Optimization of anaerobic digestion under psychrophilic conditions using plant biofilms: evaluation of biogas yield and quality in a rural tubular biodigester

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Abstract: This study evaluates the performance of a tubular biodigester operating in psychrophilic conditions, incorporating plant lianas as biofilm support to improve the anaerobic digestion of bovine manure. A 12 m³ biodigestion system was constructed in Chachapoyas, Peru, and loaded with a manure:water mixture (1:5). Physicochemical parameters, the production and quality of biogas, were monitored, and the study applied the Gompertz model to describe the kinetic behavior. The results show that, despite operating at average temperatures of 16.95 °C, the system reached its hydraulic retention time (HRT) in just 15 days, generating 3 m³ of biogas with a daily production of 0.2–0.3 m³. Purification reduced H_2S by 75 % and purified methane reached 68.18 %, its suitability for domestic energy use. The Gompertz model adequately adjusted the data (R^2 = 0.9992), projecting a potential production of 3.89 m³. The use of plant biofilms improved microbial retention and process stability, suggesting a low-cost solution with high replicability in cold rural areas.

Keywords: lianas, HRT, Gompertz, methanogenesis, biofertilizers

Introduction

Biogas, composed mainly of methane and carbon dioxide, is generated by anaerobic digestion (AD), a microbiological process that decomposes organic matter in the absence of oxygen (Ni, 2024; Gerardi, 2003). This technology contributes to mitigating climate change by capturing methane (CH_4) generated during AD, preventing its release into the atmosphere, where it has a global warming potential 23 times greater than CO_2 (Rivas-Solano *et al*., 2016), with a mitigation potential of up to 200 $MtCO_2eq$ per year (Rodríguez-Jiménez *et al*., 2022). This aligns with energy sustainability objectives by partially replacing fossil fuels and reducing emissions associated with their use.

AD is carried out in biodigesters, airtight containers that create anaerobic conditions (Ni, 2024). Their application in rural areas is efficient due to the availability of biodegradable substrates, their low cost and versatility, allowing the treatment of agricultural, food and livestock waste, the generation of clean energy and the reduction of solid waste (Aridi &Yehya, 2024).

In addition, biodigesters generate by-products such as biol and biosol, used as organic fertilizers, improving agricultural yields and rural income (Barrena *et al*., 2019; Martí-Herrero *et al*., 2014). Although this technology was introduced in Latin America in the 1970s and 1980s, its consolidation has occurred recently, with successful results in rural communities (Garfí *et al*., 2016).

There are three main types of small-scale biodigesters: fixed dome, floating drum, and tubular. Fixed dome digesters, often buried to reduce construction costs, experience variations in pressure and efficiency due to changes in temperature and volume (Kinyua *et al*., 2016). In contrast, tubular biodigesters (Taiwanese model) use inexpensive materials such as polyethylene or heat-sealed PVC, incorporate external gasometers, and are common in rural areas, where they operate with volumes of 6 to 10 m³ (Kinyua *et al*., 2016; Njoki *et al*., 2013; Barrena *et al*., 2019; Ferrer *et al*., 2011).

The efficiency of anaerobic digestion is strongly dependent on temperature. In cold environments, microbial activity is reduced, leading to the accumulation of volatile fatty acids and lower biogas production (Riau *et al*., 2010; Kashyap *et al*., 2003). This challenge is relevant in high Andean regions, where temperatures fluctuate between -15 and 20 °C and extreme events such as frost and cold occur (Álvarez & Lidén, 2008; Poveda *et al*., 2020), affecting health, livestock, and crops, especially in areas such as southern Peru and the Bolivian Amazon (Marengo *et al*., 2012; Chávez & Takahashi, 2017).

Anaerobic digestion relies on microbial communities that carry out the stages of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Abendroth *et al*., 2020). The use of biofilms on plants or synthetic supports improves microbial retention, enhancing the production and stability of biogas in cold climates (Gong *et al*., 2011).

This study evaluates a tubular biodigester operated under psychrophilic conditions, using lianas as biofilm support to optimize AD with 1:5 diluted bovine manure. Biogas production, composition and efficiency were analyzed using the modified Gompertz model, demonstrating the viability of this low-cost technology in cold rural areas.

Materials and Methods

Study Area

The experimental site was located on the central campus of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas – UNTRM (Figure 1), in Chachapoyas, Peru (6°14'1.3" S; 77°51'7.6" W). The city has a variable climate, with temperatures ranging between 11°C and 26°C, with maximums of 21°C in November and minimums of 7.5°C in August. The highest rainfall occurs in March, with 136.7 mm/month (SENAMHI, 2025).

Figure 1. Location of the experimental site

Biogas production system

The biogas production system was designed according to the criteria of Barrena *et al*. (2019) and included a biodigester, a gasometer and a purification and control panel (Figure 2).

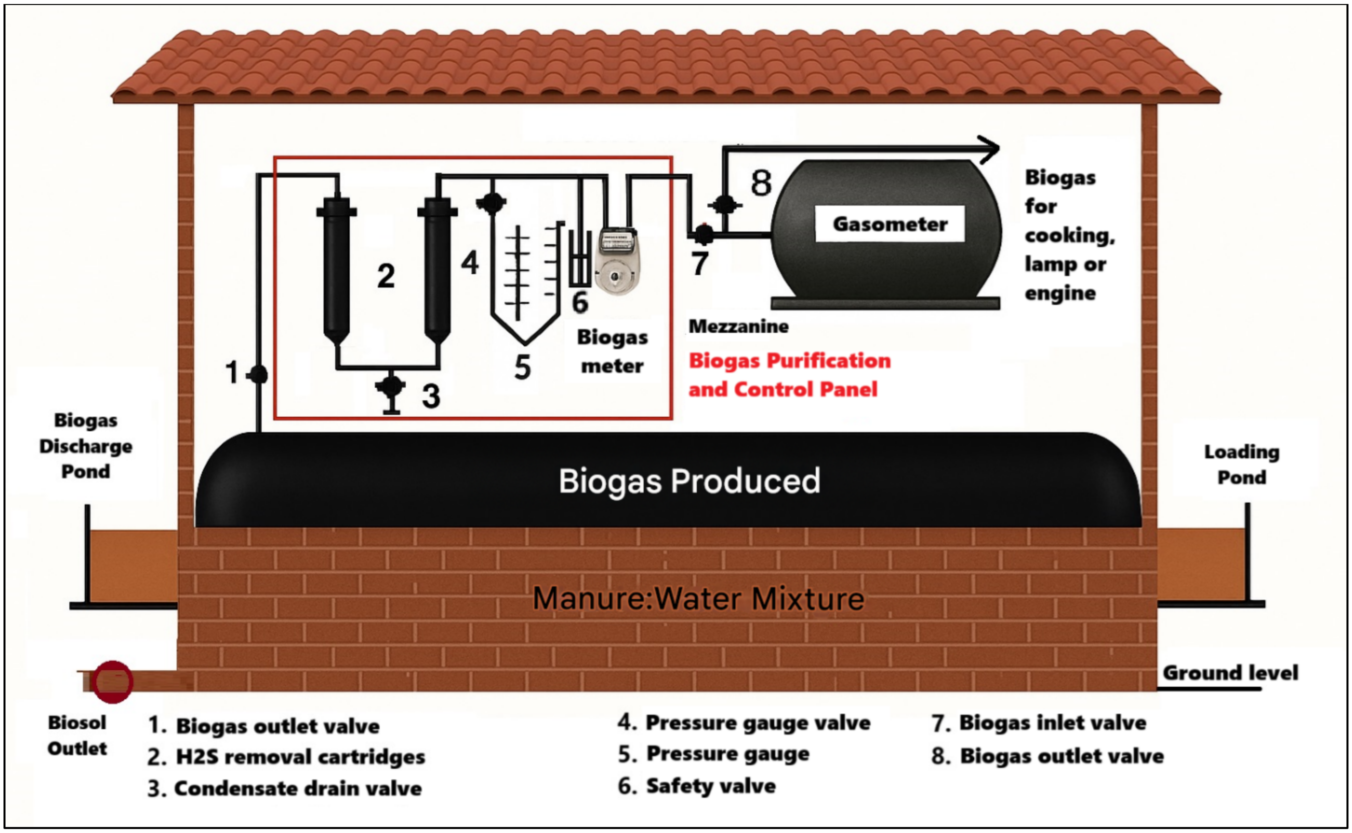

Figure 2. Biodigestion system

The tubular biodigester was built with 1.0 mm thick PVC geomembrane, with a diameter of 1.27 m, a length of 10 m and a total volume of 12 m³. It had connections for loading, discharging biol and biosol, as well as a top output of biogas by through a nipple and a 1" stopcock.

The gasometer was built with 1 mm thick PVC geomembrane, with dimensions of 1.50 m x 3 m and a volume of 3 m³. It had a 1" PVC threaded nipple and a 1" stopcock for the inlet and outlet of the biogas. The biogas purification and control panel was consisted of two heavy PVC pipe cartridges 2" and 50 cm long, with reductions to 1/2" at both ends, connected to a bridge with a valve to eliminate condensate water. Each cartridge was filled with 2.5 kg of 2" iron nails and iron sponges at both ends, to remove hydrogen sulfide (H_2S).

The pressure gauge was constructed with a U-shaped 1/4" PVC hose, partially filled with water, whose height difference indicated the biogas pressure. To regulate it, a 3 L plastic bottle with water up to 3/4 of its volume was used, into which the pipe was inserted, marked with grooves every centimeter to facilitate reading.

Installation of lianas as biofilms

Lianas are perennial, herbaceous or woody climbing plants with long and thin stems, their leaves are simple and alternate. These plants are found mainly in Tropical America, widely distributed in tropical and temperate regions (Xu & Chang, 2017).

The lianas were collected in Villa Cocochó (Camporredondo, Luya, Amazonas; 6°11'32" S; 78°19'14" W) and transferred to the experimental site. They were dried for a month to facilitate spherical weaving. Eighty structures were made in the form of bundles or compact baskets of approximately 10 cm in diameter, fixed to a rope with a 5 cm separation between them.

Before installing the biodigester in the pool, the spherically intertwined lianas were introduced using the rope attached to a 1/2" PVC pipe. This assembly was carefully inserted through the loading and unloading holes of the biodigester, ensuring adequate internal distribution of the lianas within the system.

Operating Parameters

For the loading of the biodigester, cattle manure from the UNTRM stable was used as substrate, mixed with water in a 1:5 ratio. To load 9 m³, 45 cylinders of 200 L were prepared, each containing 33.3 kg of manure. Although the mixture was calculated based on weight, the ratio corresponds to 1:5 by volume, considering that the density of manure is close to 1 kg/L (Aremanda *et al*., 2023), meaning that for each part of manure five parts of water were added. The loading was carried out daily with 4 cylinders, and was completed in 12 days. Subsequently, anaerobic digestion was allowed to produce biogas and biofertilizers (biol and biosol).

To measure ambient and biodigester temperature, four Data Loggers Yowexa SSN22ET were installed: at the inlet, center (10 cm deep) and outlet of the biodigester, and outside, on the structure of the gasometer. They recorded temperature every 15 minutes, from 6:00 a.m. to 6:00 p.m., throughout the process until the hydraulic retention time (HRT) was reached. Then, the temperatures of the three points in the biodigester and the environment were averaged. After completing the loading of the biodigester, a sample of the manure-water mixture was collected and its pH was measured with a portable multiparameter meter (model 3620 IDS, WTW brand), which measures the acidity/alkalinity of the substrate on a scale of 0 to 14.

To measure the volume of biogas produced, a diaphragm-type meter G1.6 was installed on the purification board, connected to the 1/2" PVC pipe by two universal joints of the same diameter. This high-precision, low-pressure drop equipment is widely used for reliable measurement of low natural gas flow rates. When the biodigester dome reached its gas capacity, the biogas composition was evaluated using the Multitec 545 equipment to measure CH_4, CO_2, O_2, H_2S and CO. The measurements were made at two points: upstream of the purification panel and at the connection between the gasometer and the pipe to the kitchen.

The hydraulic retention time was defined as the period, in days, from the loading of the biodigester with the manure-water mixture to the observation of biogas accumulation in the biodigester and gasometer. This value was compared with the one estimated by the formula proposed by Barrena *et al*. (2019), based on the ambient temperature conditions, as presented in equation 1:

$$HRT = -44.705\ ln(T) + 160.394 \quad \textbf{(1)}$$

Where T represents the ambient temperature of the location where the biodigester is installed.

Mathematical modelling of cumulative biogas production

The modified Gompertz model, commonly used to model biogas production because it describes the sigmoidal dynamics of biological processes, was applied (Ofon *et al*., 2025) . This model allows researchers to identify the lag phase, exponential growth and stabilization, using equation 2:

$$B(\mathrm{t}) = B_{max} * \exp\left(-exp\left(\frac{R_m * e}{B_{max}}(\lambda - t) + 1\right)\right) \quad \textbf{(2)}$$

Where B(t) is cumulative biogas production at time t, B_{max} is theoretical cumulative biogas production, R_m is maximum biogas production rate, λ lag time or lag phase, and e Euler number.

The Gompertz model was projected up to 40 days, the standard reference value in psychophilic processes with bovine manure. In mesophilic conditions, HRT typically varies between 20 and 40 days, especially when using dry or diluted manure (Song *et al*., 2023).

Energy yield of biogas

The evaluation of energy yield was carried out upon reaching the maximum HRT, using the biogas stored in the gasometer. A two-burner stove adapted for biogas was used, connected by a steel reduction from 1/2" to 3/8" and secured with a clamp. Potatoes, eggs and water (common foods in rural families) were cooked, and the system recorded the time and volume of biogas consumed.

Results and Discussion

Operating and productive parameters of the biodigester

The operating conditions of the biodigester with liana-based biofilm support were evaluated. Table 1 summarizes the main physical, chemical and productive parameters recorded during the anaerobic digestion process.

Table 1. Parameters obtained in the production of biogas.

Category	Parameter	Value	Unit	Remarks
Operating conditions	Manure-water ratio	1:5	-	Initial mix ratio
	Ambient temperature	19.91	°C	External conditions
	Biodigester temperature	16.95	°C	Thermal efficiency
	pH	7.49	-	Optimal range for methanogenesis
Retention Time	Design	34	Days	Based on theoretical design
	Real	15	Days	Observed in operation
Biogas production	Initial Volume (First 15 Days)	3	m^3	Accumulated
	Daily production	0.2	m^3/day	Average after stabilization
	Initial pressure	12	cm	Water column
	Daily pressure	2.4	cm	Water column

The 1:5 ratio (manure:water) is commonly used because it improves substrate fluidity, facilitates microbial activity, and reduces clogging. In addition, it optimizes biogas production by increasing the accumulated volume and improving its combustion quality (Tian *et al*., 2023). Proper dilution also improves the physical conditions of the system and its energy performance (Hadiyanto *et al*., 2023).

The average ambient temperature was 19.91 °C, as the experiment was carried out in September and October, during the dry season in Chachapoyas, which generates warmer days (Rascón *et al*., 2021). However, this value indicates a low mesophilic range, close to the optimal lower limit for anaerobic digestion (20–45 °C) (Feghhipour *et al*., 2024).

The internal temperature of the biodigester was 16.95 °C, indicating low psychrophilic conditions. Although mesophilic conditions are more favorable owing to the higher microbial diversity than thermophilic digesters (Liu *et al*., 2022), low temperatures reduce microbial activity and affect optimal biogas production (Hadiyanto *et al*., 2023; Liu *et al*., 2022).

Despite the suboptimal temperatures, the use of biofilm as structural support favored biogas production by providing a stable environment for microbial growth (Wu *et al*., 2023). Biofilms improve gas absorption, facilitate metabolic interactions (Zhang *et al*., 2025), and allow biomass to be retained in the reactor for longer, sustaining its performance (Cayetano *et al*., 2022).

The pH recorded (7.49) is within the optimal range for methanogenesis (6.8–8.2) (Lohani & Havukainen, 2018), indicating a good buffer effect of the substrate. This value favors the development of methanogenic archaea, sensitive to pH variations. Outside this range, methane production can be reduced or stopped (Li *et al*., 2025). The chemical equilibrium achieved allowed stable production of biogas in the system.

Although the theoretical design envisaged an HRT of 34 days, according to the formula of Barrena *et al*. (2019), in practice the biodigester dome (3 m^3 remaining) was filled in 15 days, demonstrating a significant improvement. This result is attributed to biofilm, which optimized microbial colonization and accelerated substrate degradation (Mohmed Moffit *et al*., 2025). The use of local lianas as a support represents a low-cost innovation with replicability potential.

Within 15 days of loading the biodigester with manure-water mixture, the dome reached its full capacity, indicating a production of 3 m^3 of biogas. The daily biogas generation started at 0.2 m^3/day, later stabilizing at 0.3 m^3/day, values consistent with domestic biodigesters in cold areas. For comparison, 5 m^3 rural biodigesters tipically produce around 0.2 $m^3/m^3 \cdot$day, which is sufficient to cook 3 to 4 hours a day (Ferrer *et al*., 2015). Production rates of up to 0.5 m^3/day are possible with good design and thermal management, even in cold climates (Ortega-Castro *et al*., 2025).

The biogas pressure reached 12 cm H_2O and stabilized approximately 2.5 cm H_2O during daily operation, enough for a continuous flow to the kitchen without compressors. Pressures between 15–22 cm H_2O are suitable for kitchens in fixed dome systems (Ramaiyulis *et al*., 2021). Stabilization at this lower pressure indicates a balance between production and consumption, avoiding fluctuations that interrupt the flow (Abdurrakhman *et al*., 2024).

The prior homogenization of the mixture was key to ensuring an even distribution of temperature, nutrients and microorganisms, favoring efficient anaerobic digestion (Karne & Bhatkhande, 2022; Lindmark *et al*., 2014). In addition, it avoided inactive areas and improved microbial contact with the substrate, optimizing biogas production (Babaei & Shayegan, 2019).

The use of lianas as biofilms favored a homogeneous distribution of microbial activity, improving cell retention and stabilizing anaerobic digestion (Abera *et al*., 2024). This helped maintain a uniform temperature in the biodigester (Karne & Bhatkhande, 2022) and achieve the effective HRT in 15 days (Figure 3).

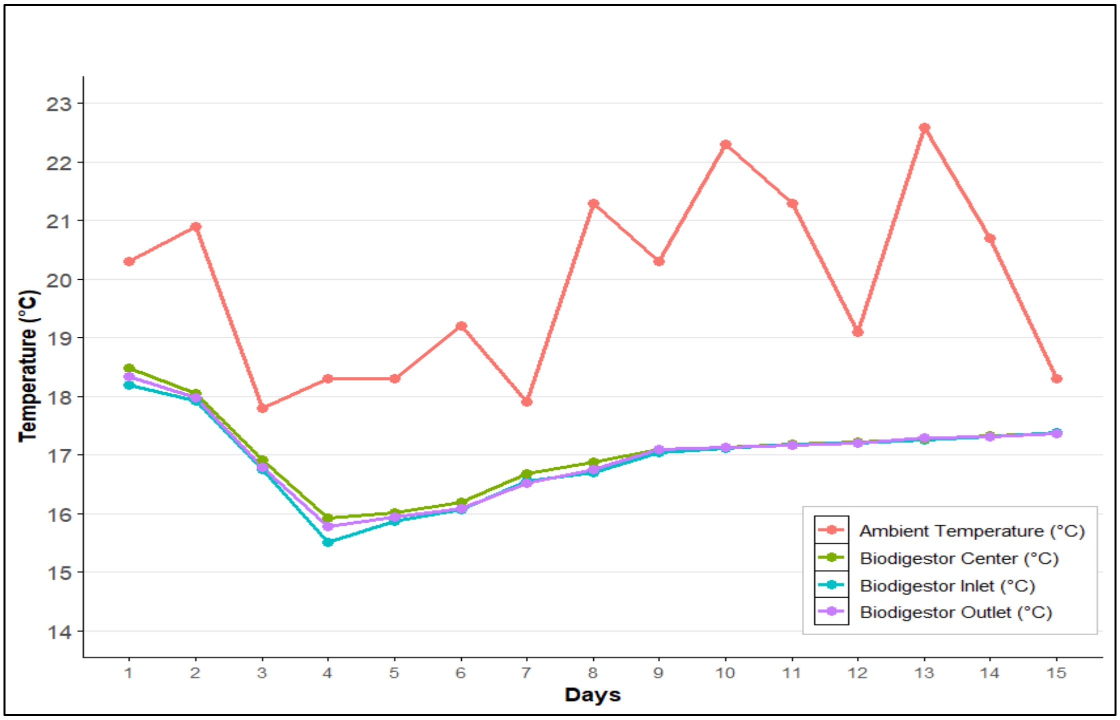

Figure 3. Temperature variation in biodigestion for 15 days

Although the ambient temperature ranged from 18 °C to 23 °C (Figure 3), the internal temperature of the biodigester remained stable between 16 and 17.5 °C until HRT was reached. This indicates that the use of biofilms and adequate homogenization of the substrate enabled an efficient and stable digestion under psychrophilic conditions (Tiwari *et al*., 2021).

Cumulative Biogas Production Curve according to the Gompertz Model

The cumulative production was evaluated until day 15, when the biogas bell was filled. However, in psychrophilic conditions, complete digestion usually requires between 20 and 40 days (Song *et al*., 2023), so the data suggest that

that the process has not reached completion. The measurement was stopped for operational reasons, not because of deplation of production potential

The Gompertz model was adjusted by nonlinear least squares using R software, assigning as initial values the maximum observed production multiplied by two to estimate Bmax, while Rm and λ were set at 0.1 m^3/day and 1 day respectively. These values follow methodological criteria reported in the anaerobic digestion modeling, to facilitate the convergence of the fit and appropriately represent the cumulative biogas production (Kavan Kumar *et al*., 2023).

Figure 4 shows the evolution of the cumulative biogas production, modeled with the Gompertz curve, which characterizes the sigmoidal behavior of anaerobic digestion and allows evaluating its performance over time.

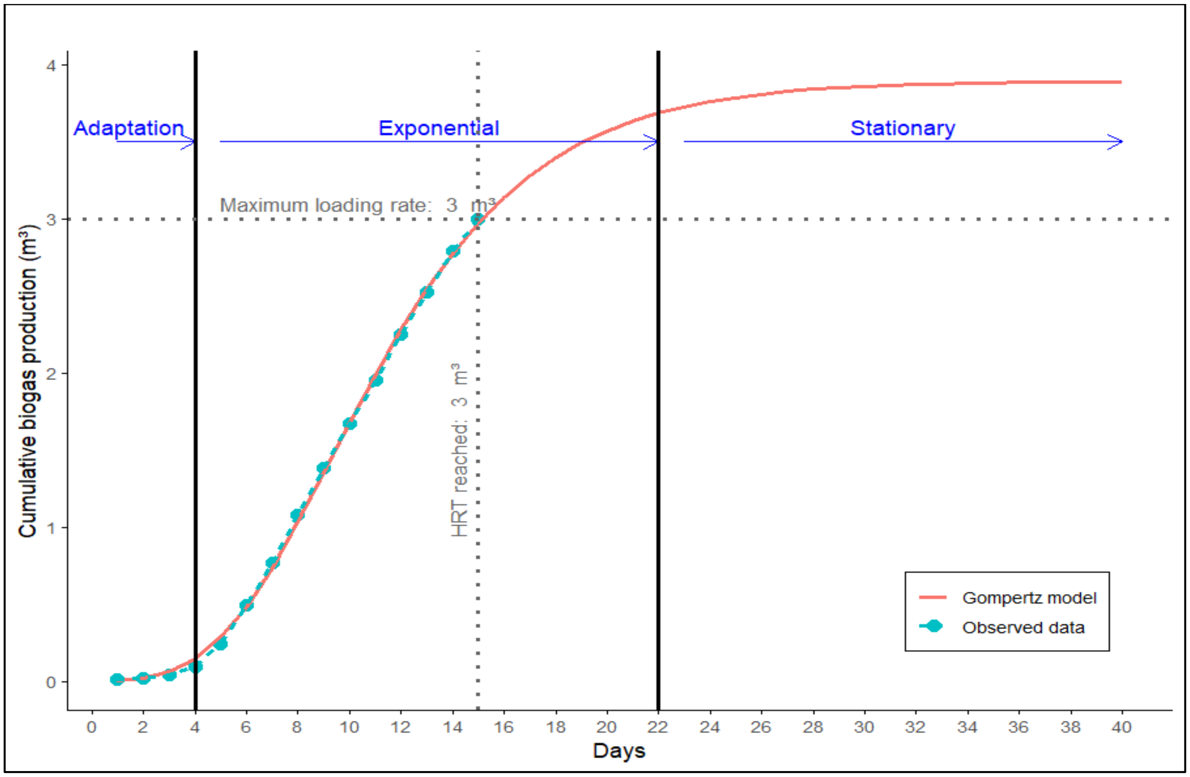

Figure 4. Cumulative growth in biogas production and adjustment with the Gompertz model

At the HRT, the Gompertz curve still displayed a positive slope, indicating that biogas production had not ended. This is common in psychrophilic conditions, where digestion can exceed 30 days (Pilarski *et al*., 2020). Therefore, in experiments where the operation period is truncated, it is necessary to apply models like this one (Esparza-Soto *et al*., 2025).

The production curve showed an adaptation phase (days 1–4) and an exponential phase (days 5–22). Gompertz's model fit well to the data, projecting a total potential of 3.89 m^3 of biogas. Although stabilization was not observed in the field, the model suggests that stabilization would occur beyond the observation window.

Table 2 presents the parameters obtained from the fit of the experimental data to the Gompertz model, which describes the cumulative production of biogas. These values allow characterizing the kinetic behavior of the process and evaluating its efficiency.

The peak generation rate (Rm) of 0.3256 m^3/day indicates an active metabolic phase during digestion. Kinetic models such as the modified Gompertz model reliably estimate this rate with high precision ($R^2 > 0.99$) on various substrates (Adamu *et al*., 2025; Ravikumar *et al*., 2020), confirming the reliability of the value and the consistency of the anaerobic process.

Table 2. Gompertz Model Parameters for Cumulative Biogas Production

Parameter	Estimated value
Bmax	3,893 m^3
Rm	0.3256 m^3/day
λ	4.8441 days
R2	0.9992
T_{80}	15.80 days
T_{90}	19.10 days

The parameter λ represents the initial period of microbial adaptation before the significant start of biogas production. In batch systems at mesophilic temperature, this time typically ranges from 2 to 6 days (Pasalari *et al*., 2021), with high levels of fit to the Gompertz model ($R^2 > 0.99$).

The times T_{80} (15.8 days) and T_{90} (19.1 days), close to the observed HRT, coincide with typical values of mesophilic digestion of organic wastes. In bovine and pig manure, 80-90% of production occurs between 12 and 28 days (Kafle & Chen, 2016), information useful to optimize HRT in continuous and batch systems.

Biogas composition before and after the purification process

Table 3 presents the composition of the biogas before and after its purification, following passage through the cartridges designed to remove hydrogen sulfide (H_2S).

Table 3. Composition and percentage of gases before and after purification.

Gases	Percentage before Purification	Percentage after Purification
CH_4 (%)	71.61	68.18
CO_2 (%)	27.50	17.90
O_2 (%)	0.18	7.99
H_2S (PPM)	624.50	157.40
CO (PPM)	3.50	496.40

Methane (CH_4), the main energy component, showed a slight decrease after purification, going from 71.61% to 68.18%. This reduction is likely attributable to losses during the passage through the cleaning system, which is common in processes where physical or absorbent filters are used (Swinbourn *et al*., 2024). Carbon dioxide (CO_2) decreased significantly from 27.50 % to 17.90 %, demonstrating the effectiveness of the purification system in improving biogas quality. This reduction implies an increase in the calorific value of the final gas, since CO_2 is not combustible (Bahrun *et al*., 2022).

Oxygen (O_2) increased from 0.18% to 7.99%, which may indicate air ingress during the purification process, possibly due to leaks or insufficient system sealing. This increase represents an operational risk, as it can promote the formation of explosive mixtures if the 12% threshold is exceeded (Biogasclean, 2016).

The hydrogen sulfide (H_2S) content was reduced by 75%, from 624.50 to 157.40 ppm, which is beneficial for preventing corrosion in equipment and emissions of toxic compounds (Arıman & Koyuncu, 2022).

Carbon monoxide (CO) increased from 3.50 to 496.40 ppm, indicating possible contamination or unwanted reaction during purification. Although CO is toxic and can affect the calorific value of biogas (Hagos *et al*., 2025; Pera *et al*., 2024), its level remains within the acceptable limit of 1000 ppm (Barrena *et al*., 2019).

Biogas Energy Efficiency Testing

Table 4 shows the three products cooked for four consecutive days. For each one, the cooking time was recorded to calculate the average number of hours required.

Table 4. Cooking time of biogas products

Food	Average Amount	Average Cooking Time (min)
Potato	0.25 kg	24.7 ± 1.2[a]
Egg	1 unit	12.3 ± 0.9[b]
Water	1 L	18.5 ± 1.1[ab]

The values are expressed as mean ± standard deviation (n = 4). Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

The energy efficiency of biogas for cooking common foods was evaluated. Potatoes required the most time (24.7 ± 1.2 min), followed by water (18.5 ± 1.1 min) and eggs (12.3 ± 0.9 min). The ANOVA and Tukey's test showed significant differences between potatoes and eggs ($p \leq 0.05$), but not between water and the other two.

The longer cooking time of the potato is due to its greater density and thermal requirement (Marle, 1997), while the egg, due to its lower mass and soft structure, cooks faster (Phisics, 2025).

Boiling 1 liter of water is a good indicator of the thermal performance of biogas, as it is directly related to its methane content (Nallamothu *et al*., 2013). The system allowed 1 liter of water to boil in less than 20 minutes, meeting the standard for domestic efficiency (Petro, 2020).

The average cooking times indicate that the biogas generated has an adequate calorific value for basic uses, comparable to LPG in rural contexts (Shinde *et al*., 2024).

Typical biogas consumption in a domestic burner is 0.34–0.45 m^3/h, with efficiencies of 59–68% (Rajendran *et al*., 2012). In countries such as China, India and Kenya, minimum consumption of 0.38, 0.45 and 0.50 m^3/h, respectively, is reported (Kasinath *et al*., 2021). Thus, the 3 m^3 generated could supply about 3.6 days of domestic use, allowing cooking for 3 to 4 hours a day for a family of 4 to 5 people.

Being a batch system, the biodigester requires daily refills after reaching HRT to maintain continuous biogas production and mass flow (Barrena *et al*., 2019). Therefore, a mixture of 200 liters should be added daily in a manure:water ratio of 1:5, equivalent to about 33.3 kg of fresh manure.

Conclusions

The use of lianas as biofilm support significantly improved microbial retention and allowed stable anaerobic digestion under psychrophilic conditions. This shortened the hydraulic retention time to 15 days, demonstrating an efficient and low-cost solution for cold rural areas.

The manure:water mixture in a 1:5 ratio ensured good substrate fluidity and optimal pH (7.49), which favored methanogenic activity. This chemical condition was essential to stable biogas production in a low-temperature environment.

The biogas produced showed high energy quality, with more than 68% methane after purification and a significant reduction in H_2S. Its use in domestic cooking showed efficiency comparable to LPG, validating its rural applicability.

Gompertz's model accurately described the cumulative biogas production ($R^2 = 0.9992$), identifying key phases of the process. Its application enables the projection of performance and optimization of operating times in similar systems.

The Gompertz curve showed that biogas production continued after day 15, indicating that the process did not fully stabilize. This interruption was due to the limited capacity of the gasometer and not to the depletion of the substrate. Removing the material at this point may result in partially digested material, with risks for its use as biofertilizer. Therefore, it is suggested to expand the storage volume and extend the operation time to fully harness the production potential and ensure stable effluent.

As it is a batch system, daily refilling is essential after TRH is reached to maintain continuous biogas production. Therefore, rural families should be aware of the need to feed the biodigester regularly with the right mixture, thus ensuring a constant and efficient supply of energy.

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