Research Report

Evaluating the effects of SLBD supplementation on biogas production from dairy cattle manure during anaerobic digestion

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1.Introduction

Livestock manure is one of the largest organic waste streams generated by agricultural systems worldwide. Improper management of manure can contribute to environmental problems, including greenhouse gas emissions, nutrient losses, water contamination, and odor pollution [1]. In dairy production systems, manure contains substantial amounts of organic matter that can be utilized as a renewable energy source through anaerobic digestion. This technology simultaneously addresses waste management challenges while producing biogas, a renewable energy source that can reduce dependence on fossil fuels [2].

Anaerobic digestion is a biological process in which microorganisms decompose organic materials under oxygen-free conditions, resulting in the production of methane (CH_4), carbon dioxide (CO_2), and stabilized digestate [3]. The efficiency of anaerobic digestion depends on several factors, including substrate composition, microbial populations, pH, temperature, and retention time [4]. Dairy cattle manure is widely used as a substrate for anaerobic digestion due to its high moisture content and abundant microbial population. However, the degradation of lignocellulosic components remains a limiting factor affecting biogas yield and methane production [5].

To improve the efficiency of anaerobic digestion, various microbial additives have been investigated. Effective Microorganisms (EM4), consisting of mixed cultures of beneficial microorganisms such as lactic acid bacteria, photosynthetic bacteria, yeasts, and actinomycetes, have been reported to enhance organic matter decomposition and improve biogas production under certain conditions [6]. Recently, microbial inoculants containing enzyme-producing bacteria have attracted increasing attention due to their ability to accelerate the hydrolysis stage, which is often the rate-limiting step in anaerobic digestion [7].

Among these microorganisms, *Bacillus amyloliquefaciens* has demonstrated considerable potential because of its ability to produce extracellular enzymes including cellulases, xylanases, proteases, and amylases that facilitate the degradation of complex organic compounds [8]. Previous studies have shown that *Bacillus*-based inoculants can improve fiber degradation, nutrient availability, and microbial activity in biological conversion processes [9]. Therefore, the SLBD strain of *Bacillus amyloliquefaciens* may offer a promising strategy for enhancing anaerobic digestion performance and increasing biogas production from dairy cattle manure.

Despite growing interest in biological additives, information regarding the application of SLBD in biogas production systems remains limited. Most previous studies have focused on commercial inoculants or conventional anaerobic sludge, while the comparative performance of SLBD against EM4 and inoculum supplementation has not been extensively evaluated. Therefore, this study was conducted to evaluate the effects of EM4, SLBD at different inclusion levels, and inoculum supplementation on biogas production from dairy cattle manure under laboratory-scale anaerobic digestion conditions. The findings are expected to provide preliminary evidence regarding the potential use of SLBD as a biological additive to improve biogas production and support sustainable manure management in dairy farming systems. The aims of this study were to evaluate the effect of SLBD supplementation on biogas production from dairy cattle manure during anaerobic digestion.

2. Materials and Methods

2.1 Experimental Design

The experiment was conducted using a completely randomized design (CRD) consisting of six treatments evaluating different microbial additives during anaerobic digestion of dairy cattle manure. The treatments were:

- **SL0**: Control (50% dairy cattle faeces + 50% water)
- **SL1**: Control + EM4 (1%)
- **SL2**: Control + SLBD (1%)
- **SL3**: Control + SLBD (2.5%)
- **SL4**: Control + SLBD (5%)
- **SL5**: Control + inoculum (5%)

Each bioreactor contained a total working volume of 70 mL. The volume of water and faeces was adjusted according to the amount of additive added to maintain a constant final volume across all treatments.

2.2 Preparation of Substrate and Additives

Fresh dairy cattle faeces were collected from a commercial dairy farm and homogenized prior to use. Water was added at a 1:1 ratio (v/v) to prepare the manure slurry. The microbial additives used in this study were Effective Microorganisms (EM4), SLBD (*Bacillus amyloliquefaciens* SLBD), and inoculum obtained from an active anaerobic digestion system.
The composition of each treatment is presented in Table 1.

Table 1. Composition of experimental treatments.

Treatment	Water (mL)	Faeces (mL)	Additive
SL0	35.00	35.00	None
SL1	34.65	34.65	EM4 (0.70 mL; 1%)
SL2	34.65	34.65	SLBD (0.70 mL; 1%)
SL3	34.13	34.13	SLBD (1.75 mL; 2.5%)
SL4	33.25	33.25	SLBD (3.50 mL; 5%)
SL5	33.25	33.25	Inoculum (3.50 mL; 5%)

2.3 Anaerobic Digestion Procedure

The prepared substrates were transferred into laboratory-scale anaerobic digesters with a working volume of 70 mL. After loading, the digesters were tightly sealed to maintain anaerobic conditions throughout the incubation period. The reactors were incubated at incubator (37^{o}C) under batch digestion conditions. Biogas production was monitored throughout the experimental period. Prior to incubation, the digesters were manually shaken to ensure homogeneity of the substrate and additives.

2.4 Measurement of Biogas Production

Biogas production was measured directly using a gas-tight syringe connected to the reactor outlet. The accumulated gas volume was recorded periodically and expressed as milliliters (mL) of biogas produced per reactor. After each measurement, the gas was released to prevent excessive pressure accumulation within the system.Cumulative biogas production was calculated as the total volume of gas generated during the digestion period.

2.5 Biogas Composition Analysis

Biogas composition was determined at the end of the anaerobic digestion period to evaluate methane (CH_4) and carbon dioxide (CO_2) concentrations. Gas samples were collected directly from each reactor using a gas-tight syringe and transferred into pre-evacuated vacuum vial bottles to prevent contamination and gas leakage. Prior to sampling, the vial bottles were checked to ensure vacuum integrity.

Approximately sufficient headspace gas from each reactor was injected into the vacuum vial bottle and immediately sealed using a butyl rubber septum and aluminum cap. The sealed vials were labeled according to treatment and transported to the Environmental Laboratory of the Environmental Research and Technology Development Center (PATI) for gas composition analysis.

Methane (CH_4) and carbon dioxide (CO_2) concentrations were quantified by PATI using gas chromatography (GC) following the laboratory's standard operating procedures. Gas concentrations were expressed as parts per million (ppm). The methane quality of the biogas was subsequently evaluated by calculating the methane proportion relative to the total measured biogas components according to the following equation:

$$\text{Methane Quality (\%)} = \frac{CH_4}{CH_4 + CO_2} \times 100$$

where:

- CH_4= methane concentration (ppm)
- CO_2= carbon dioxide concentration (ppm)

In addition, the methane-to-carbon dioxide ratio (CH_4:CO_2) was calculated as:

$$CH_4 : CO_2 = \frac{CH_4}{CO_2}$$

A higher methane proportion and CH_4:CO_2 ratio indicate better biogas quality and greater methanogenic efficiency during anaerobic digestion.

2.6 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine the effect of treatments on cumulative biogas production and other measured parameters. When significant differences were detected ($P < 0.05$), treatment means were compared using Duncan's Multiple Range Test (DMRT). Statistical analyses were performed using R statistical software.

3 Results and Discussions

Table 2. Means of gas accumulation (ml)

Treatment	Description	Mean ± SD (mL)
SL0	Control	116.00
SL1	EM4 1%	107.50 ± 19.09
SL2	SLBD 1%	119.00 ± 5.66
SL3	SLBD 2.5%	106.00 ± 25.46
SL4	SLBD 5%	124.00 ± 11.31
SL5	Inoculum 5%	116.50 ± 3.54

Source of variation	df	Sum of Squares	Mean Square	F-value	P-value
Treatment	4	468	117	0.50	0.741
Residual	5	1177	235		
Total	9	1645			

Table 2 indicates the addition of EM4, SLBD, and inoculum did not significantly affect cumulative biogas production ($P > 0.05$). The absence of significant differences may be attributed to the high variability observed among replicates, particularly in the SL3 treatment. Nevertheless, the higher average gas production observed in SL2 and SL4 suggests that SLBD supplementation may enhance anaerobic digestion performance and warrants further investigation with a larger number of experimental replicates.

Although SL4 (5% SLBD) produced the highest average cumulative gas production (124 mL), followed by SL2 (1% SLBD) (119 mL), the differences among treatments were not statistically significant due to high variation among replicates. The inoculum treatment (SL5) produced 116.5 ± 3.54 mL, which was similar to the control treatment (116 mL). The lowest cumulative gas production was observed in SL3 (2.5% SLBD) and SL1 (EM4), producing 106.0 ± 25.46 and 107.5 ± 19.09 mL, respectively.

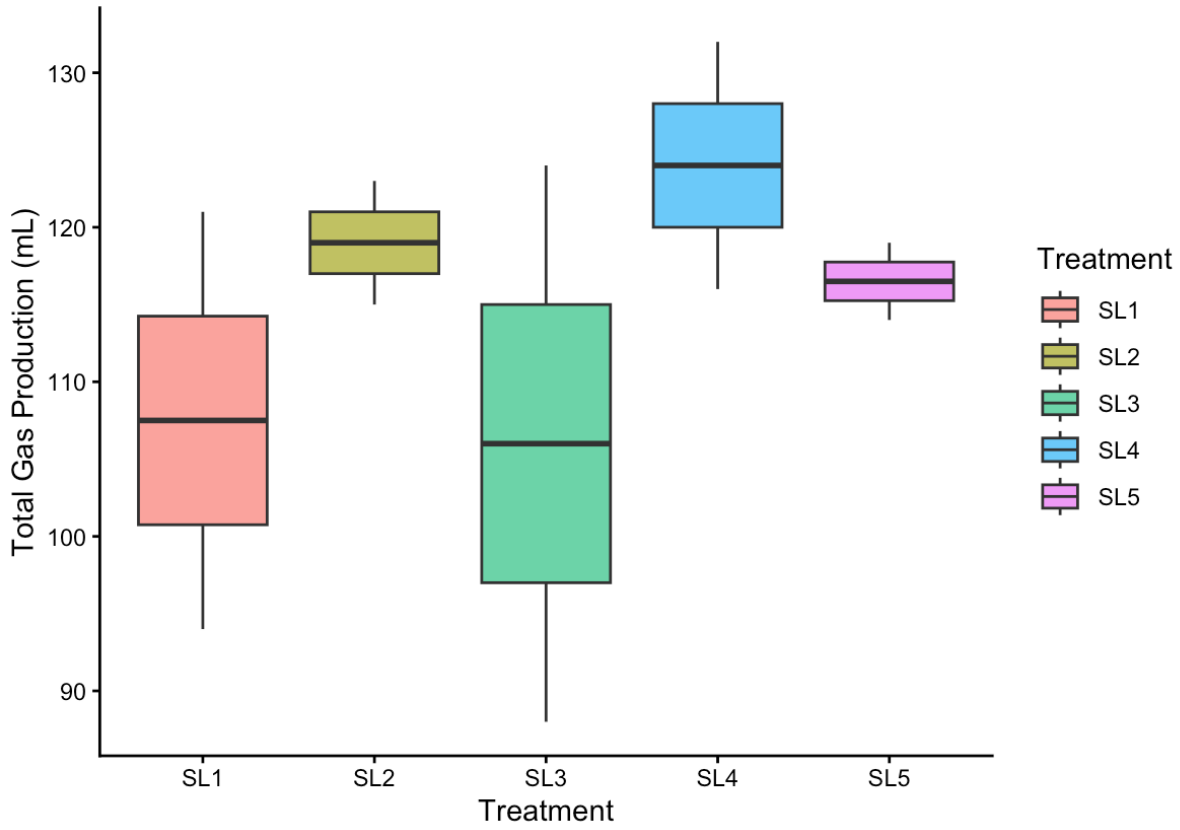


Figure 1. Total gas production (ml) among treatments

Figure 1 shows the distribution of cumulative biogas production among treatments. Numerically, the highest gas production was observed in SL4 (5% SLBD), followed by SL2 (1% SLBD). These findings suggest a potential positive effect of SLBD on anaerobic digestion performance that should be further evaluated using a larger number of replicates.

The trend suggests that **SLBD supplementation may improve anaerobic digestion performance**, particularly at the 1% and 5% inclusion levels. The higher gas production observed in SL4 could be related to enhanced degradation of organic matter by *Bacillus amyloliquefaciens*, which is known to produce extracellular enzymes involved in the breakdown of complex substrates. Interestingly, SL2 (1% SLBD) achieved gas production comparable to SL4 while exhibiting lower variability, suggesting that a lower inclusion level may already provide sufficient microbial stimulation.

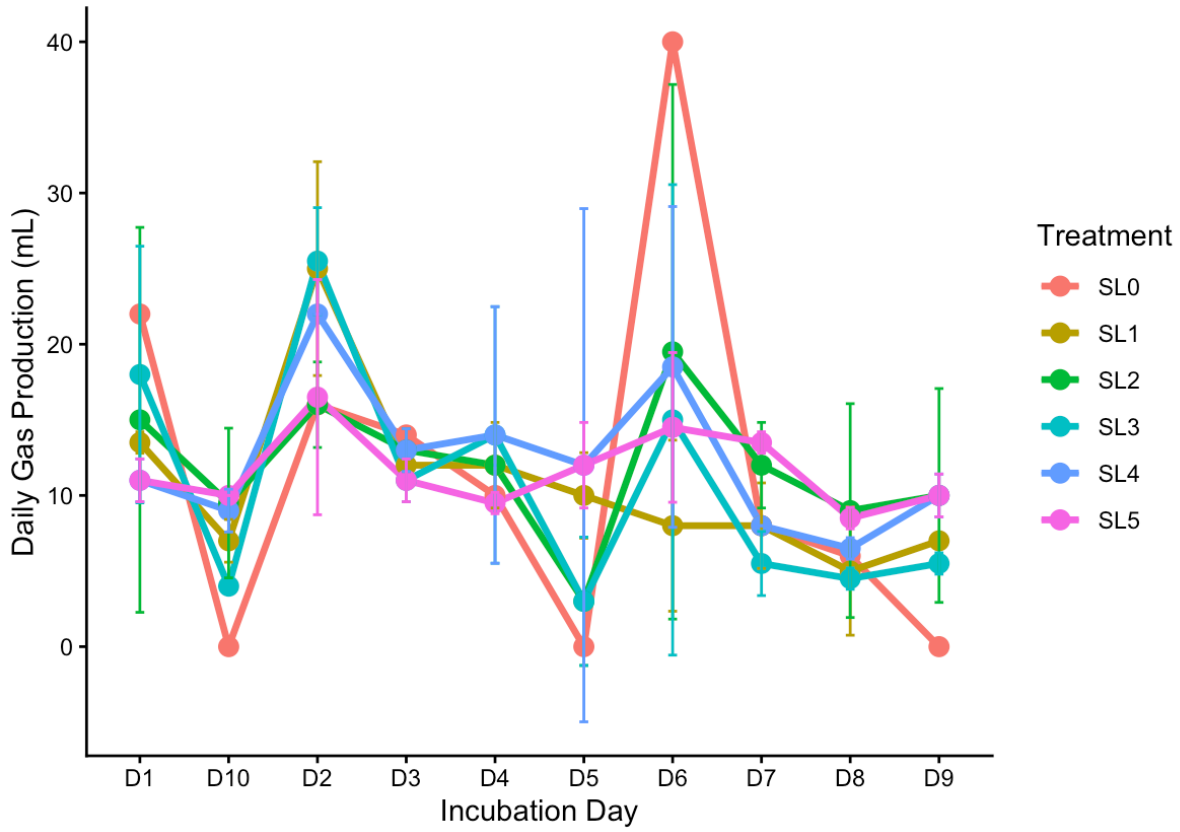


Figure 2. Daily gas production (ml) among treatments

Daily biogas production exhibited a similar pattern among treatments throughout the incubation period (Figure 2). An initial increase in gas production was observed during the first two days of digestion, followed by a gradual decline between Days 3 and 5. A secondary production peak occurred on Day 6, particularly in the control treatment, suggesting the degradation of more recalcitrant organic fractions after the depletion of readily fermentable substrates.

Among the microbial additives, SLBD treatments generally maintained higher gas production than EM4 during the later stages of incubation. The SL2 (1% SLBD) and SL4 (5% SLBD) treatments exhibited relatively stable gas production profiles and produced the highest cumulative gas yields. In contrast, the SL3 (2.5% SLBD) treatment showed greater variability between replicates. Although numerical differences among treatments were observed, statistical analysis indicated that treatment effects on cumulative gas production were not significant ($P > 0.05$). The control (SL0) produced the highest gas immediately, likely because the native microorganisms in the fresh manure were already active.

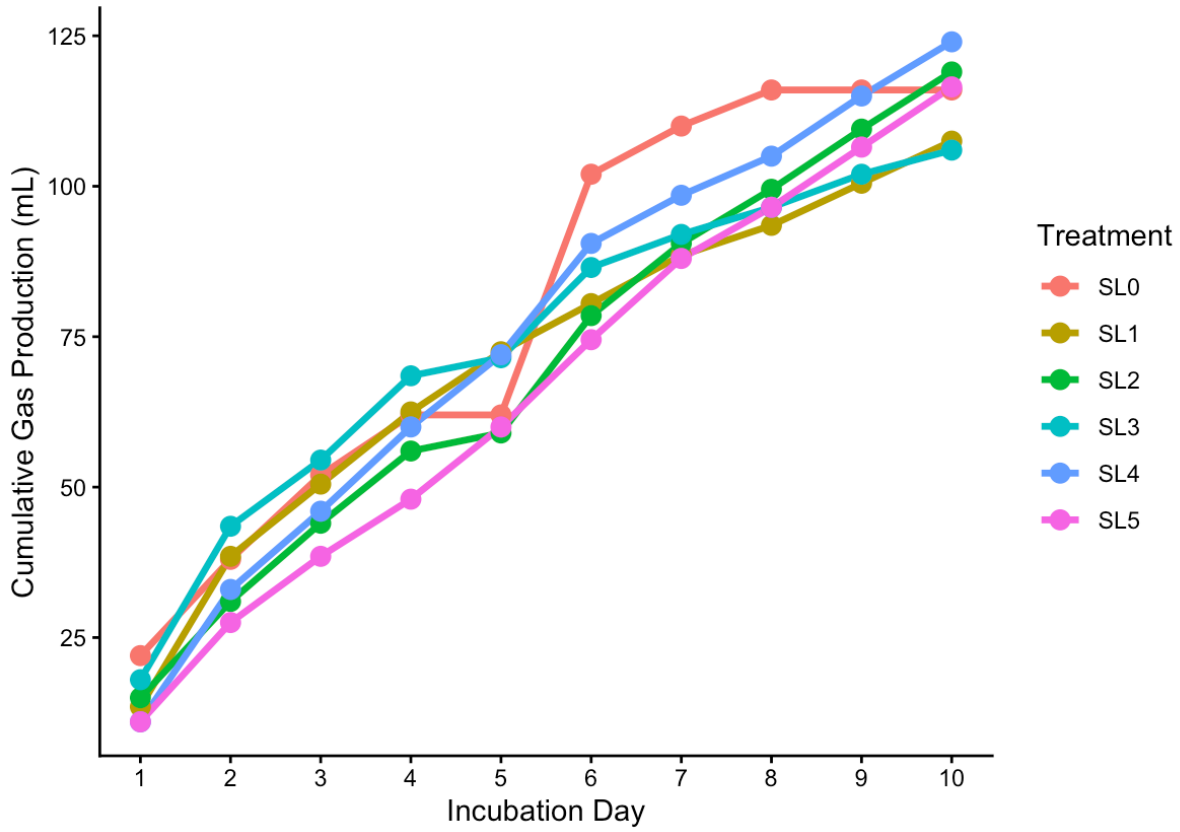


Figure 3. Cummulative gas production

The cumulative biogas production profiles of all treatments are presented in Figure 3. Gas production increased steadily throughout the incubation period, with the greatest increase occurring during the first six days of digestion. Thereafter, gas production continued to increase at a slower rate until Day 10. Among the treatments, SL4 (5% SLBD) achieved the highest cumulative gas production followed by SL2 (1% SLBD). The control treatment (SL0) and inoculum treatment (SL5) produced similar amounts of cumulative gas. In contrast, EM4 supplementation (SL1) resulted in lower cumulative gas production (107.5 mL).

The most interesting observation is around Day 6:

- SL0 increased from 62 to 102 mL
- SL4 increased from 81 to 100 mL
- SL2 increased from 79 to 88 mL

This sudden increase likely corresponds to the onset of degradation of more complex organic fractions. Because *Bacillus amyloliquefaciens* produces cellulase, xylanase, and other hydrolytic enzymes, the SLBD treatments may have facilitated the breakdown of fibrous components during this phase. The enhanced gas production observed in SLBD-treated reactors suggests that Bacillus amyloliquefaciens may have improved the degradation of organic matter during anaerobic digestion. However, analysis of variance indicated that treatment effects on cumulative gas production were not statistically significant ($P > 0.05$), likely due to the limited number of replicates and the variability among experimental units.The cumulative gas production increased rapidly during the first 5–6 days of incubation, followed by a slower increase until Day 10. This indicates that the easily degradable organic matter was utilized during the early stages of digestion, whereas the remaining gas production

was derived from the degradation of more recalcitrant organic compounds. Although SL4 produced the highest gas volume, SL2 reached approximately 96% of SL4's production while using only one-fifth of the SLBD dosage. SL1 remained among the lowest treatments throughout the incubation period and produced less cumulative gas than the control.

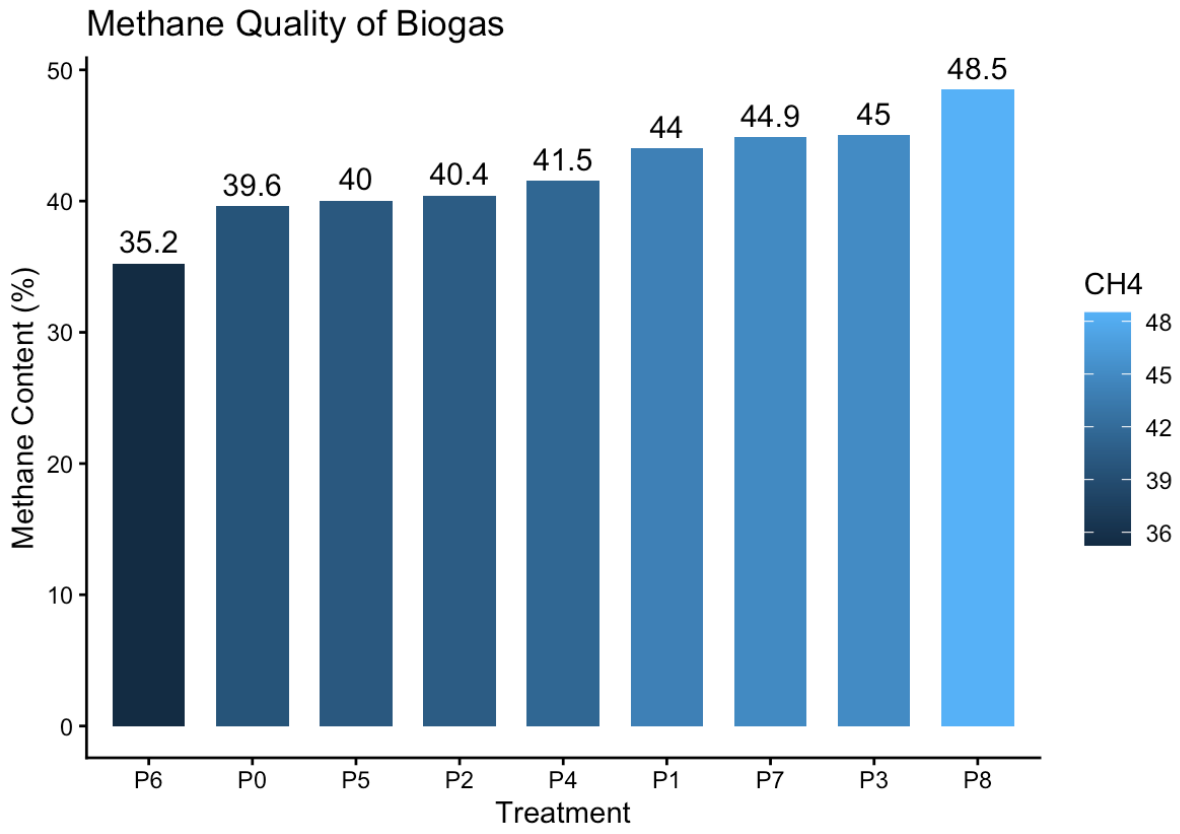
Gas quality

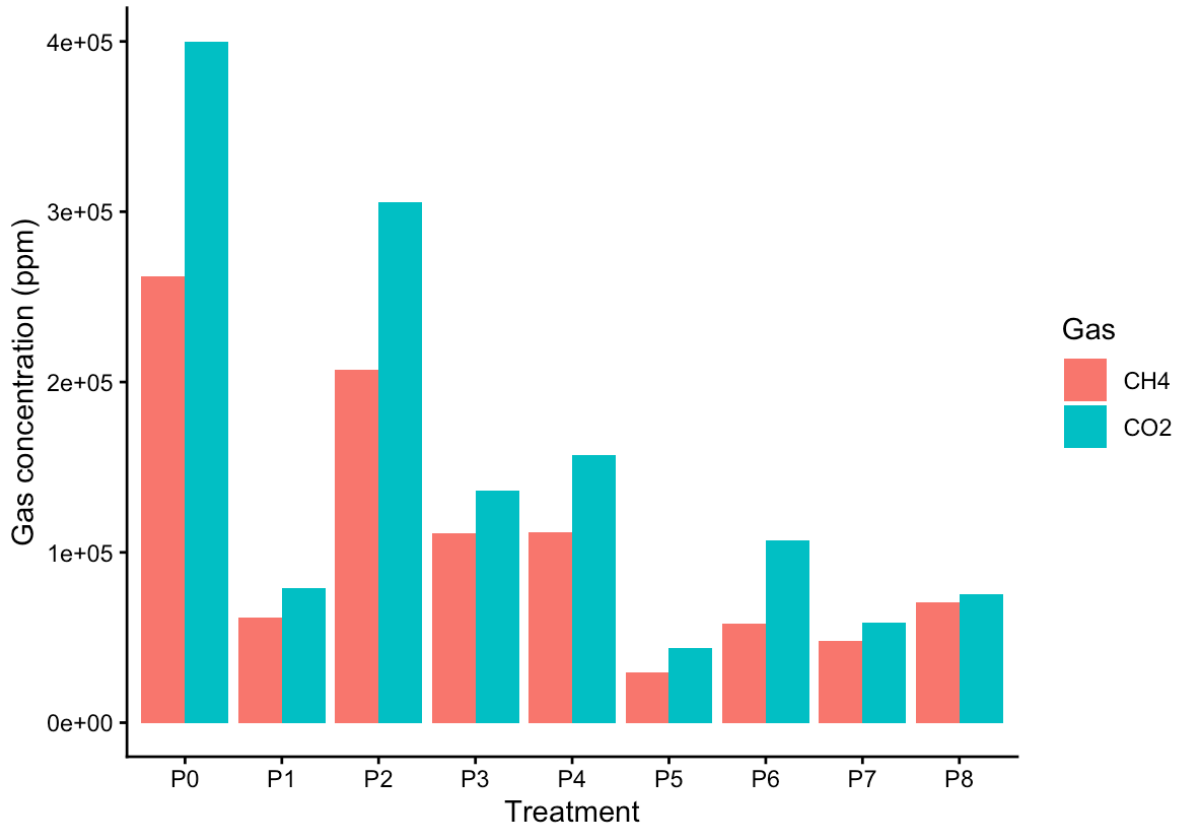
Sample	Treatment	CH_4 (ppm)	CO_2 (ppm)	CH_4:CO_2 Ratio	Estimated CH_4 (%)	Interpretation*
P1-B2	Rumen 5%, 39°C	261,963.52	399,728.92	0.66	39.59	Moderate
P2-B2	Rumen 5%, Room	61,915.97	78,732.34	0.79	44.02	Good
P3-B2	Rumen 1%, 39°C	207,410.82	305,728.75	0.68	40.42	Moderate
P4-B2	Rumen 1%, Room	111,348.55	135,986.89	0.82	45.02	Very good
P5-B2	EM4 5%, 39°C	111,701.24	157,186.75	0.71	41.54	Good
P6-B2	EM4 5%, Room	29,250.90	43,813.87	0.67	40.03	Moderate
P7-B2	EM4 1%, 39°C	58,265.06	107,020.15	0.54	35.25	Lowest
P8-B2	EM4 1%, Room	47,971.63	58,882.45	0.81	44.89	Very good
P9-B2	Rumen 5%, 39°C	70,926.31	75,314.75	**0.94**	**48.50**	Highest quality

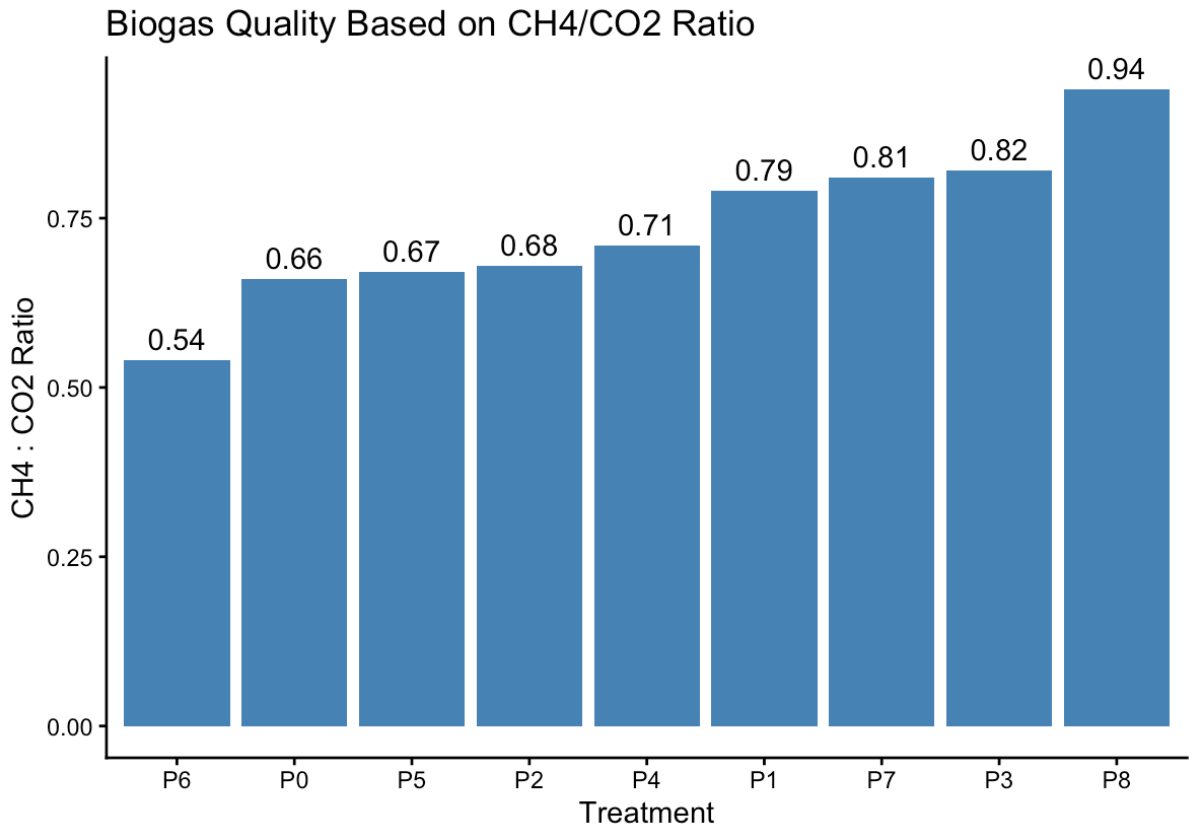
Interpretation of estimated CH4 : Very Good>45, Good 40–45, Moderate, 35–40, Poor <35

Biogas composition analysis indicated that methane quality varied considerably among treatments. The highest methane proportion was observed in P8 (EM4 1% at room temperature), reaching 48.5% CH_4, followed by P3 (1% rumen inoculum at 39°C) and P7 (EM4 1% at 39°C). In contrast, P6 (EM4 5% at room temperature) exhibited the lowest methane proportion (35.3%).

Rumen inoculum generally produced higher methane quality than EM4 at equivalent inclusion levels, particularly under incubation at 39°C. The results also suggest that increasing inoculum concentration from 1% to 5% did not improve methane quality. Overall, incubation at 39°C enhanced methane production efficiency when rumen fluid was used as an inoculum, highlighting the importance of both inoculum source and incubation temperature in anaerobic digestion performance. For future biogas experiments, **1% rumen inoculum at 39°C (P3)** may be the most efficient treatment because it achieved very high methane quality without requiring the larger inoculum dose (5%). This would reduce inoculum requirements while maintaining biogas quality.


Methane Quality of Biogas
Methane Content (%)
50
40
30
20
10
0
35.2
39.6
40
40.4
41.5
44
44.9
45
48.5
P6
P0
P5
P2
P4
P1
P7
P3
P8
Treatment
CH4
48
45
42
39
36


Gas concentration (ppm)
4e+05
3e+05
2e+05
1e+05
0e+00
P0
P1
P2
P3
P4
P5
P6
P7
P8
Treatment
Gas
CH4
CO2



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