



Effect of Copper Supplementation on Biogas Yield from Co-Digestion of Cattle Dung and *Jatropha Curcas* Fruit Coat

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ABSTRACT

Limited understanding exists regarding the role of copper as a trace additive in cattle dung–*Jatropha curcas* fruit coat (CD–JFC) co-digestion systems, particularly under ambient mesophilic conditions. This study therefore aimed to evaluate the effect of low-dose copper supplementation on biogas yield during batch anaerobic co-digestion of CD and JFC. Experiments were conducted in 2 L laboratory-scale digesters operated over 30 days at 25–35°C, comparing mono-digestion, co-digestion, and co-digestion with copper addition (4.45 g). Co-digestion improved cumulative biogas yield by approximately 47% relative to cattle dung mono-digestion, confirming enhanced substrate synergy. The inclusion of copper resulted in a further ~7.4% increase compared to co-digestion without copper. While this suggests a stimulatory effect, the relatively small absolute gain indicates that the influence of copper under the tested conditions is modest. Typical batch digestion behavior, including lag, active production, and stabilization phases, was observed; however, no direct process monitoring or yield normalization was performed, limiting mechanistic interpretation and cross-study comparability. Consequently, copper supplementation in this context should be interpreted as appearing to enhance biogas yield rather than providing definitive performance improvement. Given the laboratory-scale setup and short hydraulic retention time, further research is required to optimize copper dosage, evaluate long-term process stability, and assess potential environmental implications associated with metal accumulation in digestate.

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INTRODUCTION

Anaerobic digestion (AD) is a mature and widely implemented biotechnology for the stabilization of organic waste and production of methane-rich biogas (Bioresource Technology; Angelidaki et al., 2011). Although the biochemical stages of hydrolysis, acidogenesis, acetogenesis, and methanogenesis are well established, contemporary research has shifted from process description toward performance optimization and process intensification (Appels et al., 2008; Mata-Alvarez et al., 2014). Improving substrate utilization efficiency and methane productivity remains a central objective in advancing anaerobic digestion systems. Cattle dung (CD) is extensively used as a feedstock in both developing and industrialized biogas systems due to its buffering capacity and naturally occurring microbial consortia (Weiland, 2010). However, mono-digestion of cattle manure may suffer from suboptimal carbon-to-nitrogen (C/N) ratios and limited degradability, leading to moderate methane yields (Yenigün & Demirel, 2013). In contrast, lignocellulosic residues such as *Jatropha curcas* fruit coat (JFC) possess higher carbon content but exhibit structural recalcitrance due to lignin barriers that impede hydrolysis (Hendriks & Zeeman, 2009). Co-digestion strategies have therefore been proposed to balance nutrient composition, enhance microbial synergy, and improve methane production efficiency (Mata-Alvarez et al., 2014; Zhang et al., 2013). Beyond substrate blending, trace metal supplementation has gained attention as a strategy for enhancing methanogenic performance. Several studies have demonstrated that micronutrients such as iron, cobalt, nickel, and copper function as enzymatic cofactors in key metabolic pathways of

methanogenic archaea (Demirel & Scherer, 2011; Takashima & Speece, 1989). However, the influence of heavy metals is strongly concentration-dependent: low concentrations may stimulate enzymatic activity, whereas elevated concentrations can inhibit microbial growth or disrupt syntrophic interactions (Chen et al., 2008; Zayed & Winter, 2000).

Despite extensive investigation of trace metal effects in sludge and industrial wastewater digestion systems, comparatively limited research has focused on copper supplementation in manure–lignocellulosic co-digestion systems under ambient mesophilic conditions. Existing literature often evaluates heavy metals in isolation or in mixed-metal contexts without explicitly linking micronutrient supplementation to substrate synergy mechanisms (Demirel & Scherer, 2011; Chen et al., 2008). Consequently, the specific role of controlled copper addition in cattle dung–*Jatropha* blends remains insufficiently characterized.

In particular, there is a lack of experimental evidence addressing whether copper supplementation at defined dosage levels can modify kinetic parameters—such as lag phase duration and maximum production rate—within batch co-digestion systems. This gap limits mechanistic understanding of whether observed performance improvements arise primarily from C/N balance optimization or from trace-metal-mediated enhancement of methanogenic metabolism.

Therefore, this study investigates the combined effects of substrate co-digestion and copper supplementation on biogas production from cattle dung and *Jatropha curcas* fruit coat.

The guiding research questions are:

1. Does co-digestion of CD and JFC significantly enhance cumulative biogas yield relative to mono-digestion?
2. Does low-dose copper supplementation further modify yield?
3. Does copper addition influence lag phase duration and apparent methane production rate under batch conditions?

It is hypothesized that:

- (i) Co-digestion will improve cumulative yield due to improved substrate balance; and
- (ii) Controlled copper supplementation will enhance apparent kinetic performance without immediate inhibitory effects under short-term laboratory operation.

By explicitly testing these hypotheses, this work aims to clarify the role of copper as a trace additive in CD–JFC co-digestion systems and contribute to the optimization of micronutrient strategies in decentralized anaerobic digestion applications.

MATERIALS AND METHODS

Substrate Collection

Fresh cattle dung (CD) was collected from a livestock farm in Gidan kwano, Niger State, Nigeria. *Jatropha curcas* fruit coat (JFC) was obtained from the plant. Inoculum was prepared from a fresh cattle dung for 6 days to ensure acclimatized microbial populations.

Prior to digestion, no characterization was established due to unavailability of standard apparatus. This work is highly reliant on existing literature. The following parameters were to be determined in accordance with standard methods (APHA, 2017):

- i. Total solids (TS)
- ii. Volatile solids (VS)
- iii. Moisture content
- iv. Ash content
- v. Initial pH
- vi. Total carbon (TC)
- vii. Total nitrogen (TN)
- viii. Carbon-to-nitrogen (C/N) ratio

TS and VS were to be determined gravimetrically by oven drying at 105 °C and subsequent ignition at 550 °C. Total carbon and nitrogen were to be measured using standard Kjeldahl and combustion methods. All analyses were to be conducted in triplicate, and results reported as mean ± standard deviation.

Characterization of the substrates would have allowed proper normalization of substrate loading and facilitated interpretation of biogas yield relative to volatile solids content.

Experimental Design and Reactor Configuration

Batch anaerobic digestion experiments were conducted using laboratory-scale digesters with a total volume of 2.0 L and a working volume of 1.75 L. Reactors were fabricated from airtight high-density polyethylene containers fitted with gas-tight outlets connected to a gas collection system (in this work, water displacement was used to measure gas yield).

Four experimental conditions were evaluated:

1. CD mono-digestion (control A)
2. JFC mono-digestion (Control B)
3. CD–JFC co-digestion (Control C)
4. CD–JFC co-digestion with copper supplementation (Treatment D)

Reactors were randomly positioned to minimize positional bias.

Copper Dosing

Copper was introduced in the form of clean copper filings. The selected concentration was chosen randomly.

Operating Conditions and Monitoring

All digesters were operated under mesophilic conditions (25–35 °C). Ambient laboratory temperature was not continuously monitored, and no daily averages were recorded.

Initial pH was not adjusted to 7.0 ± 0.2 neither was pH monitored periodically throughout the duration of experiment. Digesters were manually agitated once daily to prevent stratification and ensure uniform microbial contact. The hydraulic retention time (HRT) was 30 days.

Biogas Measurement

Biogas production was measured daily using the water displacement method.

Methods

Preparation of Inoculum

The fresh cattle dung collected was mixed with tap water and digested for six days to serve as inoculum.

Fabrication of Digesters

The four containers (digesters) of size 2litres were bored on the cork. Iron rod was heated to red hot and used to bore the holes on the corks. One yard of hose was cut for each digester inserted into the holes. Silicon sealant was applied to the hole-hose assembly to prevent gas leakages.

Preparation of Substrates

Four substrate samples were prepared in this experiment. Sample A contains cattle dung alone, sample B contains *Jatropha* fruit coat alone, sample C contains cattle dung and *Jatropha* fruit coat and sample D contains cattle dung, *Jatropha* fruit coat and Copper filing.

Cattle Dung alone (Sample A)

Dry cattle dung was mixed with clean tap water in the ratio of 1:1. The mixture was stirred until fine slurry was obtained. 150ml of inoculum was then added to the slurry and stirred again for about one minute. The slurry was afterward poured into digester A.

Jatropha Fruit Coat Alone (sample B)

Jatropha fruit coat was mixed with clean tap water in the ratio of 2:1 respectively. The mixture was stirred thoroughly. 150ml of inoculum was then added to the mixture and stirred for about one minute. The whole mixture was then poured into the digester B.

Cattle Dung and Jatropha Fruit Coat (Sample C)

Dry cattle dung and *Jatropha* fruit coat was mixed in the ratio of 3:1. The cattle Dung–*Jatropha* coat mixture was mixture was mixed with water in the ratio of 1:1 and stirred until fine slurry was obtained. The 150ml of inoculum was added to the whole mixture and stirred thoroughly and then poured into the digester labeled C.

Cattle Dung, Jatropha Fruit Coat and Copper Filing (sample D)

Dry cattle dung, *Jatropha* fruit coat, and water were mixed in the same ratio as sample C. copper filing was then added to the mixture. The whole mixture was also stirred until fine slurry was obtained and then poured into digester D.

Reactor's Setup and Operation

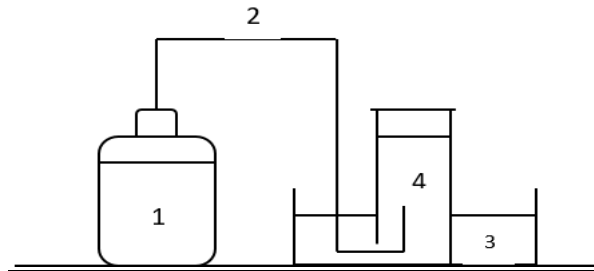


Figure 1: Experimental Device. Here: 1. Reactor; 2. Gas Collector hose; 3. Water Bath; 4. Gas Jar

A total of four improvised batch digesters (A, B, C and D) each having a capacity of 2L with 1.75L working volume were used in this work. A 6mm internal diameter hose were fixed on each cap of the batch digesters, silicon sealant was applied to ensure no air entrapment. After charging the digesters with substrate, the gas jar was filled with water at a marked level and inverted into the water bath. The experiments were carried out under ambient temperature. The first two digesters (A and B) were charged with cattle dung and *Jatropha* fruit coat for mono-digestion respectively. Digester C was charged with a mixture of 12.5% *Jatropha* fruit coat + 37.5% cattle dung + 50% water, i.e., 250ml JT + 750ml CD + 1000ml water. Digester D was charged with a mixture of 12.5% JT + 37.5% CD + 50% Water + 0.00445kg Cu for co-digestion. The hose from the digester (gas collection hose) was then passed into the gas jar. As the gas is produced in the digester, it passes through the hose and is collected over the water in the gas jar. The gas generated was recorded 4 times daily (8am, 11am, 2pm and 5pm) using water displacement method by reading the volume of water displaced in the gas jar which is equal to the volume of gas generated. Similarly, agitation was done daily at the anaerobic digesters manually. This was to enhance the digester process

by transferring heat uniformly throughout the digester and preventing formation of surface crust and scum (Alawi et al, 2009)

RESULTS AND DISCUSSION

Result

This experiment was carried out for a period of 30 days under ambient temperature of between 25°C and 35°C. Readings of the gas yield were taken four times daily (8am, 11am, 2pm and 5pm) and recorded. Figure 2 shows, biogas yield during 30 days of anaerobic digestion for sample A containing cow dung alone. Figure 3, shows biogas yield during 30 days of anaerobic digestion for sample B containing *Jatropha* alone. Figure 4 shows Biogas yield during 30 days of anaerobic digestion for sample C containing Cattle dung + *Jatropha* fruit coat. Figure 5 shows Figure 5. Biogas yield during 30 days of anaerobic digestion for sample D containing Cattle dung + *Jatropha* fruit coat + Copper filing. And Figure 6 shows a combined graph of biogas yield during 30 days of anaerobic digestion for sample A, B, C and D. Figure 7. shows a bar chart showing cumulative biogas yield during 30 days of anaerobic digestion for sample A, B, C and D.

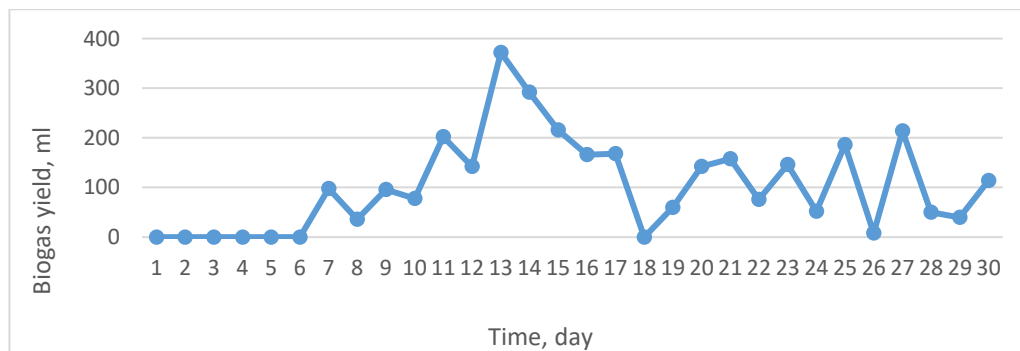


Figure 2: Biogas Yield during 30 days of Anaerobic Digestion for Sample A Containing Cow dung alone

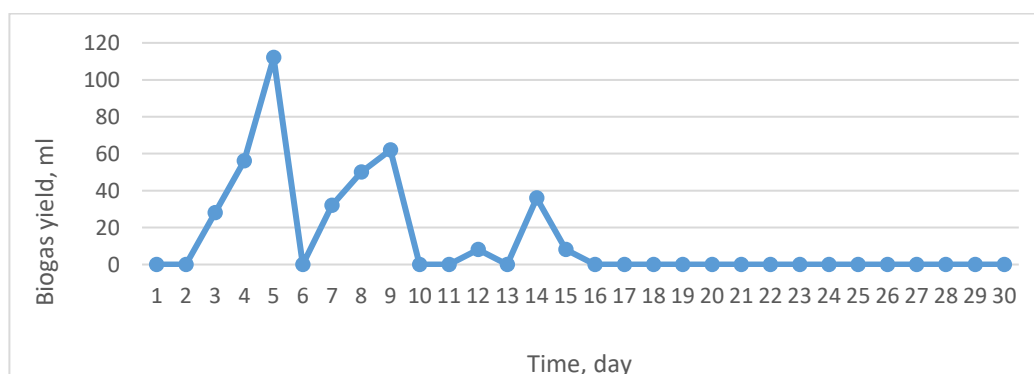


Figure 3: Biogas Yield during 30 days of Anaerobic Digestion for Sample B Containing *Jatropha* alone

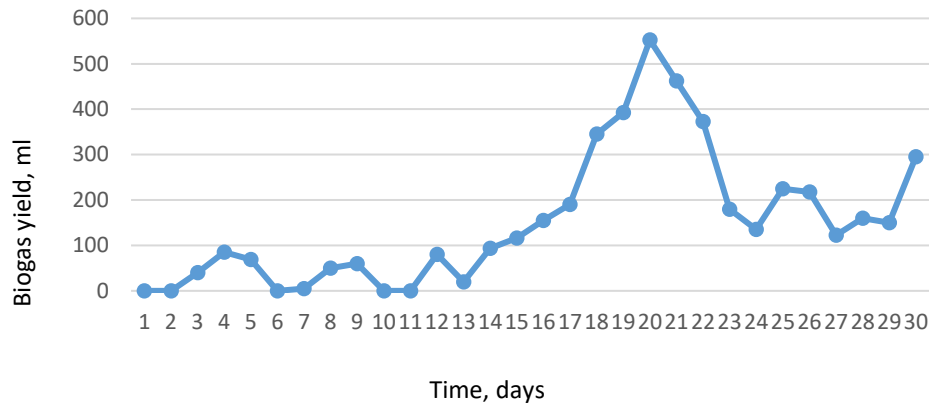


Figure 4: Biogas Yield during 30 days of Anaerobic Digestion for Sample C Containing Cattle dung + Jatropha Fruit Coat

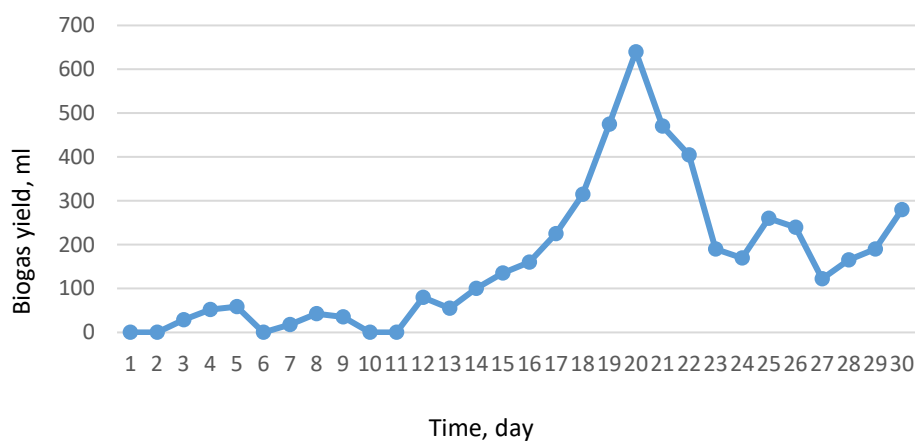


Figure 5: Biogas Yield during 30 days of Anaerobic Digestion for sample D Containing Cattle dung + Jatropha Fruit Coat + Copper Filing

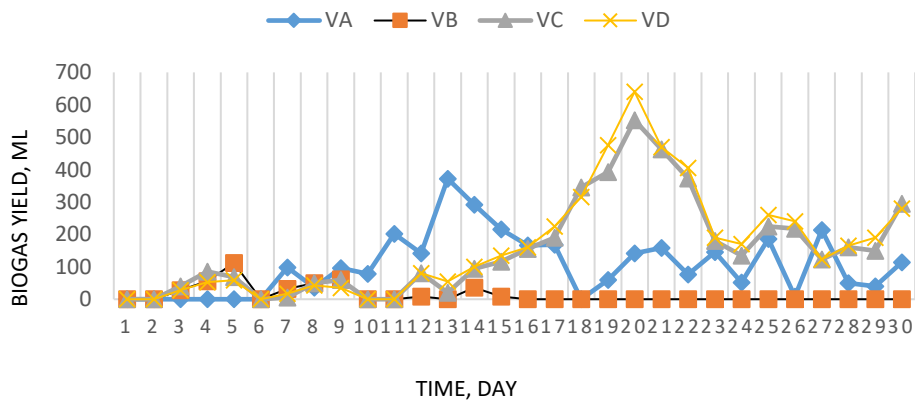


Figure 6: Biogas Yield during 30 days of Anaerobic Digestion for Sample A, B, C and D

Table 1: Cumulative Biogas Yield during 30 days for Sample A, Sample B, sample C and Sample D

Sample	Cumulative gas yield, ml
A(cattle dung alone)	3112
B(Jatropha fruit coat alone)	392
C (Cattle dung + Jatropha fruit coat)	4574
D (Cattle dung + Jatropha fruit coat + Copper filing)	4912.5

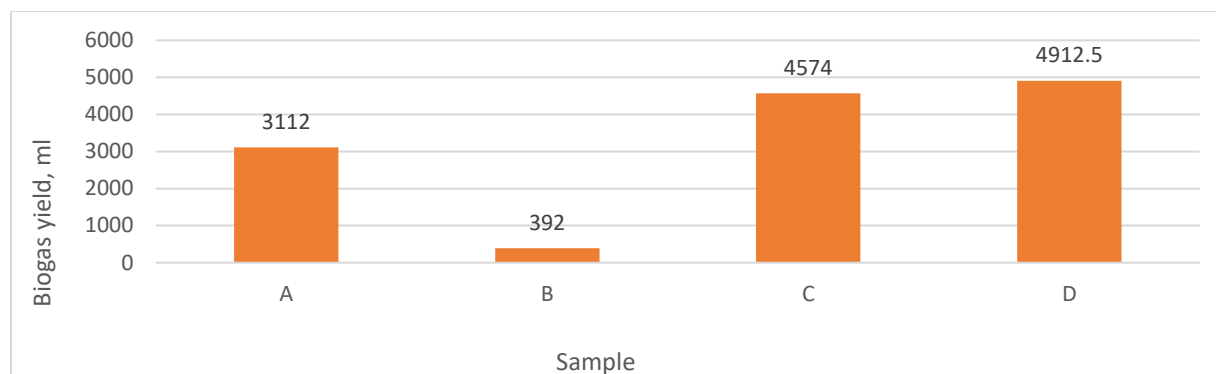


Figure 7: Bar Chart showing Cumulative Biogas Yield during 30 days of Anaerobic Digestion for Sample A, B, C and D

Discussion

The anaerobic digestion experiment was conducted for 30 days under ambient temperature conditions ranging between 25 °C and 35 °C. Cumulative biogas yields obtained from Samples A, B, C, and D were 3112 mL, 392 mL, 4574 mL, and 4912.5 mL respectively as shown in Figure 2-Figure 7.

Biogas Production Trends

All samples exhibited the typical pattern of batch anaerobic digestion, characterized by an initial lag phase, a period of accelerated gas production, and eventual stabilization.

Sample A (cattle dung alone) showed delayed gas production, with noticeable yield beginning around day 7, likely due to the time required for microbial acclimatization and hydrolysis of lignocellulosic components.

Sample B (Jatropha fruit coat alone) produced significantly lower gas volumes, indicating its relatively low biodegradability when digested as a sole substrate. In contrast, Sample C (co-digestion of cattle dung and Jatropha fruit coat) demonstrated a marked increase in cumulative yield, suggesting improved nutrient balance and synergistic microbial activity.

Sample D (co-digestion with copper addition) yielded the highest cumulative biogas volume. While this suggests a potential stimulatory role of copper, the magnitude of improvement relative to Sample C is modest.

Quantitative Comparison of Yields

To better interpret the results, relative increases in cumulative yield were evaluated:

- i. Sample C produced approximately 47% more biogas than Sample A, confirming the advantage of co-digestion.
- ii. Sample D produced approximately 7.4% more biogas than Sample C, corresponding to an absolute increase of about 338 mL over 30 days.

Although percentage differences suggest improvement, the relatively small absolute difference between Samples C and D indicates that the practical impact of copper addition under the tested condition may be limited. Therefore, the observed enhancement should be interpreted with caution.

Limitations in Yield Representation

The biogas yields are reported in absolute volume (mL), without normalization to substrate properties such as volatile solids (VS), total solids (TS), or reactor working volume.

This limits the ability to:

- i. Compare results across samples on an equal basis
- ii. Benchmark against existing literature
- iii. Assess scalability from an engineering perspective

For improved comparability, future analysis should express yield in standardized units such as mL/g VS added or m³/kg TS.

Statistical and Experimental Uncertainty

The study does not include replicate experiments or statistical indicators such as standard deviation or confidence intervals. As a result, it is not possible to determine whether observed differences, particularly the small increase between Samples C and D—are statistically significant or within normal experimental variability.

Consequently, the reported improvements should be regarded as *indicative trends rather than definitive performance differences*.

Effect of Copper Addition

The addition of copper (0.00445 kg) in Sample D appears to have a positive influence on biogas production. However, this conclusion is constrained by several factors:

- i. Only a *single copper dosage* was tested
- ii. The dosage was not normalized (e.g., mg Cu per kg substrate)
- iii. No investigation of inhibitory thresholds was conducted

Copper is known to play a *dual role* in anaerobic digestion: it can act as a micronutrient at low concentrations but becomes inhibitory at higher levels. Without testing multiple concentrations, it is not possible to determine whether the observed effect represents an optimal, suboptimal, or potentially inhibitory range.

Therefore, the role of copper in this study should be interpreted as *preliminary and exploratory*.

Mechanistic Interpretation

The discussion attributes enhanced biogas production to factors such as microbial acclimatization, lignin degradation, and increased methanogenic activity. While these explanations are consistent with established anaerobic digestion theory, they remain **speculative** in the context of this study due to the absence of supporting measurements such as:

- i. pH and alkalinity
- ii. Volatile fatty acids (VFA)
- iii. Methane composition of biogas
- iv. Temperature variation within digesters

Without these parameters, the proposed mechanisms cannot be directly validated.

Comparison with Existing Studies

The observed improvement in biogas yield through co-digestion aligns with previous studies reporting enhanced

digestion performance due to improved carbon-to-nitrogen balance and nutrient availability. However, the magnitude of improvement observed in this study appears modest when compared with some literature reports under controlled mesophilic conditions.

Similarly, while previous studies have shown that trace metals such as copper can enhance microbial activity, the lack of dosage optimization and process monitoring in this work limits direct comparison.

Figure Interpretation

Collectively, the figures distinguish between process kinetics (lag, peak, stabilization phases) and overall yield performance, enabling clearer interpretation of substrate synergy and copper supplementation effects.

CONCLUSION

The study indicates that co-digestion of cattle dung and *Jatropha* fruit coat improves biogas yield compared to mono-digestion. The inclusion of copper at the tested dosage showed a modest increase in cumulative gas production; however, due to the absence of replicate experiments, process monitoring, and dosage variation, this effect should be considered indicative rather than conclusive and also as a preliminary observation. Further studies incorporating standardized yield metrics, statistical validation, and multiple copper concentrations are required to confirm the role of copper in enhancing anaerobic digestion performance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

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