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Circular valorization of nopal (*Opuntia spp*.) waste through anaerobic digestion: biofertilizer quality, agronomic response and carbon footprint

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Abstract. *Opuntia* spp. is a strategic crop for arid and semi-arid regions due to its high drought tolerance and widespread cultivation; however, the management of postharvest and pruning residues remains a challenge for sustainable production systems. This study aimed to evaluate the circular valorization of nopal (*Opuntia* spp.) waste through semi-continuous anaerobic digestion, with a primary focus on digestate quality, agronomic relevance, and environmental implications rather than on energy maximization. Reactor operation exhibited a clearly defined pseudo-steady-state (PSS) period characterized by stable methane production (1.85-2.35 L CH_4 d^{-1}), methane concentration (65-67%), and reactor pH (≈7.6), enabling reliable digestate characterization. Digestate produced during the PSS met physicochemical, maturity, and microbiological criteria established by Mexican regulatory standards, supporting its suitability for agricultural reuse. Agronomic validation showed that partial substitution of synthetic fertilizers with digestate improved crop performance when applied at optimized rates; in hydroponic lettuce, a 50% substitution level increased fresh biomass by 15% compared to the control, whereas higher substitution rates reduced growth. A cradle-to-gate carbon footprint assessment indicated a net reduction in greenhouse gas emissions, primarily driven by avoided synthetic fertilizer production. Overall, this integrated evaluation provides a proof-of-concept for the circular valorization of Opuntia-derived residues through anaerobic digestion, supporting sustainable nutrient management strategies for CAM crops and agricultural systems in arid and semi-arid regions.

Keywords: *Opuntia* spp.*, anaerobic digestion, digestate, biofertilizer, circular valorization, carbon footprint, arid and semi-arid regions*

Introduction

The increasing reliance on synthetic fertilizers has contributed substantially to soil degradation, groundwater contamination, and greenhouse gas emissions in agricultural systems worldwide. Nitrogen use efficiency in conventional fertilization practices rarely exceeds 50%, leading to significant nutrient losses through leaching and volatilization, particularly in intensive horticultural systems (Tilman *et al.,* 2002; Zhang *et al*., 2023). Nopal (*Opuntia ficus-indica* (L.) Mill.) cladodes have recently gained increasing attention as an emerging biomass resource for agro-energy applications due to their sustainable production and wide availability in arid and semi-arid regions (Espinosa-Solares *et al*., 2022).

In addition, the manufacture and distribution of mineral fertilizers are highly energy-intensive processes, representing an important source of carbon emissions and raising concerns about the long-term sustainability of current nutrient management strategies (FAO, 2021). These challenges are especially pronounced in arid and semi-arid regions, where water scarcity, low soil organic matter content, and limited nutrient retention capacity constrain agricultural productivity. Mexico exemplifies this situation as large agricultural areas operate under chronic hydric stress while simultaneously generating considerable volumes of organic residues with limited valorization pathways. Recent national estimates indicate that less than 15% of agricultural organic waste is effectively reused, despite its potential for nutrient recycling and renewable energy production (INEGI, 2022; Pérez-Castañeda *et al.,* 2023). Developing integrated approaches that simultaneously address waste management, soil fertility, and environmental impact is therefore a priority for sustainable agriculture in water-scarce regions.

Within this context, nopal (*Opuntia* spp.) represents a strategic crop for arid environments due to its high drought tolerance, crassulacean acid metabolism (CAM), and widespread cultivation. Mexico hosts more than 100 species of *Opuntia* and produces over 800,000 tons of cladodes annually, generating residues from pruning, postharvest handling, and processing activities (CONABIO, 2021; SAGARPA, 2023). These residues are characterized by high moisture content, readily fermentable carbohydrates, and soluble fibers, properties that make them suitable substrates for anaerobic digestion processes (Díaz-García *et al.,* 2020; Rojas-Molina *et al.,* 2022). Nevertheless, most studies on the anaerobic digestion of nopal residues have focused primarily on biogas production under batch or short-term operational conditions, with limited attention to digestate quality, agronomic performance, or environmental implications.

Anaerobic digestion offers a relevant pathway for the circular valorization of agricultural residues by simultaneously producing renewable energy in the form of biogas and generating digestates rich in plant-available nutrients. The agronomic value of digestates depends not only on their nutrient content but also on their stability, maturity, and microbiological safety, which must comply with regulatory standards to ensure safe agricultural application (Möller and Müller, 2012; López-López *et al.,* 2023). In Mexico, organic fertilizers are regulated under standards such as NMX-FF-109-SCFI-2017 and NOM-113-SSA1-1994, which establish criteria for maturity, phytotoxicity, and microbiological safety. However, digestates derived from arid-region substrates, particularly *Opuntia* spp*.,* have rarely been evaluated under operational conditions that allow a clear assessment of their agronomic suitability and regulatory compliance.

Agronomic validation remains a critical gap in many anaerobic digestion studies. Demonstrating crop response under controlled conditions is essential to move beyond theoretical nutrient recovery and to assess the feasibility of partially substituting synthetic fertilizers. Previous studies have shown that digestates derived from fruit and vegetable residues can enhance plant growth when applied at appropriate dilution rates, whereas excessive application may induce salinity stress or nutrient imbalance (Gutiérrez-Miceli *et al.,* 2021; Petric *et al.,* 2022). For digestates obtained from nopal residues in particular, systematic dose-response evaluations under horticultural and semi-arid cropping conditions are still scarce.

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In addition to agronomic performance, the environmental implications of residue valorization strategies are increasingly relevant. Life cycle assessment provides a framework for quantifying the carbon footprint associated with fertilizer production and use, as well as the potential climate benefits derived from nutrient recycling and renewable energy generation (ISO 14067:2018; Alvaro-Fuentes *et al.,* 2021). However, integrated assessments combining anaerobic digestion performance, digestate quality, agronomic response, and carbon footprint remain limited for *Opuntia*-based systems in arid and semi-arid regions.

Therefore, the objective of this study was to evaluate the circular valorization of nopal (*Opuntia* spp.) waste through semi-continuous anaerobic digestion, with a primary focus on digestate quality and agronomic relevance rather than on energy maximization. Specifically, this work aimed to: (i) characterize reactor performance and biogas production under a well-defined pseudo-steady-state (PSS) operational period, ensuring stable and representative digestate generation; (ii) assess the physicochemical, maturity, and microbiological quality of the resulting digestate in relation to Mexican regulatory standards for agricultural reuse; (iii) evaluate crop response to the partial substitution of synthetic fertilizers with digestate under controlled horticultural conditions; and (iv) estimate the associated cradle-to-gate carbon footprint linked to nutrient recycling. By integrating process stability, regulatory compliance, agronomic validation, and environmental assessment, this study provides a proof-of-concept for sustainable nutrient management based on *Opuntia*-derived residues, particularly relevant for CAM crops and agricultural systems in arid and semi-arid regions.

Material and Methods

Study site and substrate characterization

The study was conducted at the Universidad Politécnica de la Región Laguna (UPRL), located in San Pedro de las Colonias, Coahuila, Mexico (25°47′ N, 103°11′ W; 1,429 m a.s.l.). The region is characterized by a semi-arid climate (Köppen BWh), with a mean annual temperature of 22.3 °C and average annual precipitation of approximately 312 mm. Fresh nopal cladodes (*Opuntia ficus-indica*) were collected from the university's experimental plantation. The plants were managed under rainfed conditions without the application of chemical fertilizers or pesticides. The cladodes were harvested approximately 120 days after pruning. Thirty representative cladodes were randomly selected for physicochemical characterization. Samples were washed with potable water, manually chopped, homogenized, and stored at 4 °C for no longer than 24 h prior to analysis.

Moisture content was determined gravimetrically by oven drying at 105 °C for 24 h. Total solids (TS) and volatile solids (VS) were quantified according to APHA standard methods (APHA, 2017). Total sugars were analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1260 Infinity system (Agilent Technologies, USA) equipped with a Hi-Plex Ca column (300 × 7.7 mm) operated at 80 °C. Ultrapure water was used as the mobile phase at a flow rate of 0.6 mL min^{-1}. Sugars were detected using a refractive index detector (RID). Quantification was performed using external calibration curves prepared with analytical standards of glucose, fructose, and sucrose. Crude protein was determined by the Kjeldahl method, while total carbon and nitrogen contents were measured by elemental analysis using a Thermo Scientific® Flash

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2000 CHNS analyzer (Thermo® Fisher Scientific, USA). The C/N ratio was calculated on a dry weight basis. The physicochemical characteristics of the nopal substrate used in this study are summarized in Table 1, including moisture content, total and volatile solids, soluble sugars, elemental composition, and C/N ratio. These baseline characteristics were used to establish feeding rates and operational conditions during anaerobic digestion.

Table 1. Physicochemical characterization of nopal (*Opuntia* spp.) substrate used for anaerobic digestion.

Parameter	Unit	Value (mean ± SD)	Method / Reference
Moisture content	% (wb)	92.5 ± 0.6	Gravimetric (105 °C, 24 h)
Total solids (TS)	% (wb)	7.5 ± 0.6	APHA 2540 G
Volatile solids (VS)	% TS	86.2 ± 1.4	APHA 2540 G
Total sugars	% (wb)	2.3 ± 0.2	HPLC
Total carbon (C)	% (dw)	38.6 ± 1.2	Elemental analysis (CHNS)
Total nitrogen (N)	% (dw)	4.8 ± 0.3	Elemental analysis (CHNS)
C/N ratio	–	8.0 ± 0.7	Calculated

Values correspond to the mean of 30 representative cladodes. wb: wet basis; dw: dry weight.

Anaerobic digester design and inoculum preparation

Anaerobic digestion was carried out in a laboratory-scale semi-continuous reactor with a total volume of 50 L and a working volume of 20 L. The reactor was constructed of high-density polyethylene (HDPE) and operated under mesophilic conditions. Temperature was maintained at 35 ± 1 °C using a submersible aquarium-grade heater (200 W), ensuring stable thermal conditions throughout the experimental period. The reactor was initially inoculated with anaerobic sludge obtained from an operating municipal wastewater treatment plant. The inoculum was acclimated to the nopal substrate for seven days prior to the start of the experimental feeding regime. During this acclimation period, the reactor was fed with diluted substrate at low organic loading rates to promote microbial adaptation and avoid acidification.

Once acclimated, the reactor was operated under a semi-continuous feeding mode with daily substrate addition and an equivalent volume of digestate withdrawal to maintain a constant working volume. The hydraulic retention time (HRT) was set at 10 days. Based on the physicochemical characteristics of the nopal substrate (Table 1), the organic loading rate (OLR) applied during the experiment was 3.5 ± 0.2 kg VS m^{-3} d^{-1}. The reactor was manually mixed for approximately 2 min prior to feeding to ensure homogeneity of the reactor contents. No external pH control or chemical buffering agents were applied during the operation, allowing reactor stability to be governed by the intrinsic buffering capacity of the system. Reactor pH and temperature were monitored daily using a calibrated multi-parameter probe (YSI ProDSS®, Xylem Inc., USA). Biogas produced during digestion was conveyed through gas-tight tubing to a gas collection system for subsequent volumetric and compositional analysis. All reactor connections were periodically checked to prevent gas leakage and to ensure consistent operation over the experimental period.

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Operational protocol and monitoring

The anaerobic digester was operated under a semi-continuous feeding regime throughout the experimental period. Daily feeding consisted of the addition of the prepared nopal substrate, followed by the withdrawal of an equivalent volume of digestate to maintain a constant working volume and a hydraulic retention time (HRT) of 10 days. Based on the defined operational conditions, the organic loading rate (OLR) was maintained at 3.5 ± 0.2 kg VS m^{-3} d^{-1}. The main operational parameters applied during reactor operation are summarized in Table 2.

Table 2. Operational parameters of the semi-continuous anaerobic digester

Parameter	Unit	Value
Reactor total volume	L	50
Working liquid volume	L	20
Headspace volume	L	30
Operating temperature	°C	35 ± 2
Feeding regime	–	Semi-continuous (daily)
Substrate input	kg fresh matter d^{-1}	1.0 ± 0.02
Dilution ratio (substrate:water)	–	1:1 (w/w)
Hydraulic retention time (HRT)	d	10
Organic loading rate (OLR)	kg VS m^{-3} d^{-1}	3.5 ± 0.2
Inoculum-to-substrate ratio	VS basis	2:1
Reactor mixing	–	Manual (daily)
Biogas measurement method	–	Water displacement (ISO 14853:2016)

Biogas volume was measured daily using a calibrated water displacement system, following ISO 14853:2016 guidelines. Measurements of biogas production and reactor operational parameters were recorded at a fixed time each day (09:00 h) to ensure consistency throughout the monitoring period. Biogas volumes are reported as measured under laboratory temperature and pressure conditions. Methane (CH_4) and carbon dioxide (CO_2) concentrations were determined by gas chromatography using an Agilent 7890A gas chromatograph (Agilent® Technologies, USA) equipped with a thermal conductivity detector (TCD). Hydrogen sulfide (H_2S) concentrations were monitored using electrochemical sensors suitable for low-ppm detection ranges. Reactor pH and temperature were monitored daily using a calibrated multiparameter probe (YSI ProDSS®, Xylem Inc., USA) to verify stable mesophilic operating conditions.

The operational phases of the digestion process were identified based on the temporal evolution of methane production, biogas composition, and reactor pH. An initial start-up phase was characterized by low methane content and variable pH values, followed by a transition phase marked by increasing methane concentration and progressive stabilization of reactor performance. A pseudo-steady-state (PSS) period was subsequently identified as the operational phase during which daily methane production rate, methane concentration, and reactor pH exhibited minimal variation.

For quantitative consistency, the pseudo-steady-state (PSS) period was defined as the phase in which methane production rate, methane concentration, and reactor pH presented a coefficient of variation below 10% over at least five consecutive days. Only data corresponding to this PSS period were used for digestating characterization, agronomic validation, and carbon footprint assessment, ensuring that downstream analyses were based on stable and representative reactor operation.

Digestate characterization and regulatory compliance

Digestate samples were collected during the pseudo-steady-state (PSS) period and analyzed for physicochemical, agronomic, and microbiological properties. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were determined following APHA standard methods (APHA, 2017). The BOD_5/COD ratio was used as an indicator of digestate stability. Total nitrogen was determined by the Kjeldahl method, ammonium nitrogen by spectrophotometry using a UV-Vis spectrophotometer (Jenway 5610, Cole-Parmer®, UK), total phosphorus by colorimetric analysis, and potassium by flame photometry using a digital flame photometer (Jenway PFP7, Cole-Parmer®, UK).

Digestate maturity and phytotoxicity were evaluated using a germination index (GI) test with barley (*Hordeum vulgare* L.) seeds, following the criteria established in NMX-FF-109-SCFI-2017. Germination percentage and root elongation were measured after 72 h of incubation at 25 °C under controlled conditions, and the GI was calculated relative to a distilled water control.

Microbiological safety was assessed using the criteria established in NOM-113-SSA1-1994 as a general sanitary reference framework for evaluating microbial contamination in organic materials intended for agricultural use. Total coliforms and *Escherichia coli* were quantified by membrane filtration using selective media, while *Salmonella* spp. detection was performed through pre-enrichment, selective enrichment, and plating on xylose lysine deoxycholate (XLD) agar.

Agronomic validation experiments

Agronomic performance of the digestate was evaluated through controlled greenhouse experiments to assess its potential for partial substitution of synthetic fertilizers under horticultural conditions. Lettuce (*Lactuca sativa* L.) seedlings were cultivated in a deep-water culture (DWC) hydroponic system under greenhouse conditions (25 ± 2 °C; natural photoperiod). The treatments consisted of the partial substitution of a standard nutrient solution with digestate at different substitution levels (0, 25, 50, and 75%, v/v). A randomized complete block design was applied, with three replicates per treatment. The electrical conductivity and pH of the nutrient solutions were monitored throughout the experiment to avoid excessive salinity stress and to ensure that plant responses were attributable to nutrient composition rather than uncontrolled physicochemical conditions.

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Carbon footprint assessment

A cradle-to-gate carbon footprint assessment was performed following ISO 14067:2018 guidelines, using 1 kg of fresh lettuce at farm gate as the functional unit. System boundaries included substrate transport, anaerobic digester operation, digestate application, and avoided emissions associated with the partial substitution of synthetic fertilizers. Infrastructure construction and end-of-life stages were excluded from the system boundaries.

Emission factors for nitrogen, phosphorus, and potassium fertilizers were obtained from IPCC guidelines. Carbon footprint calculations were performed using spreadsheet-based modeling. Uncertainty analysis was conducted using Monte Carlo simulation with 10,000 iterations to evaluate the robustness of the estimated emission values.
Results derived from the carbon footprint assessment are intended for comparative purposes within the defined system boundaries.

Statistical analysis

All data were analyzed using R software (version 4.3.1). Analysis of variance (ANOVA) was performed to identify significant differences among treatments, followed by Tukey's HSD test at a significance level of $\alpha = 0.05$. Data are presented as mean ± standard deviation unless otherwise stated.

Results and Discussions

Reactor performance and biogas production

The semi-continuous anaerobic digestion of nopal (*Opuntia* spp.) waste exhibited a clear progression through start-up, transition, pseudo-steady-state (PSS), and post-PSS operational phases. During the start-up phase (days 0-4), methane concentrations remained below 45% and reactor pH values were lower than 6.8, indicating the predominance of acidogenic activity and the incomplete establishment of methanogenic populations. This response is characteristic of anaerobic systems fed with readily fermentable substrates and has been widely reported for fruit and vegetable residues (Li *et al.,* 2020). Between 5 and 8 days, a transition phase was observed, characterized by a rapid increase in methane concentration (from approximately 52 to 64%) and progressive stabilization of reactor pH toward neutral values. During this phase, hydrogen sulfide concentrations reached their maximum values and subsequently declined, reflecting the gradual establishment of a more balanced microbial consortium and the stabilization of sulfur-transforming microbial pathways. Similar transitional dynamics have been reported in semi-continuous digesters treating carbohydrate-rich biomass (Wang *et al.,* 2021). Based on daily methane production rates, a pseudo-steady-state (PSS) period was clearly identified between 10 and 24 days of operation, as illustrated in Figure 1. During this period, methane production remained within a narrow range (1.85-2.35 L CH_4 d^{-1}), and the coefficient of variation for daily methane production remained below 10%, indicating stable reactor performance under the applied semi-continuous feeding regime.

The stabilization of biogas composition and reactor pH during the PSS period further confirms the establishment of stable methanogenic conditions (Figure 2). Methane concentration

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remained stable between 65 and 67%, while reactor pH was buffered at approximately 7.6 throughout this operational phase.

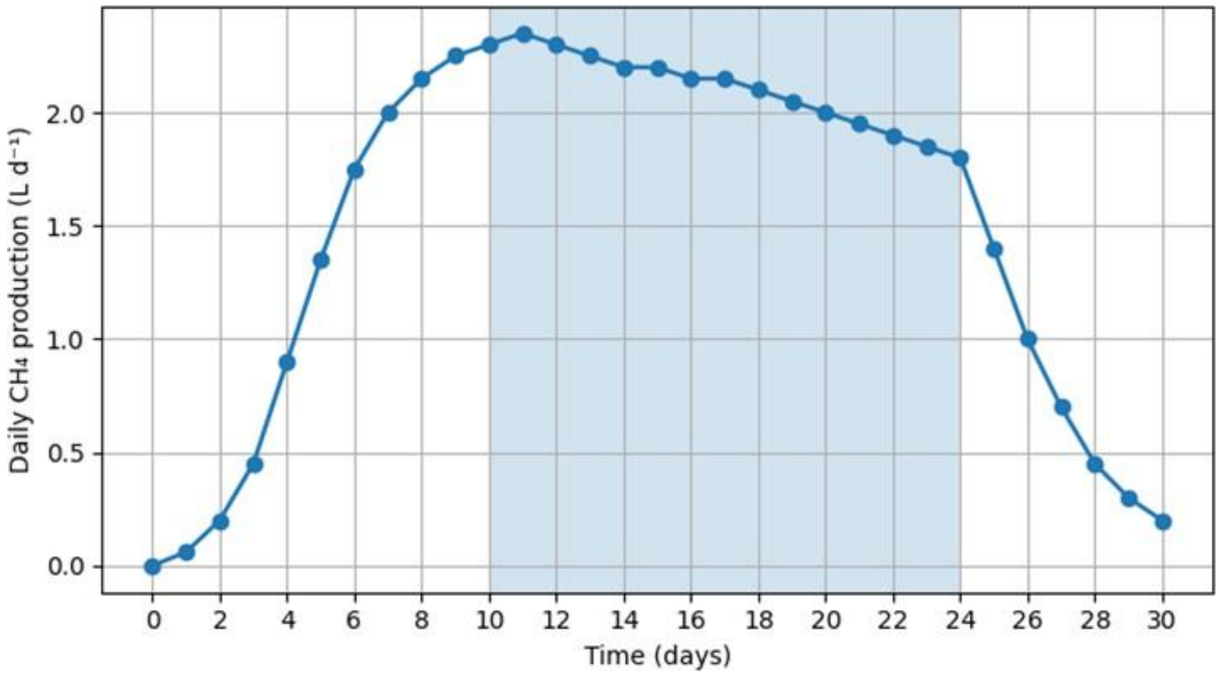

Figure 1. Daily methane production during semi-continuous anaerobic digestion of nopal (*Opuntia* spp.) waste.

Average reactor performance parameters during the PSS period, including methane production rate, gas composition, hydrogen sulfide concentration, pH, and temperature, are summarized in Table 3. These values provide a representative snapshot of reactor stability under the applied operating conditions.

Table 3. Average reactor performance parameters during the pseudo-steady-state (PSS) period (days 10-24).

Parameter	**Unit**	**Value (mean ± SD)**
Methane production rate	L CH_4 d^{-1}	2.12 ± 0.18
Methane content	% (v/v)	66.1 ± 0.8
Carbon dioxide content	% (v/v)	33.2 ± 0.9
Hydrogen sulfide	ppm	295 ± 18
Reactor pH	–	7.6 ± 0.1
Temperature	°C	36.0 ± 0.2

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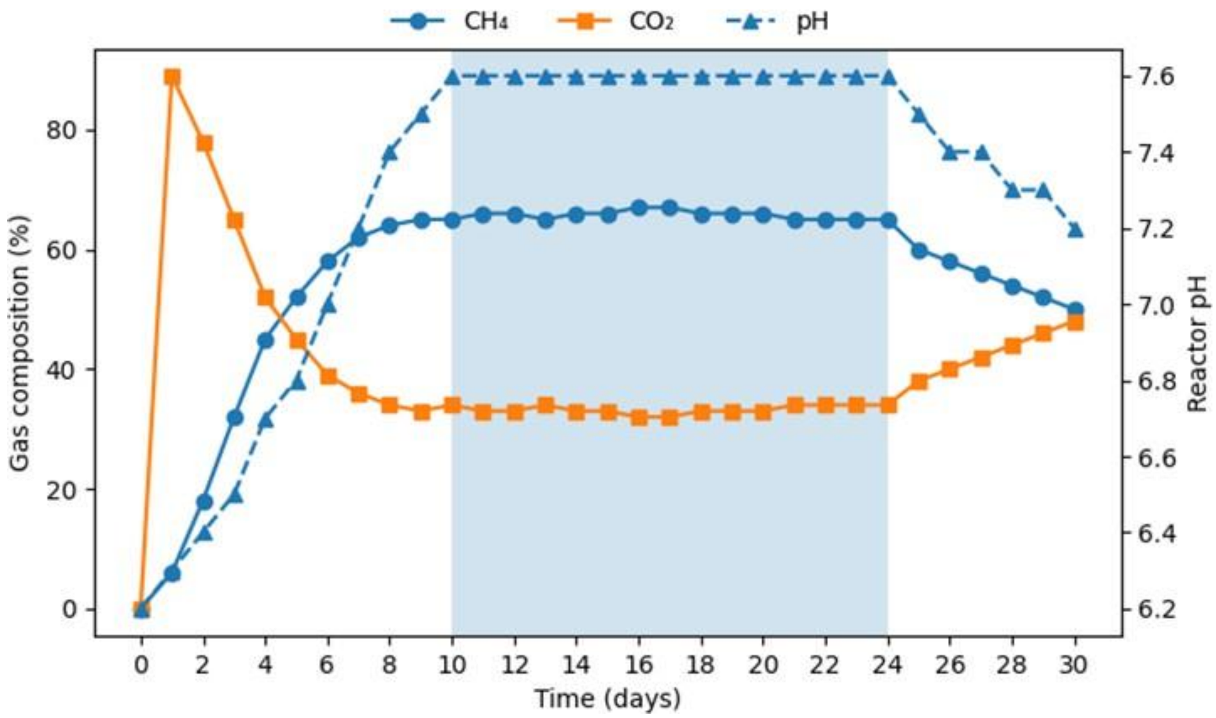

Figure 2. Biogas composition and reactor pH during semi-continuous anaerobic digestion of nopal (*Opuntia* spp.) waste.

After day 24, methane productivity gradually declined, accompanied by a decrease in methane concentration and a slight increase in CO_2 proportion. Given the semi-continuous feeding regime, this behavior cannot be attributed to substrate depletion. Instead, it is more likely associated with the progressive accumulation of poorly degradable solids and sedimented biomass, which may reduce the effective working volume and limit mass transfer within the reactor. Nopal residues are rich in mucilage and soluble fibers, which can promote flocculation and sediment formation in small-scale digesters, as previously reported for fiber-rich substrates (Monlau *et al.,* 2020). This post-PSS decline does not compromise the objectives of the present study, as the PSS period provided a sufficiently stable operational window for reliable digestate characterization and downstream agronomic evaluation.

Digestate quality and regulatory compliance

Digestate samples collected during the pseudo-steady-state (PSS) period exhibited physicochemical characteristics suitable for agricultural reuse. Digestate pH remained within the neutral range (7.5-7.7), which is favorable for nutrient availability and soil application. The BOD_5/COD ratio averaged 0.61 ± 0.05, exceeding the maturity threshold established in NMX-FF-109-SCFI-2017 and indicating a stabilized organic matrix with low phytotoxic potential. Comparable values have been reported for mature digestates derived from vegetable residues under mesophilic conditions (Gutiérrez-Miceli *et al.,* 2021).

The digestate showed a C/N ratio close to 8, reflecting effective nitrogen mineralization during anaerobic digestion. While low C/N ratios are generally associated with enhanced nitrogen

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availability, they also require controlled application rates to avoid excessive ammonium inputs (López-López *et al.,* 2023). Nutrient concentrations further confirmed the potential of the digestate as a partial substitute for synthetic fertilizers rather than as a complete nutrient source. A summary of digestate physicochemical and microbiological quality parameters, together with their corresponding regulatory reference standards (Table 4).

Table 4. Physicochemical and microbiological quality of digestate collected during the PSS period.

Parameter	Unit	Value (mean ± SD)	Reference standard
pH	–	7.6 ± 0.1	NMX-FF-109
BOD_5/COD	–	0.61 ± 0.05	NMX-FF-109
C/N ratio	–	8.1 ± 0.6	NMX-FF-109
Total nitrogen (N)	$g\ L^{-1}$	2.7 ± 0.2	–
Phosphorus (P_2O_5)	$g\ L^{-1}$	1.2 ± 0.1	–
Potassium (K_2O)	$g\ L^{-1}$	4.2 ± 0.3	–
Germination index	%	96 ± 2	NMX-FF-109
Salmonella spp.	Presence/absence	Absent	NOM-113
E. coli	CFU mL^{-1}	<10	NOM-113

Microbiological analysis confirmed compliance with Mexican safety standards. No strains of *Salmonella spp*. were detected, and *Escherichia coli* counts remained well below the limits established in NOM-113-SSA1-1994. These results are consistent with previous studies demonstrating effective pathogen reduction during mesophilic anaerobic digestion operated at sufficient retention times (Alburquerque *et al.,* 2021). Phytotoxicity evaluation using the germination index (GI) yielded values above 90%, confirming digestate maturity and the absence of inhibitory effects on seed germination. GI values above 80% are widely accepted as indicative of digestates suitable for agricultural application (Petric *et al.,* 2022).

Agronomic response to digestate application

In hydroponic lettuce cultivation, partial substitution of the standard nutrient solution with digestate significantly affected plant growth, revealing a clear dose-response pattern across treatments (Table 5). Intermediate substitution levels enhanced vegetative development, whereas excessive substitution resulted in growth inhibition. Among the evaluated treatments, the 50% substitution level produced the highest fresh biomass, representing a 15% increase compared with the control receiving only synthetic fertilizer. Plant height and number of leaves showed similar trends among treatments; however, statistical analysis indicated no significant differences for these variables according to Tukey's HSD test ($\alpha = 0.05$). This response suggests improved nutrient use efficiency at moderate digestate concentrations, likely associated with the combined availability of readily assimilable mineral nutrients and low-molecular-weight organic compounds present in the digestate. In contrast, higher substitution rates (≥75%) negatively affected plant growth, indicating that nutrient imbalance and osmotic stress can outweigh potential biofertilization benefits when digestate is applied at excessive proportions in hydroponic systems.

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Table 5. Effect of digestate substitution on hydroponic lettuce growth parameters

Digestate substitution (%)	Fresh biomass (g plant^{-1})	Plant height (cm)	Number of leaves
0 (control)	28.5 ± 1.2^{b}	18.4 ± 0.8	14.2 ± 1.0
25	30.1 ± 1.3^{b}	19.1 ± 0.7	15.0 ± 0.9
50	32.8 ± 1.1^{a}	20.3 ± 0.9	16.4 ± 1.1
75	26.9 ± 1.3^{c}	17.6 ± 0.8	13.5 ± 0.8

Different superscript letters within a column indicate significant differences according to Tukey's HSD test ($\alpha = 0.05$).

The observed dose-response pattern reveals a clear optimum at intermediate digestate concentrations. This response may be associated with improved nutrient use efficiency at moderate substitution levels, potentially related to the presence of organic nitrogen forms and low-molecular-weight organic compounds in the digestate that can enhance nutrient uptake and plant metabolic activity (Rivas-García *et al*., 2021). The overall biomass response trend is illustrated in Figure 3 and is consistent with previous reports on digestate-based fertilization strategies for leafy vegetables under hydroponic systems (Poveda *et al.,* 2021).

In contrast, higher substitution rates (≥75%) resulted in reduced biomass production and diminished vegetative growth. This inhibitory effect is likely attributable to increased electrical conductivity and nutrient imbalance, both of which are recognized constraints when digestates are applied at excessive concentrations in hydroponic systems (Gutiérrez-Miceli *et al.,* 2021). These findings highlight the importance of optimizing digestate dilution to balance nutrient availability and osmotic stress in soilless cultivation systems.

Carbon footprint assessment

The cradle-to-gate carbon footprint assessment indicated that the circular valorization of nopal waste through anaerobic digestion can contribute to a reduction in net greenhouse gas emissions when digestate is used to partially substitute synthetic fertilizers. Avoided emissions associated with mineral fertilizer production represented the main contribution to emission reduction, outweighing the emissions derived from digester operation and substrate transport. A detailed breakdown of emission sources and credits is shown in Table 6.

Table 6. Cradle-to-gate carbon footprint of lettuce production with digestate substitution

Contribution	kg CO_2-eq kg^{-1} lettuce
Digester operation	0.013
Substrate transport	0.005
Avoided N fertilizer production	−0.048
Avoided P_2O_5 fertilizer production	−0.012
Avoided K_2O fertilizer production	−0.010
Net carbon footprint	−0.015 ± 0.005

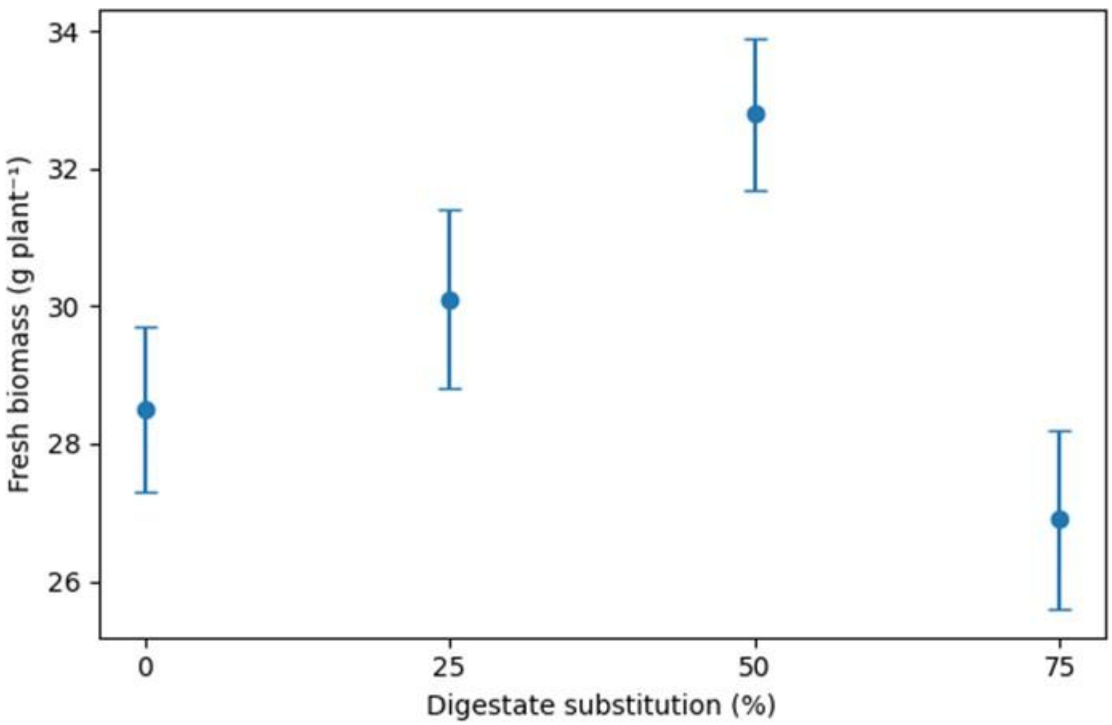

Figure 3. Effect of digestate substitution on fresh biomass of hydroponic lettuce (*Lactuca sativa* L.).

The estimated net carbon footprint was negative (−0.015 ± 0.005 kg CO_2-eq kg^{-1} lettuce), primarily due to the avoided production of N, O and K fertilizers. These results are consistent with previous life cycle assessments reporting that nutrient recycling is a key driver of climate benefits in anaerobic digestion systems, particularly when digestates are effectively reused in agricultural production (Alvaro-Fuentes *et al.,* 2021).

It should be noted that the present carbon footprint assessment represents a simplified cradle-to-gate approach and is intended for comparative purposes within the defined system boundaries. Negative carbon footprint values reflect avoided emissions associated with the partial substitution of synthetic fertilizers and should not be interpreted as net-negative food production systems beyond the farm-gate boundary. Infrastructure construction, downstream post-harvest stages, and long-term soil carbon dynamics were excluded from the analysis and may influence absolute emission values. Nevertheless, within the applied methodological framework, the results provide a robust indication of the potential climate benefits derived from integrating anaerobic digestion and digestate-based fertilization strategies in arid and semi-arid agricultural systems.

Implications and limitations

The results of this study demonstrate that semi-continuous anaerobic digestion of nopal (*Opuntia* spp.) waste can sustain stable methanogenic conditions over a well-defined pseudo-steady-state (PSS) period, enabling the generation of a digestate that complies with regulatory quality and safety criteria and delivers measurable agronomic and environmental benefits.

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Beyond demonstrating process feasibility, the integrated evaluation of reactor stability, digestate quality, crop response, and carbon footprint highlights the relevance of anaerobic digestion as a circular nutrient management strategy rather than solely an energy recovery process. The gradual decline in reactor performance observed beyond the PSS period represents an operational limitation linked to solids accumulation and mass transfer constraints in mucilage- and fiber-rich substrates, such as nopal residues, and does not undermine the validity of the agronomic and environmental assessments derived from stable operation.

The gradual decline in reactor performance observed after the PSS period represents an operational limitation rather than a constraint to the objectives of the present study. This behavior highlights the importance of solids accumulation and mass transfer effects in semi-continuous digesters treating mucilage- and fiber-rich substrates, such as nopal residues. Although long-term operational optimization was beyond the scope of this work, future studies could evaluate management strategies such as co-digestion with complementary substrates or periodic solids removal to mitigate performance decline and improve process stability.

Conclusions

This study demonstrates that semi-continuous anaerobic digestion of nopal (*Opuntia* spp.) residues can be stably operated under a well-defined pseudo-steady-state (PSS) period, enabling the production of a digestate that complies with physicochemical and microbiological regulatory standards for agricultural reuse. Digestate characterization confirmed adequate stabilization and maturity, while agronomic validation under hydroponic conditions showed that partial substitution of synthetic fertilizers with digestate-particularly at intermediate substitution levels-can enhance lettuce growth without adverse effects. In addition, the cradle-to-gate carbon footprint assessment indicated that avoided emissions associated with synthetic fertilizer production can offset emissions derived from digester operation, resulting in a net reduction in greenhouse gas emissions within the defined system boundaries. Overall, these findings provide an integrated proof-of-concept for the circular valorization of *Opuntia*-derived residues through anaerobic digestion, supporting sustainable nutrient management strategies for CAM crops and agricultural systems in arid and semi-arid regions.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article, but are available from the first author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

J.L.R.-P. and S.S.M.-R. contributed to the conceptualization of the study. J.L.R.-P., R.Z.-V., and J.G.L.-O. developed the methodology. The investigation was carried out by J.L.R.-P., A.G.-T., and M.V.P.-G. Data analysis was performed by J.L.R.-P. and R.Z.-V. S.S.M.-R. supervised the research and was responsible for project administration. The original draft of the manuscript was prepared by J.L.R.-P., and the manuscript was reviewed and edited by S.S.M.-R., R.Z.-V., and J.G.L.-O. S.S.M.-R. served as the corresponding author. All authors read and approved the final manuscript.

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