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Biogas Production from Cattle Manure in Southern Benin: Effects of Inoculum, Temperature and Agitation

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Abstract:
Background: To assess the potential for biogas production from cow dung in the specific context of southern Benin, by identifying the physico-chemical parameters (temperature, agitation, addition of inoculum) likely to optimise yield.
Materials and methods: *Four laboratory-scale digesters were set up using three 750 ml plastic water bottles, with a slurry concentration of 1,500 g of cow dung per 3,000 cm³ of distilled water, over a retention period of three weeks, varying the inoculum, temperature (25 or 35 °C) and agitation. The pH and gas volume were monitored daily. Biogas production began on the fourth day of fermentation and followed an upward trend. Three replicates were carried out for each experimental condition.*
Results: *The results show that mechanical agitation is the most important factor: the agitated digester (IV) produced 187 ml compared with 134 ml for the non-agitated control (III), representing a gain of 53 ml. The inoculum reduced the start-up time by two days (141 ml versus 136 ml). Temperature alone (35 °C without agitation) did not improve yield (134 ml compared to 136 ml at 25 °C). It is the combination of temperature and agitation that yields the best results.*
Conclusion: *To maximise biogas production, it is essential to combine temperature and agitation. Mechanical agitation is the key factor*
Key Word*: Bio methanation, cattle manure, inoculum, agitation, biogas production.*

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I. Introduction

Bio methanation is a simple process in which microorganisms convert organic waste into biogas and digestate, which can be used as fertilizer. This technology is particularly useful in rural or remote areas where access to energy is limited. Furthermore, it helps reduce greenhouse gas emissions by capturing methane that would otherwise be released during open-air decomposition [1, 2, 9]. In Benin, the majority of residents still rely on firewood and charcoal for cooking. This dependence places considerable pressure on forests, leading to accelerated deforestation. It is therefore urgent to develop alternative, local, and renewable energy sources [3,21]. Biogas offers numerous advantages: it is clean, inexpensive to operate, and can be produced from locally available waste. In southern Benin, particularly in the municipalities of Zè, Allada, and Toffo, cattle farming is widespread. Cattle manure is abundant there, easily accessible, and therefore constitutes a potential feedstock for biogas production [4,5,22]. Other types of organic waste, such as municipal waste [6], slaughterhouse waste [12,37,38], or fruit and vegetable waste [11], have also been successfully studied elsewhere. Replacing energy crops with organic waste for biogas production is a promising approach [13]. However, despite these advantages, biogas remains relatively unknown in our regions and is not part of current energy practices. Yet it could represent an integrated solution for waste management, decentralized energy production, and environmental protection [5,24]. Recent technological advances make it possible to consider enhanced anaerobic digestion to improve yields [20]. For the bio methanation process to function effectively, several physicochemical conditions must be met: an appropriate pH, an adequate temperature, sometimes an inoculum (pre-digested material rich in microorganisms), and possibly agitation of the reaction medium [8,19,25]. The pH must remain between 6.5 and 7.5 to avoid inhibiting methanogenic bacteria [10,14]. The inoculum provides a diverse microbial population from the start, reducing the lag phase [23,25]. Bioaugmentation strategies can also improve the process [39]. Agitation homogenizes the medium, prevents sediment formation, and improves mass and heat transfer [28]. Finally, temperature controls the rate of biological reactions, with an optimum generally in the mesophilic range (30–40°C) [22,32]. The use of heating may be beneficial in certain contexts [16]. The objective of our study was to assess in practice how these parameters influence biogas production from cattle manure under local conditions in southern Benin. We set up laboratory digesters, varied one parameter at a time (inoculum, temperature, agitation), and measured the pH and the volume of gas produced on a daily basis. The ultimate goal was to determine the optimal operating conditions to maximize biogas production from this abundant local resource.

II. Material And Methods

2.1 Experimental site

The study focuses on the implementation of an experiment to produce biogas from cattle manure collected from farms located in the municipality of Zè, using batch fermentation in pilot digesters installed in the Laboratory of Geosciences, Environment, and Applications at the National University of Science, Technology, Engineering, and Mathematics of Benin.

2.2. Testing equipment

2.2.1. Feedstocks for fermentation

The bio methanation process combines a substrate of fresh cattle manure, sourced from available animals, with an inoculum of black manure collected from a septic tank. The physicochemical characteristics of these feedstocks are presented in Table 1.

Table no1: Physicochemical characteristics of the bovine manure used

Type of cattle manure	% DM	pH
Fresh manure	31,5	6,5
Black manure	9,4	7,2

2.3 Experimental setup

Four 500 mL pilot digesters made of borosilicate glass were used, in accordance with recommendations for biogas potential testing [29,30,31]. This allowed for the monitoring of the physicochemical properties and the quantitative production of biogas generated by the bovine biomass. Each digester was equipped with a water seal system to ensure anaerobic conditions. The experimental conditions are summarized in each condition was performed twice to ensure the reproducibility of the measurements [33].

Table 2: General data on the digesters used

Digester	Cattle manure	Temperature (°C)	Agitation
I	Fresh	25	Without agitation
II	Fresh and black	25	Without agitation
III	Fresh	35	Without agitation
IV	Fresh	35	With agitation

2.4 Digester feeding

A single initial feed was added (batch mode) [30]. The mixture followed a ratio of 2 parts water to 1 part manure, commonly used for cattle manure [7,8]. Table 3 shows the exact quantities added.

Table 3: Quantities of substrate, inoculum, and water introduced (mL)

Digester	Substrate (ml)	Inoculum (ml)	Water (ml)
I	100	–	200
II	60	30	180
III	100	–	200
IV	100	–	200

2.5 Physicochemical analyses

The pH was measured daily using a laboratory pH meter (Hanna Instruments), which had been previously calibrated with buffer solutions at pH 4.0, 7.0, and 10.0. pH control is essential because the optimal range is between 6.5 and 7.5 [10,14]. Methods for controlling pH during fermentation have been described elsewhere [15]. When the pH fell below 6.5, an adjustment was made by adding a sodium hydroxide (NaOH 2.5 N) solution drop by drop.

Dry matter (DM) was determined by oven drying at 105°C until constant weight (24 h), according to VDI 4630 [40] and the recommendations of Raposo et al. [30]. The temperature was monitored daily using an immersed thermometer.

2.6 Monitoring of biogas production

The volume of biogas produced was measured by the water displacement method [29,31]. Each digester was connected via a silicone tube to an inverted graduated column filled with acidified water (pH < 2) to limit CO_2 dissolution [34,35]. The gas, being less dense than water, accumulated at the top of the column and forced the water downward. The volume was recorded daily at ambient pressure and temperature for 19 days.

III. Result

3.1 pH monitoring

pH control is essential because the optimal range is between 6.5 and 7.5. Above or below this range, methanogenic activity may be inhibited.

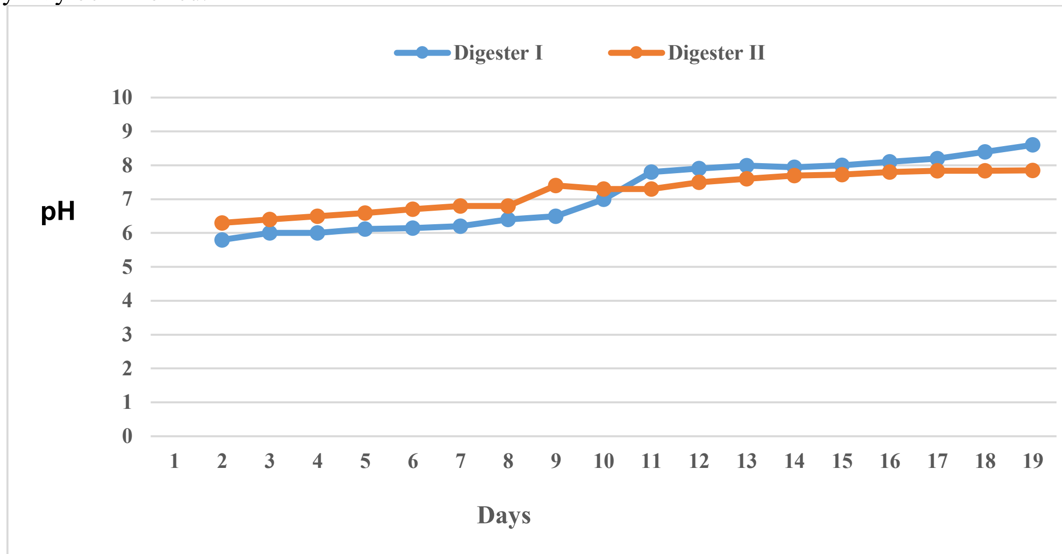

Fig. 1. Shows the pH variation for digesters I and II.

According to Figure 1, the pH profiles are similar during the first week, indicating that the inoculum has little influence on pH in the short term. However, starting on the 8th day, a slight increase in pH is observed in digester II (with inoculum) compared to digester I.

3.2 Evaluation of the effect of agitation on the fermentation process

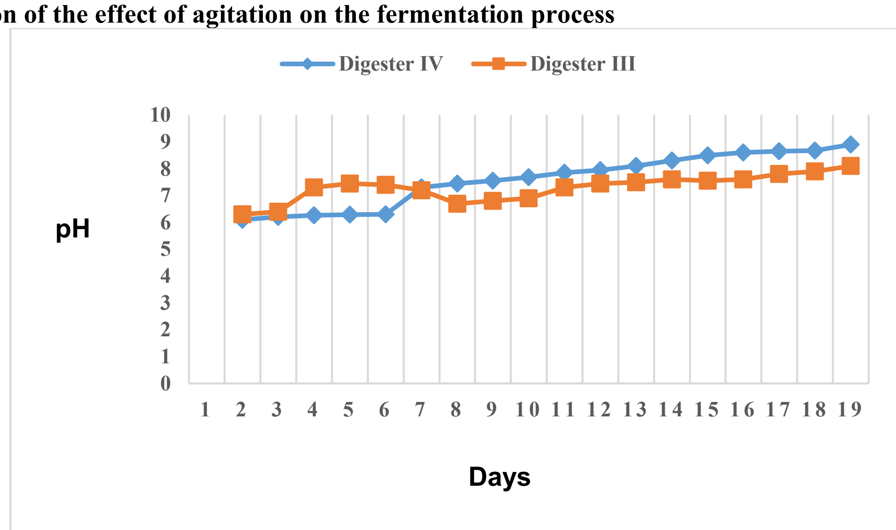

Fig. 2. pH variation over time (Digesters III and IV)

Figure 2 shows the effect of agitation (digesters III and IV). Mechanical agitation optimizes fermentation by ensuring better pH regulation starting on the 7th day. The pH was initially adjusted with NaOH to prevent it from falling below 6.5. A rapid increase to pH 8.0 was observed in Digester IV (agitated) as early as the 13th day, and biogas production continued at pH 8.2 until the 18th day in Digester III (unagitated).

3-3 Impact of temperature on fermentation start-up kinetics

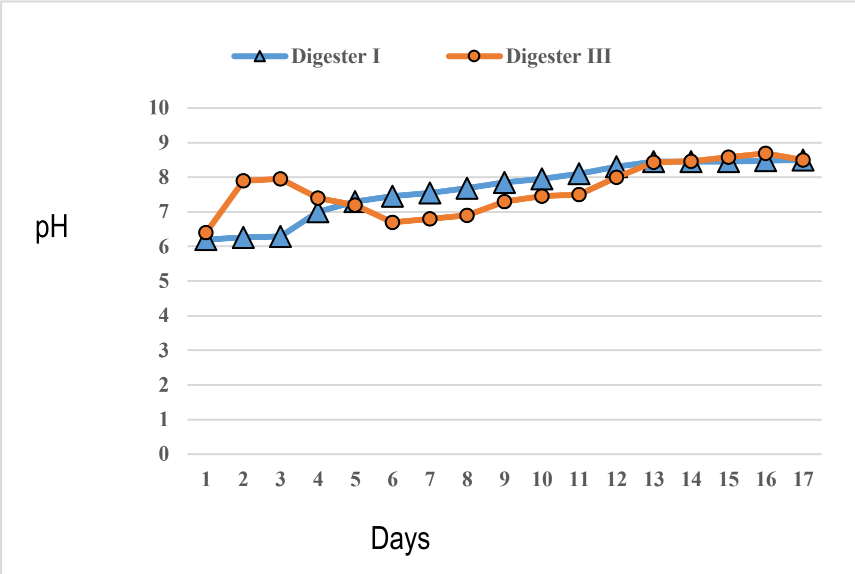

Fig. 3. pH variation over time (Digesters I and III)

This figure compares the effect of temperature (Digester I at 25°C and Digester III at 35°C). Digester III (35°C) shows a continuous linear increase in pH starting on the 7th day, peaking at 8.25, while digester I (25°C) follows a nearly linear trend interrupted by a drop on the 16th day, also reaching 8.25 at the end of the experiment.

3.4 Determination of dry matter

Table 4. Dry matter content of the introduced mixtures

Digester	Dry matter (%)
I	15,8
II	13,6
III	16,6
IV	15,3

Analysis of the table reveals similar DM levels, ranging from 13.6% to 16.6% for all systems, with digester II having the lowest value. The study was based on a comparison of bio methanation by varying key parameters (agitation, temperature, inoculum), while standardizing operating conditions (DM, pH) through targeted adjustments.

3.5 Biogas production in pilot reactors

After a start-up phase, the experimental batch digesters show an initial increase in production, followed by a stable plateau, before declining (Figures 4, 5, and 6).

3.5.1 Effect of initial inoculum load on biogas yield

Biogas production in Digester II began on the 5th day, as soon as the pH threshold was reached. In contrast, although Digester I showed favorable pH levels earlier, production did not begin until the 7th day (Figure 4). This discrepancy is likely due to a delay in the growth of bacterial populations.

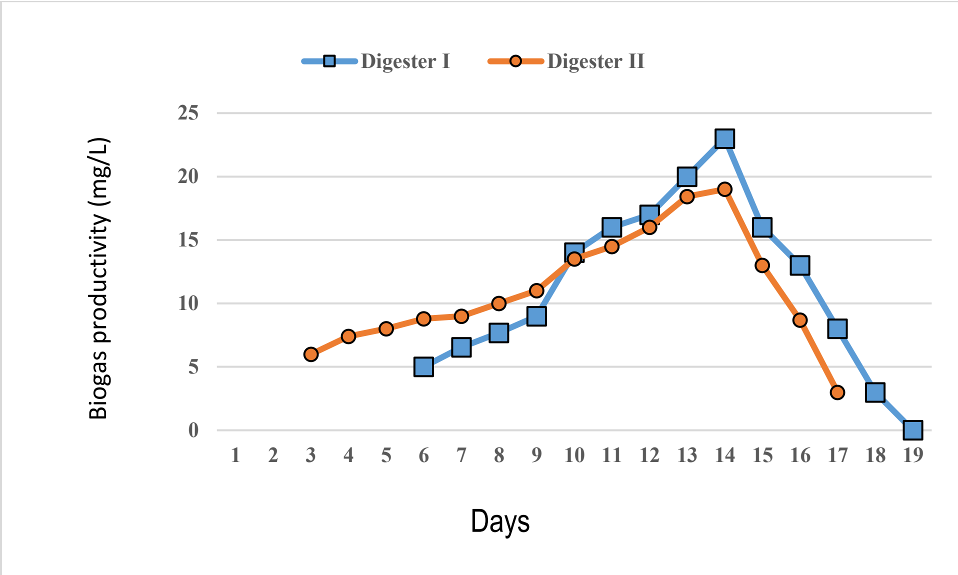

Fig. 4. Variation in gas productivity as a function of inoculum (Digesters I and II).

The early start of Digester II is due to the use of black manure, whose high bacterial content allowed for a two-day reduction in the start-up phase. However, this acceleration resulted in a shorter production phase. Despite this reduced duration, Digester II showed a slightly higher yield with 141 ml of biogas, compared to 136 ml for Digester I (Figure 4)

3.5.2 Influence of agitation on gas production kinetics

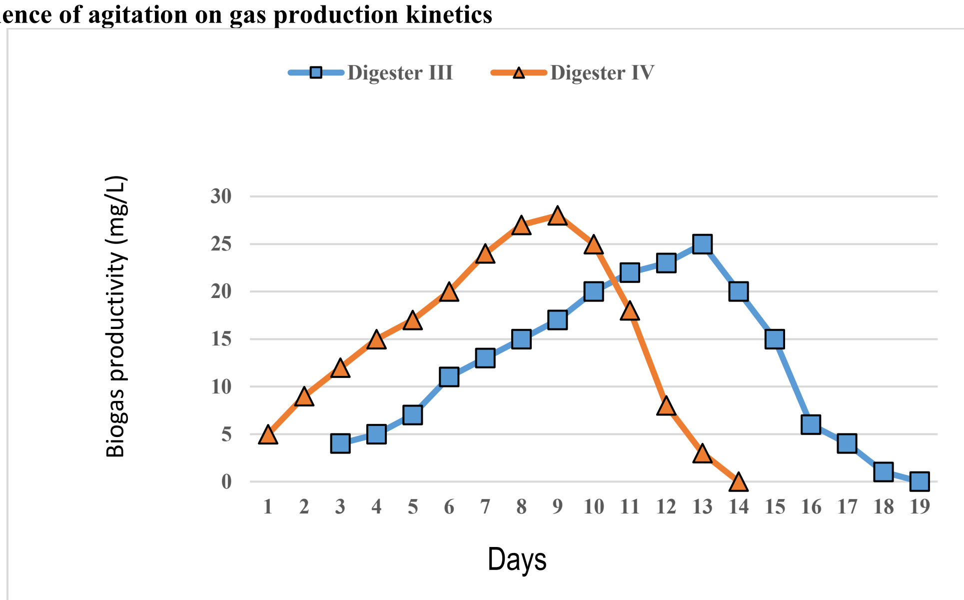

Fig. 5. Variation in gas productivity as a function of agitation (Digesters III and IV)

Agitation influences the methanization process in a manner similar to the introduction of an inoculum, but with a more pronounced effect on productivity.

By optimizing mass transfer between microorganisms and organic matter, agitation boosted the productivity of digester IV. The results show an increase of 53 mL compared to the unagitated digester III: 187 mL for digester IV versus 134 mL for digester III. Digester IV also recorded a maximum activity of 27 mL/day on the 10th day.

3.5.3 Influence of temperature on gas yield

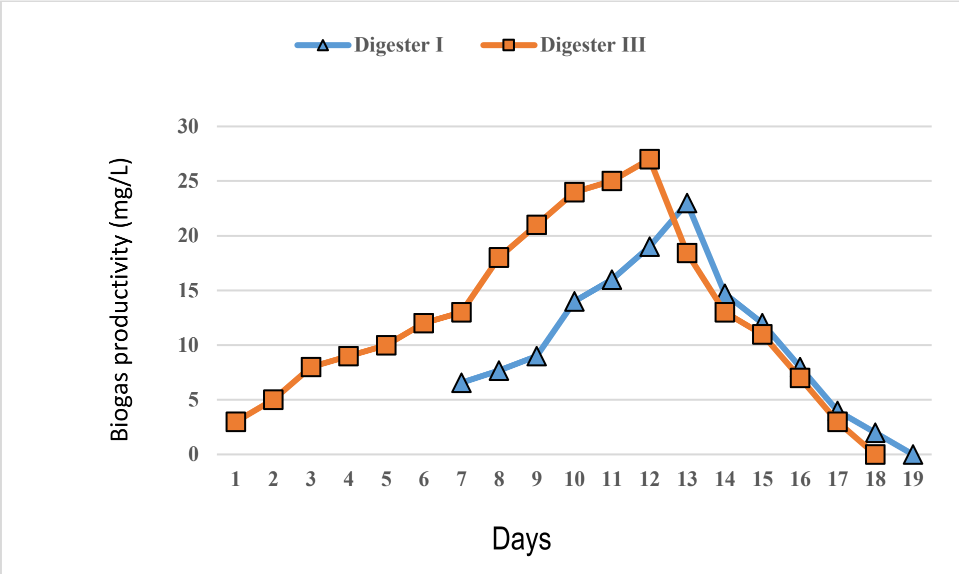

Fig. 6. Variation in gas productivity as a function of temperature (Digesters I and III)

Figure 6 compares digesters I (25°C) and III (35°C). Digester III (35°C) produced 134 mL, a volume slightly lower than that of digester I (136 mL). Contrary to what might be expected, the higher temperature did not improve volumetric yield under our non-agitated conditions. However, the production kinetics were faster at 35°C: the peak in production occurred earlier, and the lag phase was shorter.

IV. Discussion

4.1 Results interpretation

Three key findings emerge from our experiments.

Firstly, mechanical agitation is the most influential factor. Digester IV (agitated at 35°C) produced 187 mL, which is 53 mL more than Digester III (unagitated at the same temperature). This nearly 40% increase confirms the importance of homogenizing the medium. Agitation promotes contact between microorganisms and organic matter, prevents the formation of sediments and crusts, and improves heat and nutrient transfer. This result aligns with those of Stinner and al. [28] and Polastri and al. [27], who also observed significant improvements with mechanical agitation. The co-digestion of bovine rumen waste and brewery spent grain also demonstrated interesting synergies [26].

Secondly, the inoculum accelerates startup but does not significantly increase the total volume. Digester II (with inoculum) completed the latency phase two days faster than Digester I. However, the total volume reached only 141 mL, compared to 136 mL for Digester I amodest difference (+5 mL). This is likely due to the quality of the inoculum used: the black manure collected from a septic tank was already partially digested and contained fewer active bacteria than fresh inoculum from an operational digester. Niya and al. [25] recently demonstrated that the microbial composition of the inoculum plays a key role in successful start-up, and that not all inocula are created equal.

Thirdly, temperature alone is not sufficient to improve yield. At first glance, this result is surprising. Digester III (35°C, no agitation) produced 134 mL, a volume slightly lower than that of Digester I (25°C, 136 mL). In other words, raising the temperature from 25 to 35°C without agitation did not increase the total volume. However, the reaction rate was faster at 35°C. It was the combination of temperature and agitation (digester IV) that yielded the best result. Under mesophilic conditions (35°C) with agitation, the viscosity of the medium decreases, which facilitates mixing and microbial activity.

4.2 Comparison with the literature

Our results are comparable to those reported by Moyo and al. [22] in South Africa, where yields of 1.5 to 2.2 L of biogas per liter of digester were obtained from agricultural waste. Paes and al. [23] observed higher yields (2.5 L/L) with co-digestion (a mixture of cattle manure and sewage sludge), suggesting that adding other substrates (market waste, agricultural residues) could improve our performance. N'guessan and al. [24], working with swine manure in Côte d'Ivoire, obtained 1.2 L/L, a yield lower than that of our digester IV (1.87 L/L).

The absence of a significant effect of temperature alone has already been observed in some batch-mode studies [34,35], especially when the duration of the experiment is limited (19 days in our case) and the feedstock exhibits natural variability. It is possible that differences may emerge over a longer period or in continuous mode. Studies on digestion using temperature steps [36] have shown that larger temperature variations can have significant effects.

4.3 Practical implications for rural benin

Our findings have direct implications for the design of small-scale biogas digesters in rural areas:

- Agitation is strongly recommended. Even manual agitation (stirring once or twice a day) could improve performance in simple digesters.
- Inoculum is useful for saving time, but it is not essential. If one is willing to wait a few extra days, the process starts naturally.
- The optimal temperature is 35°C, but in our tropical climate (ambient temperature 25–30°C in Abomey-Calavi), acceptable results are obtained without heating. Investing in a heating system is likely not cost-effective on a small scale.

Technical and economic guidelines exist to guide the deployment of small-scale anaerobic digestion units [17]. The quality of the digestate produced, comparable to compost, is an additional benefit for local agriculture [18].

4.4 Limitations of the study

Our work has several limitations:

- Limited number of replicates (n=2 per condition), which reduces statistical power and does not allow for robust statistical tests.
- Duration of the experiment (19 days): some digesters may not have completed their production.
- Lack of analysis of biogas composition (methane, CO_2, H_2S content), which is essential for assessing energy quality.
- Discontinuous batch mode: this does not reflect the continuous operation of actual facilities.
- Variability in manure composition depending on animal feed and season, which was not controlled.

These issues will be addressed in future work.

V. Conclusion

This 19-day experimental study shows that biogas production from cattle manure in southern Benin depends primarily on mechanical agitation, and to a lesser extent on temperature and the use of an inoculum.

The main results are as follows:

- Digester IV (fresh manure, 35°C, with agitation) yielded the best results: 187 mL of biogas from 100 mL of substrate, representing a yield of 1.87 L per liter of digester.
- Agitation alone resulted in a gain of 53 mL (+39.5%) compared to the non-agitated digester at the same temperature.
- The inoculum reduced the start-up time by two days but did not significantly increase the total volume (141 mL versus 136 mL).
- Temperature alone (35°C without agitation) did not improve yield (134 mL vs. 136 mL at 25°C). It is the combination of temperature and agitation that is decisive.

Moving forward, we plan to:

- Compare the batch process to the continuous mode
- Characterize the composition of the biogas (CH_4, CO_2, H_2S content) using gas chromatography
- Test the effect of co-substrates (market waste, crop residues)
- Evaluate the quality of the digestate as a fertilizer in agronomic trials
- Conduct a preliminary economic analysis for smallholders

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