the potential presence of inhibitors influence the methane production of the OF cultivars evaluated in the present study.

In contrast, no statistical difference was found among all treatments for the methane production rate (μ_m,) kinetic parameter (Table 3), which ranged from 1.4 to 2.6 mL CH_4 g^{-1} $_{VSfed}$ d^{-1}. Similar behavior was observed for the lag phase (λ) time, where no statistical difference was found among most treatments. However, it was observed that OFA and OFM treatments showed low λ values, which is an indicator of fast microorganism adaptation to the substrate. Possibly because the inoculum used for the present study was obtained from digesters that were fed with OFM as substrate. Interestingly, OFC82 showed the highest λ value (Table 3). These observations could be an indicator of a lack of coordination between trophic groups during the anaerobic digestion process. These observed differences may be attributed to diverse factors, including substrate characteristics, which could lead to the variability in the structure and function of the microbial population (Zhang *et al*. 2016), as well as to the fact that different substrate ratios (environmental gradients for microbial populations) result in the dynamics of syntrophic populations being highly selective and thus leading to differences in methanogenic activity (Werner *et al*. 2011).

Conversely, the results evidenced an accumulation of mainly acetate, and to a lesser extent of propionate, at the beginning of the experiments in all treatments (Figure 2). In most cases, when the above-mentioned volatile fatty acids (VFAs) reached their maximum production, depending on the treatment, at around day 15 to 30, methane production started. This observed behavior can be attributed to acetate consumption for methane production since it is used by methanogenic microorganisms. Concerning the VFA production of the OFC82 and OFM75 treatments, the behavior of their methane production curves shows prolonged (large) λ values, suggesting that these treatments follow different VFA accumulation pathways, with the main routes being carbohydrate fermentation during acidogenesis and, to a lesser extent, amino acid fermentation and long-chain fatty acid oxidation (Batstone *et al*. 2002). Accordingly, the proportion and OF cultivar used influenced BMP. To the best of our knowledge, this is the first time the influence of different OF cultivars on BMP has been described.

According to this, the treatments with the higher methane yield (OFC75 and OFM75) were those with the lowest accumulation of these VFAs, which suggests their consumption for methane production. On the other hand, treatments OFA82 and OFC82 had a greater accumulation of those VFAs, which is following their lower methane production. Interestingly, it can be observed that regardless of the OF cultivars, the treatments with a 75:25 OF: CM proportion had better methane production. Accordingly, these treatments showed low VFA accumulation.

Figure 3 shows that there is a trend for treatments with similar C: N ratios to form groups, with the treatments with the higher C: N values being those with the higher BMP. It is also important to note that as the treatments with low C: N ratios (OFA and OFC) may be affected by inhibitors and considering that all treatments had the same source of N, a dilution effect may have occurred.

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Table 3. Estimated parameters of the Modified Gompertz model from the experimental data.

Substrates	λ* mean [d]	μ_{m^*} mean [mL CH_4 g^{-1} $_{VSfed}$ d^{-1}]	BMP* mean [mL CH_4 g^{-1} $_{VSfed}$]	R^2
OFM75	19.8^{ab}	1.4^{a}	71.4^{a}	0.986 0.992 0.980
OFC75	14.5^{b}	2.6^{a}	66.5^{a}	0.978 0.978 0.975
OFM82	10.0^{b}	1.9^{a}	64.6^{a}	0.995 0.99 0.994
OFC82	34.6^{a}	2.4^{a}	59.0^{a}	0.998 0.970 0.996
OFA75	8.4^{b}	2.3^{a}	52.8^{b}	0.980 0.987 0.989
OFA82	8.5^{b}	1.9^{a}	41.5^{b}	0.972 0.988 0.989

* Different letters in the same column are significantly different at α = 0.05. **OFA**, **OFC** and **OFM** codes are *Opuntia ficus-indica* (L.) Mill. (OF) cladode cultivar Atlixco, Copena V1 and Milpa Alta, respectively. The ratio between **OF** and cow manure for each treatment is defined in Table 2.

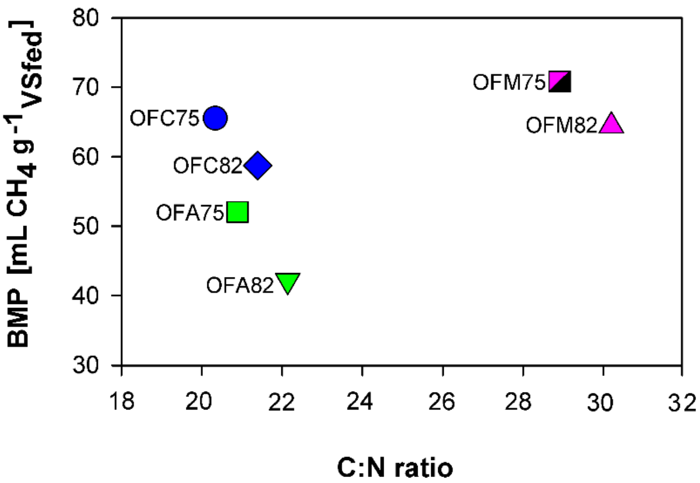

Figure 3. BMP as a function of different C: N ratios at the endpoint. **OFA**, **OFC** and **OFM** codes are *Opuntia ficus-indica* (L.) Mill. (OF) cladode cultivar Atlixco, Copena V1 and Milpa Alta, respectively. The ratio between **OF** and cow manure for each treatment is defined in Table 2.

Conclusions

To the best of our knowledge, this is the first report describing the influence of *Opuntia ficus-indica* (L.) Mill cultivar on biochemical methane potential. The results indicated that the best biochemical methane potential (mL CH_4 g^{-1} $_{VSfed}$) was obtained by Milpa Alta 75:25 (71.4), followed by Copena V1 75:25 (66.5), whereas the Atlixco treatments (75:25 and 82:18) had the lowest biochemical methane potential. These results demonstrate that BMP is dependent on the co-digestion proportion and *Opuntia ficus-indica* (L.) Mill cultivar. Overall, the results suggest that the chemical composition of the cultivar, mainly its lipid and S and N contents, influences methane production. Thus, for bioenergy applications and follow-up research, it is suggested to use *Opuntia ficus-indica* (L.) Mill Milpa Alta or Copena V1 cultivars in co-digestion with cow manure at a 75:25 ratio.

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Compliance with ethical standards

Not apply

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution statement (CRediT)

T. Espinosa-Solares – **Formal analysis, Project Administration**., J.E. Aguilar-Toalá – **Writing, Validation**., C. Barrales-Fernández – **Investigation**, **Methodology**., J.C. Meneses-Reyes – **Supervision**., C. Gallegos-Vázquez – **Resources**., G. Hernández-Eugenio – **Conceptualization, Data curation.**

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