Comparison of Biomethane Production Process Using Two Different Types of Reactors from Dairy Wastewater

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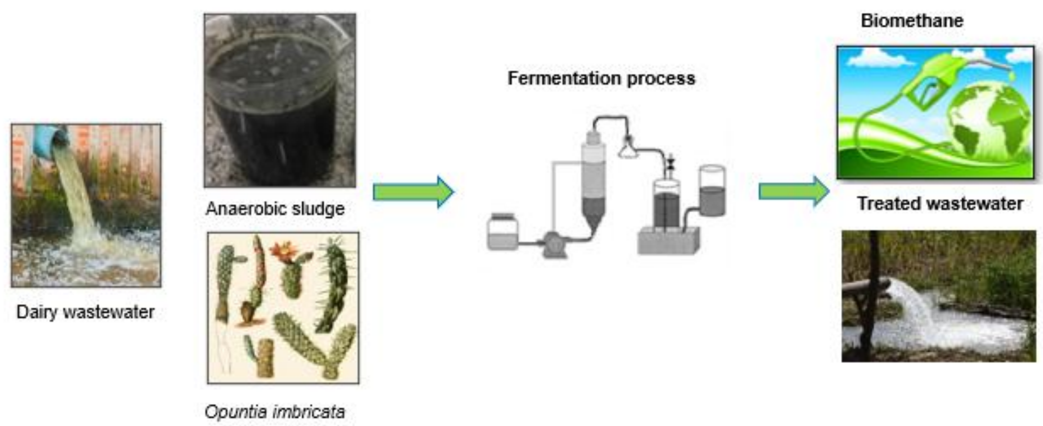
DOI: 10.15376/biores.20.2.2574-2586

GRAPHICAL ABSTRACT



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The production of biomethane (Bio-CH_4) from dairy wastewater was evaluated using two types of reactors: an upflow anaerobic sludge blanket (UASB) reactor and a batch reactor, using dairy wastewater, anaerobic sludge (as inoculum), and *Opuntia imbricata* (as biomass substrate). The latter is a cactus known as coyonoxtle and is considered an invasive plant in northern Mexico. The wastewater was characterized in accordance with NOM-001-SEMARNAT-2021. The UASB reactor having a capacity of 4.5 L, was charged with 350 mL of sludge, 24 g of *Opuntia imbricata*, and 3.5 L of dairy wastewater (20.1 g/L of O_2) at pH 7.0. Batch reactors with a volume of 120 mL, were charged with 72 mL of dairy wastewater (20.1 g/L of O_2), 8 mL of sludge, and 3 pieces of *O. imbricata*. The results of the UASB reactor: Total specific production was 21.2 mmol of Bio-CH_4 and an efficiency in the degradation of organic matter of 70.7%, with a hydraulic retention time of 4.8 h and a total duration of 720 h. For the batch reactors: Total specific production was 11.6 mmol of Bio-CH_4 and 97.95% efficiency in the removal of organic matter, with a total duration of 192 h. The results showed an economic, efficient and sustainable way of producing Bio-CH_4.

DOI: 10.15376/biores.20.2.2574-2586

Keywords: Biomethane; Diary wastewater; Pollution

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INTRODUCTION

Due to population growth, there has been an increase in the consumption of industrial products, which mostly come from petroleum derivatives. Such production generates a greater amount of pollution. Another effect is increased consumption of fossil fuels, which causes problems in the environment such as climate change, global warming, economic conflicts, social imbalances, and resource depletion (Hernández *et al*. 2022). High oil prices, environmental deterioration, and restrictions on gas emissions into the atmosphere have led governments and the scientific community to look for clean energy alternatives to replace fossil fuels (Orozco-Ramírez *et al*. 2022).

A solution is the generation of biofuels such as biomethane (Bio-CH_4). These can be obtained from renewable resources such as biomass and generate clean energy (Duarah *et al*. 2022). These can be used in various applications, such as non-polluting fuel in vehicles and as a domestic fuel (Baquero and Monsalve 2023). According to the literature, Bio-CH_4 produced by microbial consortia from dairy wastewater has the necessary conditions to be used as renewable energy. They are energy sources of the future, they have several advantages over commonly used fossil fuels, among which are: High conversion efficiency, recyclability, capable of removing pollutants and suitable for energy production (Cabrera 2022).

Mexico ranks 16th in the world in milk production (Serrano *et al*. 2024); therefore, the volume of wastewater produced in the dairy industry is abundant. The production process is wet, and a large volume of water is required for production, cleaning, and sanitization of areas and equipment. It is estimated that a dairy company can generate a discharge volume of between 2 to 5 liters of wastewater for each liter of milk processed (Contreras *et al.* 2019). Dairy wastewater is characterized by its dark gray color with a milky appearance and has an unpleasant odor. Such wastewater has a high chemical oxygen demand, inorganic and organic particles, biological demand for oxygen and nutrients, in addition to having a highly variable pH (Díaz *et al*. 2021). Wastewater contains solid waste that is eliminated through a filter, to later be treated properly; otherwise, it may pollute water bodies and greatly affect the ecosystem and biodiversity (Kaur 2021).

Today, dairy industries are forced to resort to robust strategies to reduce the amount and load of wastewater. These strategies include biological treatments (Ahmad *et al*. 2019). The use of *Opuntia imbricata* has been implemented for the generation of biofuels. This because it has been used as a natural support for the immobilization of microorganisms due to its high porosity (Mari *et al*. 2020; Moreno-Dávila *et al*. 2011). This forms biofilms (BPs), a very dynamic sessile community of microorganisms, characterized by cells irreversibly adhered to a substrate. In general, BPs are comprised of life forms that are adapted to survive in hostile environments (Cárdenas *et al*. 2022). Currently, the use of biofilms is becoming an alternative for the generation of biofuels.

Biomethane is a sustainable energy source obtained by processing biological waste and organic materials. It is often used as a high-quality substitute for fossil natural gas and offers a wide range of environmentally and climate-friendly applications (Sanyal *et al*. 2024). Bio-CH_4 is a gaseous renewable fuel, composed of methane and carbon dioxide, in a proportion of 65% to 35% (Zhao *et al*. 2020). It comes from the refining of biogas obtained either by thermal or biological technologies, whose raw material is organic matter (biomass). Its use as energy has a wide variety of applications and meets various environmental requirements such as: it does not contribute to the formation of greenhouse gases, does not generate acid rain, and prevents the depletion of the ozone layer (Hidalgo and Martín-Marroquín 2020). That is why it is considered an option for the generation of clean and sustainable energy. Bio-CH_4 is an attractive energy vector for the future due to its high efficiency in its conversion into usable energy (Ramos *et al*. 2017).

With this biofuel production system, the aim is to implement the use of simple technologies for the generation of biomethane and with this dairy food production plants can produce clean energy, thus avoiding the depletion of fossil energy sources, while reducing polluted effluents. The objective of this work was to determine the production process of Bio-CH_4 from wastewater from the dairy industry, using two different types of reactors. This work contributes to the Sustainable Development Goals (SDGs): 6 clean water and sanitation and 7 affordable and clean energy, in addition to contributing to national strategic programs (PRONACES); water, energy, and climate change.

EXPERIMENTAL

Inoculum

As an inoculum, 600 mL of a mixed microbial anaerobic culture was used, which was provided by the Model brewery in Torreón, Coahuila, Mexico, obtained from a upflow anaerobic sludge blanket (UASB).

Substrate

Wastewater from a dairy industry established in the city of Saltillo, Coahuila, Mexico, was used as a substrate. The dairy wastewater was stored at 3 °C and characterized in accordance with NOM108 001-SEMARNAT-2021. The results appear in Table 1.

Table 1. Characterization of Dairy Wastewater

Characterization	Content
Fats and oils (g/L)	8.3 ± 0.318
Ph	9.4 ± 0.197
COD (0_2/L)	20.1 ± 0.273
TDS (ppm)	125 ± 0.149
Temperature (ºC)	33 ± 0.199
Sulfates (mL/L)	143.53 ± 0.375
Chlorides (mg/L)	426.82 ± 0.381

Substratum (*Opuntia imbricata*)

The dried stem of a cactus (*Opuntia imbricata*) was washed with distilled water and cut into small rectangular pieces (1.4 x 0.6 x 0.5 cm). The descriptions of the cactus are presented in Table 2.

Table 2. Characterization of the Substratum

Parameter	Specification
Identification	*Opuntia imbricata*
Origin	Natural
Density	0.799 (g/cm^3)
Dimensions	1.4 x 0.6 (cm)
Specific surface area	0.487 (m^2/g)
Dried weight	11 ± 0.017 (g)
Configuration	Packed bed

Assembly of Upflow Anaerobic Sludge Blanket (UASB)

The experiment was carried out in an upflow anaerobic sludge blanket (UASB) with the following characteristics: 4.5 L capacity, 62 cm height, and 36 cm diameter. 3.5 L of dairy wastewater, 24 g (dry weight) of *Opuntia imbricata*, and 350 mL of mixed anaerobic microbial culture were added, under the following initial conditions: 20.1 g/L O_2, 25 ± 2 °C, pH 7.0, and hydraulic retention time (HRT) 4.8 h. The bioreactor was fed by a peristaltic pump (MANOSTAT - Barnant Company Division 132, Simon Varistaltic Pump, USA). Three cycles of 240 h each were performed, with a total of 720 h. During this period, monitoring was carried out every 24 h to determine the production of Bio-CH_4 and the removal of COD. Figure 1 shows the schematic design of the reactor.

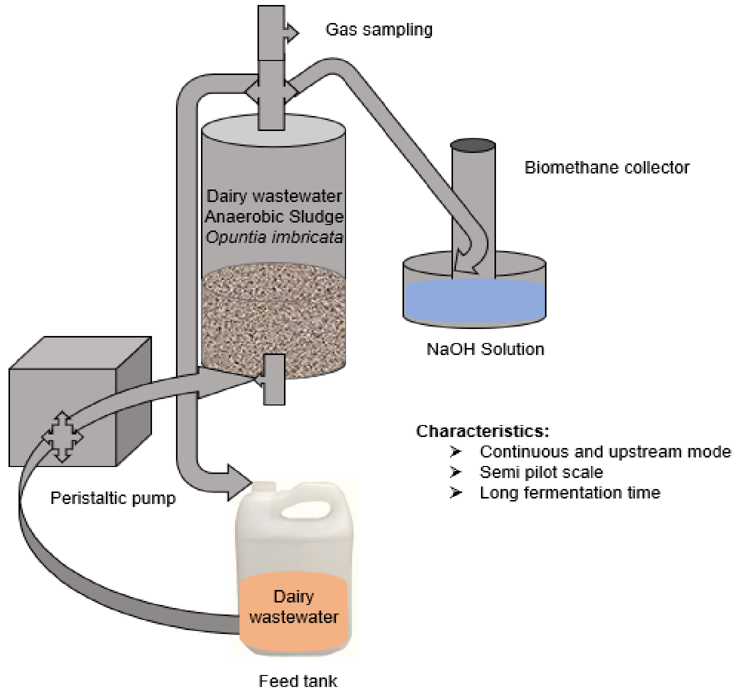

Fig. 1. Schematic design of the upflow anaerobic sludge blanket (UASB)

In the UASB reactor it is necessary to control various factors that intervene in the process such as: Organic loading speed, HRT, pH control, and alkalinity control. These directly influence the biomethane production process and the removal of organic matter.

Assembly of Batch Reactors

The experiment was carried out in batch reactors with a volume of 120 mL (transparent glass bottles with narrow mouths with phenolic caps), to which 72 mL of dairy wastewater (20.1 g/L of O_2) and 8 ± 0.012 mL of substrate (0.550 ± 0.10 g of biomass were added) and 3 pieces of *Opuntia imbricata* at an initial pH of 7.0 were added. The reactors were hermetically sealed with silicone rubber and standard seals to prevent gas leaks. The parameters measured were gas production and COD removal, samples were taken every 24 h. This experiment was performed in triplicate in the period of 192 h. Fig 2 shows the schematic design of the reactors.

Fig. 2. Schematic design of batch reactors (triplicate)

Statistical Analysis

The statistical evaluation of Bio-CH_4 production in UASB reactors and batch reactors was evaluated to determine the comparison of means of these two processes by means of an analysis of variance (ANOVA), with a P value of 0.05 (Camarena-Martínez *et al*. 2020). All data were processed with Minitab 17.1.0® software. Under pre-established conditions (pH, temperature, and HRT). The ANOVA showed results based on experimental error, number of factors and sum of squares, as well as the values resulting from Fisher's F test and the reliability of the P test.

Analytical methods

The amount of methane was determined by gas chromatography (GC TCD) VARIAN 3400, equipped with a Molecular Sieve 5ª packed column injecting 25 µL of sample. GC conditions were as follows: injector and column 50 °C, detector 200 °C, using argon as carrier gas with flow rate 6 mL/min. Removal of COD was determined according to standard methods (APHA 1998). The pH was determined by extracting liquid sample (4 mL) from effluent and measured by potentiometer (APERA INSTRUMENTS pH 700 benchtop lab pH meter). All reactors were monitored every 24 h. All data presented represents the means from three replications for each experiment.

RESULTS AND DISCUSSION

Biomethane Production in UASB Reactor

Wastewater from the dairy industry was treated in a UASB reactor. The conditions of the reactor are shown in Table 3. The reaction time was established as the time when no organic matter remained and no more Bio-CH_4 was produced. This run lasted 240 h; to carry out the experiment in triplicate, three cycles of 240 h each resulted in a total fermentation time of 720 h. Under these conditions, the biomethane production (Bio-CH_4) and COD removal were calculated, and their kinetics were evaluated.

Table 3. Conditions of the Anaerobic Upflow Reactor (UASB)

Parameters	Measurement
Reactor volume (m^3)	0.00375
Caudal (m^3/d)	0.0302
Hydraulic holding time (TRH) (h)	4.8
Volumetric hydraulic load (m^3 (m^3 d)$^{-1}$)	4.992
Volumetric organic load (Kg DQO/ m^3 d)	9.98
Fermentation temperature (°C)	25 ± 3

Regarding the production of biomethane in the UASB reactor, Table 4 shows the production of Bio-CH_4 expressed in mmol, the specific production (mmol/g of COD $_{removed}$), and the cumulative COD removal effficiency (%), during the three cycles of 240 h, which gave a total of 720 h, which was the duration of the entire fermentation process. During the first days, the production of Bio-CH_4 increased, with the highest production being reflected at 120 h. From then on, production decreased, to start with the new feeding of the new cycle. The same behavior was observed in each cycle because the microorganisms present adapted to the new conditions of the reactor. The pH remained at 6.5, which was reported by Yang *et al*. (2024) as the optimal condition for methane generation.

Table 4. Specific Biomethane Production and COD Removal Efficiency in a UASB Reactor in a Fermentation Time of 720 h

Time (h)	mmol Bio-CH_4	Specific production of Bio-CH_4 (mmol/g COD $_{removed}$)	Cumulative COD removal effficiency (%)
0	0	0	0
24	1.509	0.426809653	8.5
48	1.811	1.280570381	14.625
72	1.891	1.337138923	23.9
96	2.092	0.739633693	29.75
120	2.374	1.678671499	34.05
144	2.052	1.450983115	40.45
168	1.851	1.308854652	49.55
192	1.65	1.166726189	54.35
216	1.408	0.995606348	59.725
240	1.046	0.739633693	64.425
0	0	0	0
24	1.569	1.10945054	6.76
48	1.931	0.682711597	15.73
72	2.092	0.739633693	24.355
96	2.213	1.564827307	28.725
120	2.616	1.84979134	34.63
144	2.374	1.678671499	43.315
168	2.213	1.564827307	50.865
192	1.77	0.312894751	59.04
216	1.287	0.455023214	64.715
240	1.167	0.825193614	69.935
0	0	0	0
24	1.65	1.166726189	9.94
48	1.851	1.308854652	18.74
72	1.891	1.337138923	24.825
96	2.052	1.450983115	29.29
120	2.857	2.020204074	36.23
144	2.575	0.910399981	48.71
168	2.213	1.564827307	55.665
192	2.052	1.450983115	65.415
216	1.811	1.280570381	74.39
240	1.328	0.939037805	77.78

At the end of the experiment, a total specific production of 21.2 mmol of Bio-CH_4 was achieved at 240 h. These results coincide with those reported by Parra Huerta and Campos Montiel (2014), who worked with a UASB reactor with different organic loads, for a period of 20 d, thus obtaining a production of 20 mmol of Bio-CH_4 and a 78.8% removal of organic matter. In relation to the elimination of COD, it was observed that during the experiment COD removal was achieved, but at 216 h removal became stalled. Such behavior was observed during the three cycles. At the end of the experiment, a total COD removal efficiency of 70.7% was achieved. This coincides with a previous report (Kongjan *et al*. 2014) that assessed the anaerobic digestion of serum for the generation of hydrogen and methane in an UASB using anaerobic sludge as inoculum; 23.2 mmol of Bio-CH_4 was obtained with an organic matter removal efficiency of 62%. Ordaz-Díaz and Bailón-Salas (2020) carried out a study for the identification of microbial consortia in the production of methane from vinasse remains, by anaerobic digestion at pH 7.0, finding a microbial diversity in the vinasse residues. These results are directly related to the efficiency and quality of the methane production they generated.

Based on the results obtained to produce Bio-CH_4 in UASB reactor, the experimental data were adjusted with the help of the Gompertz model, which indicated the maximum accumulation of Bio-CH_4 (H_{max}) = 154.7 mL, the maximum reaction rate (R_{max}) = 6.3 mL/d, and with an R^2 = 0.97

Figure 3 shows the production of Bio-CH_4 and the removal of COD, of the three cycles that have a duration of 240 h each, with respect to the production of Bio-CH_4. Initially, there is an increase in production, reflecting a greater obtaining at 120 h. With more time, there is a decrease in the production of Bio-CH_4 because there is less organic matter remaining. For COD removal, the percentage of removal increases over time until the maximum percentage of removal is reached.

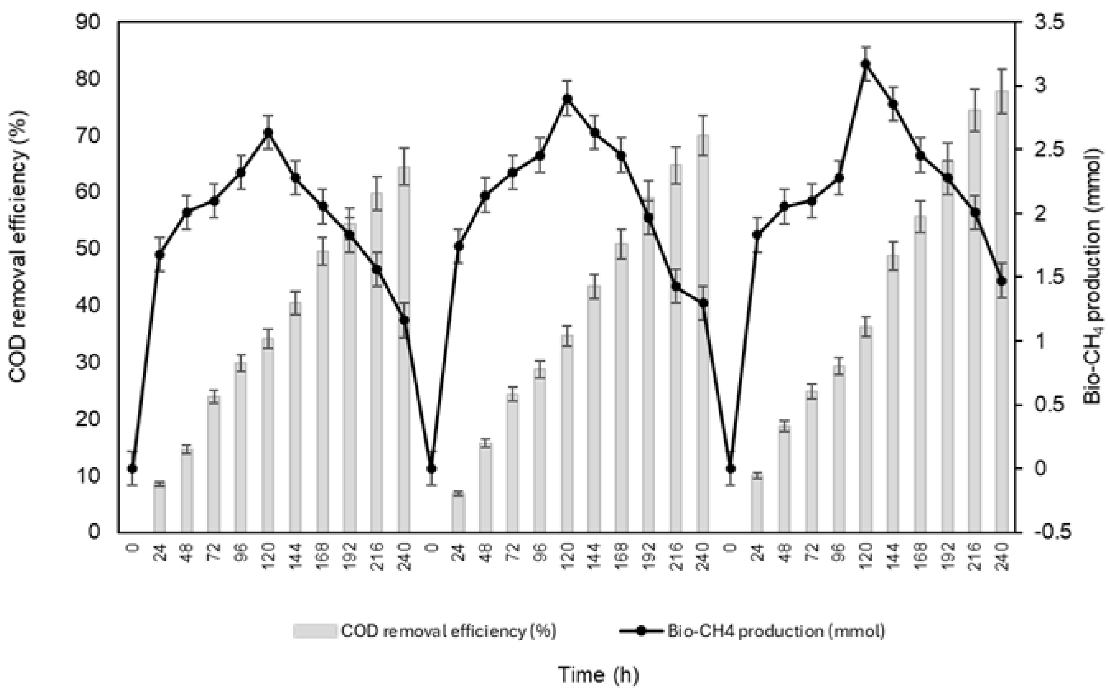

Fig. 3. Production of Bio-CH_4 and COD removal, by means of a UASB reactor, at an initial substrate concentration (20.1 g/L O_2), 3 feed cycles and a total time of 720 h

Biomethane Production in Batch Reactors

Biomethane was produced in batch reactors for a total duration of 192 h, and the experiment was carried out in triplicate. Table 5 shows the specific production of Bio-CH_4 and the efficiency in COD removal. The production of Bio-CH_4 and COD removal increased to 168 hours and then plateaued. This was attributed to the fact that microorganisms no longer have enough substrates for their food, so the amount of biomethane decreases and reaches its maximum point of removal of organic matter. At the end of the fermentation time, a final specific production of 11.6 mmol of CH_4 was obtained. The results are like those achieved by (Xiao *et al*. 2021), who worked on obtaining biomethane by anaerobic co-digestion with sewage sludge. The tests were carried out in batch reactors and a production of 11.8 mmol of Bio-CH_4 was obtained; however, they obtained a low percentage of organic matter removal by presenting only 56.1%. These results are consistent with what was achieved by Sillero *et al*. (2022), who studied the potential for methane generation, using sewage sludge as substrate, through dark fermentation, resulting in a production of 7.4 mmol of accumulated CH_4, with 79.5% of organic matter removal. These results coincide with those reported by de Siqueira *et al*. (2022), who evaluated a microbial consortium and the methanogenic potential through the co-digestion of wastewater from the dairy industry using anaerobic sludge as an inoculum. The experiment was carried out in an anaerobic upflow reactor (UASB) on a laboratory scale, and as a result they reached a total production of 13.73 mmol of Bio-CH_4 g/$COD_{removed}$ and a removal of organic matter of up to 81%. Ren *et al*. (2019) worked on obtaining Bio-CH_4, using wastewater from the dairy industry. As an inoculum they used anaerobic sludge that was collected from an anaerobic upflow reactor (UASB) at laboratory scale. They also generated a biofilm and as a result a final production of 82.1 ± 5.0% higher than in conventional methanogenesis processes was obtained, thus achieving an effective way to treat dairy wastewater and at the same time generate energy.

Table 5. Biomethane Production and COD Removal Efficiency in Batch Reactors at a Substrate Concentration (20.1 g/L O_2) over a Period of 192 h

Time (h)	mmol Bio-CH_4	Specific Production of Bio-CH_4 (mmol/g $COD_{removed}$)	COD Removal Efficiency (%)
0	0	0	0
24	1.086	0.76791796	13.1
48	1.529	0.54058313	30.94
72	1.891	1.33713892	57.81
96	2.495	0.88211571	74.66
120	2.133	1.50825876	87.83
144	1.448	0.51194531	95.85
168	0.764	0.54022958	97.25
192	0.281	0.19869700	97.95

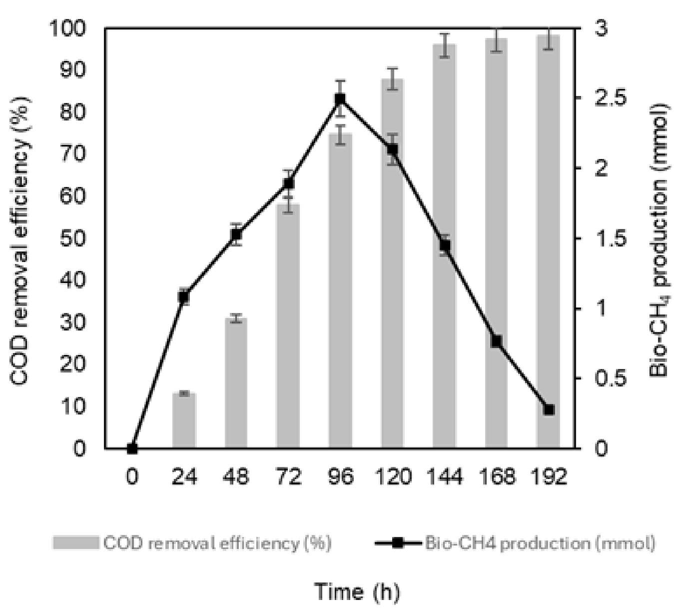

Fig. 4. Production of Bio-CH_4 and COD removal, in batch reactors, at an initial substrate concentration (20.1 g/L O_2) with a fermentation time of 192 h

Based on the results obtained to produce Bio-CH_4 in batch reactors, the experimental data were adjusted with the help of the Gompertz model, which indicated the maximum accumulation of Bio-CH_4 (H_{max}) = 107.4 mL, the maximum reaction rate (R_{max}) = 4.6 mL/d, and with an R^2 = 0.96

Figure 4 shows the production of Bio-CH_4 and the removal of COD in batch reactors, in relation to the fermentation period. Bio-CH_4 production increased over time up to 96 h and then decreased. This is attributed to the decreased quantity of organic matter. Organic matter removal increased over time until the maximum percentage of removal is reached.

The production of Bio-CH_4 and the removal of organic matter in batch reactors was favored because it was possible to maintain the pH at 6.5. This value coincides with those found by Yang *et al*. (2024), who worked on obtaining methane in batch reactors through anaerobic digestion, obtaining efficient results in the production of this biofuel under this condition.

Analysis of Variance (ANOVA)

The statistical evaluation was performed with Fisher's F test, obtaining that the means of the Bio-CH_4 production process in batch reactors and UASB reactors were the same, which means that there was no statistically significant difference between the means of these two processes that are being compared (Table 6). If there were any difference observed in the means, it could be due to chance and not to a real difference between the processes.

Table 6. Results of the Analysis of Variance (ANOVA) of the Production of Bio-CH_4, in Batch Reactors and UASB Reactor

Source	DF	Sum of Squares (SS)	Mean of Squares (MS)	F-Value	P-Value
Factor	1	526.4	526.4	2.45	0.137
Error	16	3441.5	215.1		
Total	17	3967.9			

Table 7 shows the results obtained from the production of Bio-CH_4 in batch reactors and in UASB, for the batch reactors a production of 11.6 mmol was obtained and for the UASB reactor it was 21.2 mmol. In the same way it shows the results of the percentage of efficiency of the COD removal, where in the discontinuous reactors there was a 98.0% removal and for the UASB reactor it presented only 70.7%. The results coincide with those obtained by (Vu and Min, 2019), who worked on the production of methane in an anaerobic digester through fermentation, using sludge from a wastewater treatment plant as an inoculum, and obtained as a result a Bio-CH_4 production of 8.48 mmol and a percentage of removal of organic matter of 89%. Similarly, Pomdaeng *et al*. (2024), evaluated the generation of biomethane using an immobilized mixed microflora as an inoculum and organic food waste as a substrate in batch reactors, achieving a production of 16.7 mmol of Bio-CH_4 and an elimination of 90.6% of organic matter.

Table 7. Comparison of Bio-CH_4 Production in Batch and UASB Reactors

	Production of Bio-CH_4 (mmol)	Production of Bio-CH_4 /h (mmol·h)	Specific production (mmol/g $COD_{removed}$)	Specific production/h (mmol/gCOD $_{removed}$·h)	Specific production (mmol/g $COD_{removed}$ ·VSS)	COD removal effficiency (%)
Batch reactors 192 h	11.63	0.060	0.5904	0.0307	0.046	97.95
UASB reactor 240 h	21.15	0.0881	1.4922	0.0062	0.0048	70.72

Biomethane generation was inconsistent during the fermentation period, but it was observed continuously. These results could be attributed to different factors such as, the presence of Bio-CH_4 consumers and/or inhibitors, the conditions of the reactor, and the hydraulic retention time. *Castello et al*. (2020) stated that in methanogenic reactors, after the start-up period, methane production remains within a certain range that allows for further use. This is evidenced by the large number of large-scale applications.

Additionally, the wastewater generated in both reactors was characterized at the end of the production processes, in accordance with the NOM-001-SEMARNAT-2021 standard. The results are shown in table 8.

Table 8. Characterization of Dairy Wastewater at the End of Biomethane Production Processes

Characterization	Reactor UASB	Batch Reactors
Fats and oils (g/L)	1.6	1.5
pH	5.5	5.1
COD (g/L)	5.87	0.44
TDS (ppm)	175.3	123.5
Temperature (ºC)	30	30
Sulfates (mL/L)	39.1	38.4
Chlorides (mg/L)	97.1	83.2

The values were evaluated through NOM-001-SEMARNAT-2021. It was observed that the values for reuse in the environment were not fully met; however, there are methods that would help to comply with the permitted values, within these methods physical (filtration) and chemical (coagulation-flocculation) treatments are recommended. With any of these subsequent treatments, the NOM would be complied with. Thus, it appears feasible to reuse the wastewater generated.

CONCLUSIONS

1. The work demonstrated the feasibility of producing Bio-CH_4 from wastewater from the dairy industry using *Opuntia imbricata* as a natural support for the immobilization of microbial consortia, because they work better than free cells.
2. Regarding the comparison, these tested biomethane production processes were considered efficient in both processes, verifying that the substrate is appropriate for this

production process, observing that the UASB reactor presented a final specific Bio-CH_4 production of 21.2 mmol, while the batch reactor showed a final specific production of 11.6 mmol. Regarding the efficiency in the degradation of organic matter, the UASB reactor showed 70.7%, while the batch reactors showed 98.0% removal. Verifying according to these results that the UASB reactor is a very appropriate system for the generation of biomethane, in the same way it contributes to the removal of organic matter with results above 70%, it also offers various advantages such as: low energy consumption by operating at room temperature and not requiring any mechanical agitation system, high organic loading speed, low nutritional requirements due to feeding only on dairy wastewater, low operating costs, small assembly space, and low waste production.

3. In general, the generation of Bio-CH_4 is possible under these production processes. However, much remains to be done to improve operating conditions and increase production.

ACKNOWLEDGMENTS

The authors thank the National Council of Humanities, Sciences and Technologies (CONAHCYT) for the support provided to this research and likewise the Faculty of Chemical Sciences of the Autonomous University of Coahuila.

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Article submitted: Nov. 13, 2024; Peer review completed: January 21, 2025; Revised version received and accepted: February 2, 2025; Published: February 10, 2025.
DOI: 10.15376/biores.20.2.2574-2586