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Optimization of Biogas Production from Rice Husk and Cow Manure Using Liquid Anaerobic Digestion (L-AD) with Response Surface Methodology

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ABSTRACT

Human activities, particularly fossil fuel use, have increased greenhouse gas (GHG) emissions and contributed to climate change. Renewable energy from organic waste, such as biogas, is one potential mitigation strategy. This study aimed to optimize total gas production from rice husk and cow manure using a liquid anaerobic digestion (L-AD) system. A laboratory-scale batch experiment was conducted using response surface methodology with central composite design (RSM-CCD). The independent variables were total solids (TS) and substrate mixture volume, while the response was gas productivity measured by the water displacement method. The results showed that the optimum condition was obtained at 14% TS and 750 mL substrate mixture volume, equal to 75% of the digester capacity. Under this condition, the model predicted gas productivity of 51.89 mL/g, while the actual experimental productivity reached 56 mL/g. The cumulative gas production under the optimum condition was 428 mL, consisting of 85 mL during acclimatization and 343 mL during the active fermentation phase. The RSM quadratic model was statistically significant, with mixture volume and the interaction between TS and mixture volume showing important effects on gas productivity. Since this study measured only total gas volume and did not analyze methane concentration, the results should be interpreted as optimization of biogas volume production rather than confirmed biomethane production. Future studies should include CH_4 composition, energy yield, and CO_2-equivalent mitigation analysis to better evaluate the environmental benefits of the process.

1. INTRODUCTION

Climate change is one of the most imminent threats globally, and its impacts span ecological, economic, and social domains. This has been highlighted in previous studies [1-3]. Another study has also emphasized the connection between human activity and climate change, particularly the role of increasing emissions of greenhouse gases (GHG) from fossil fuel combustion [4]. In response to these challenges, the international community endorsed the Paris Agreement at the United Nations Framework Convention on Climate Change (UNFCCC) conference in 2015, which seeks to limit global average temperature increases to below 2 ℃ above pre-industrial levels, with an aspirational target of 1.5 ℃ [5, 6]. To support this goal, nations are urged to implement detailed Nationally Determined Contributions (NDCs) that directly contribute to GHG reductions. Achieving net zero emissions and other ambitious targets requires substantial investment in sustainable energy systems, aligning with the United Nations Sustainable Development Goals (SDGs) [7], including affordable and clean energy (SDG 7), responsible consumption and production (SDG 12), and climate action (SDG 13).

Indonesia has adhered to these global frameworks by ratifying the Paris Agreement through Law No. 16 of 2016, committing to voluntary GHG reductions of 29%, potentially increasing to 41% with international support [7]. Given its status as the world’s fourth most populous country and 16th largest economy, Indonesia faces rising energy demands alongside its development needs. However, fossil fuels remain predominant, and domestic natural gas reserves decreased from 100 TSCF in 2013 to 36 TSCF in 2022 [7]. Projections suggest that global energy demand will rise by 45% within the next decade, with fossil fuels still providing roughly 80% of energy. Indonesia’s National Energy Policy (Government Regulation No. 79 of 2014) mandates increasing new and renewable energy to 23% by 2025 and 31% by 2050 [8].

One viable solution to address energy and waste challenges is the conversion of organic waste into renewable biogas energy, which provides environmental benefits and serves as a fossil fuel substitute [9]. Biogas can also be produced through liquid anaerobic digestion (L-AD) or Solid-State Anaerobic Digestion (SS-AD), with L-AD demonstrating efficiency at total solids (TS) levels of approximately 5–15% [10]. Other research highlights that biogas production from organic materials is feasible and environmentally beneficial [5, 11, 12]. Recent studies have further shown that the optimization of L-AD systems is strongly affected by TS concentration, substrate loading, liquid availability, and pretreatment strategy [13-15]. In L-AD, lower TS values may improve mixing and microbial contact, but they can also reduce organic loading per reactor volume. In contrast, higher TS values may increase available organic matter, but excessive solids can limit mass transfer, reduce hydrolysis efficiency, and increase the risk of volatile fatty acid accumulation. Therefore, TS optimization should not be evaluated independently, but together with substrate mixture volume and liquid-phase availability. For lignocellulosic substrates, pretreatment is also a critical factor because the complex structure of cellulose, hemicellulose, and lignin can restrict microbial access during hydrolysis. Previous studies have reported that mechanical, chemical, thermal, biological, and combined pretreatments can improve biogas yield by increasing substrate accessibility and accelerating organic matter degradation [16-18]. Chemical pretreatment is often effective in disrupting lignin structure, but it may require additional chemical input and pH adjustment. Biological pretreatment is more environmentally friendly, but it usually requires a longer processing time. Mechanical pretreatment, such as grinding, is simpler, easier to operate, and more suitable for community-scale application, although its effect may be lower than intensive chemical pretreatment. These comparisons indicate that the selection of pretreatment should consider not only biogas yield but also operational simplicity, cost, and field applicability.

As an agrarian nation, Indonesia generates significant agricultural waste, including rice husks from milling, which consist of 50–63.5% milled rice, 20–30% husk, and 8–12% bran [13]. If disposed of improperly, such as by rooftop burning, these residues contribute to environmental pollution and GHG emissions like CH_4 and CO_2. Managing livestock waste also poses challenges; in 2022, Indonesia's beef cattle population exceeded 19 million, each producing about 87 kg of wet manure daily, totaling roughly 570 million tons/year [7]. This unmanaged waste significantly contributes to GHG emissions, since CH_4 has 25 times the global warming potential of CO_2, and the methane potential of cow manure is estimated at 17.1 billion m³/year, valued at up to IDR 64.3 trillion/year [14]. The co-digestion of rice husk and cow manure is therefore relevant because rice husk provides abundant lignocellulosic carbon, while cow manure and rumen inoculum can supply microbial communities and nutrients that support anaerobic digestion. However, because rice husk has low biodegradability and a relatively high C/N ratio, process optimization is required to improve total gas production.

Focusing on Bandongan District in Magelang Regency, which comprises 44.1% (1332 ha or 20.19 km² of 45.79 km²) agricultural land, 52 Rice Mill Units (RMUs) produce substantial rice husk, yet much remains unused. Additionally, five Organic Fertilizer Processing Units (UPPOs) for organic fertilizer production exist, and their efficiency depends on the utilization of cattle manure. Previous research by Saputri et al. [15] demonstrated that lignocellulosic substrates like rice husk achieved maximum biogas output with NaOH pretreatment at a TS of 7%, within the tested range of 7–9% [15]. However, studies addressing L-AD biogas production at TS levels between 9.5–12% are lacking. Therefore, this study aims to examine TS values from 7–14% to determine optimal operational conditions for biogas production, integrating findings through quantitative analysis to define ideal TS parameters and enhance understanding of L-AD biogas production from lignocellulosic agricultural waste. Specifically, this study evaluates the combined effects of TS and substrate mixture volume on total gas productivity from mechanically pretreated rice husk and cow manure under L-AD conditions.

2. METHODOLOGY

2.1 Study area

The location of this research is in Bandongan District, Magelang Regency, Central Java Province, Indonesia. The district has a total area of 45.79 km² and a maximum population density of 1,373 inhabitants per square kilometer, distributed across 14 villages as seen in Figure 1. Land area data show that 44.1% of the district consists of agricultural land (2,012.29 ha), and the average dry harvested grain (Gabah Kering Panen, GKP) production in 2024 exceeded the provincial average of Central Java, which was 56.69 quintals/ha, reaching 62.08 quintals/ha. This productivity indicates that rice husk has strong potential as a surplus substrate for biogas production.

The total number of Rice Processing Units (UPP) in the regency is 52 units, while the number of UPPO is 5 units. Based on four criteria, namely: (1) the presence of RMUs, (2) the presence of UPPOs, (3) agricultural land area, and (4) total village area, three villages were selected for further investigation. The selected villages were Sukosari (229.38 ha of agricultural land, 11 RMUs, and 1 UPPO), Tonoboyo (135.44 ha, 6 RMUs, and 1 UPPO), and Kebonagung (119.87 ha, 6 RMUs, and 1 UPPO), as shown in Table 1.

Table 1. Selected villages for the study focus area

Village	Total Area (km²)	Agricultural Land (ha)	No. of RMUs	No. of UPPOs
Sukosari	2.45	229.38	11	1
Tonoboyo	2.16	135.44	6	1
Kebonagung	2.50	119.87	6	1

Note: RMU: Rice Mill Units, UPPO: Organic Fertilizer Processing Units

2.2 Substrate collection and characterization

Substrates were collected from the three study villages. Rice husk was procured from RMUs located in each village (Bandongan, Sukosari, Kebonagung, and Tonoboyo), and cow dung manure was obtained from the UPPO livestock units existing in each of these villages. Rumen fluid was used as an inoculum starter and was taken from Penggaron slaughterhouse in Semarang, which has a high, rich & active methanogenic microbial community.

Initial laboratory characterization of all substrates was performed to determine their physicochemical properties. Moisture content and TS content were determined gravimetrically according to SNI 01-2891-1992. Organic carbon content was determined using the Walkley–Black wet oxidation–spectrophotometry method, while total nitrogen was analyzed using wet digestion–spectrophotometry. The C/N ratio was then calculated based on the measured organic carbon and total nitrogen values. The C/N ratios reported in this study represent the measured initial C/N ratios of the individual substrates before urea addition. Because the post-adjustment C/N ratio after urea addition was not directly re-measured for each experimental run, the urea addition should be interpreted as a nitrogen adjustment step based on substrate characterization and mass calculation, not as a directly verified final C/N ratio.

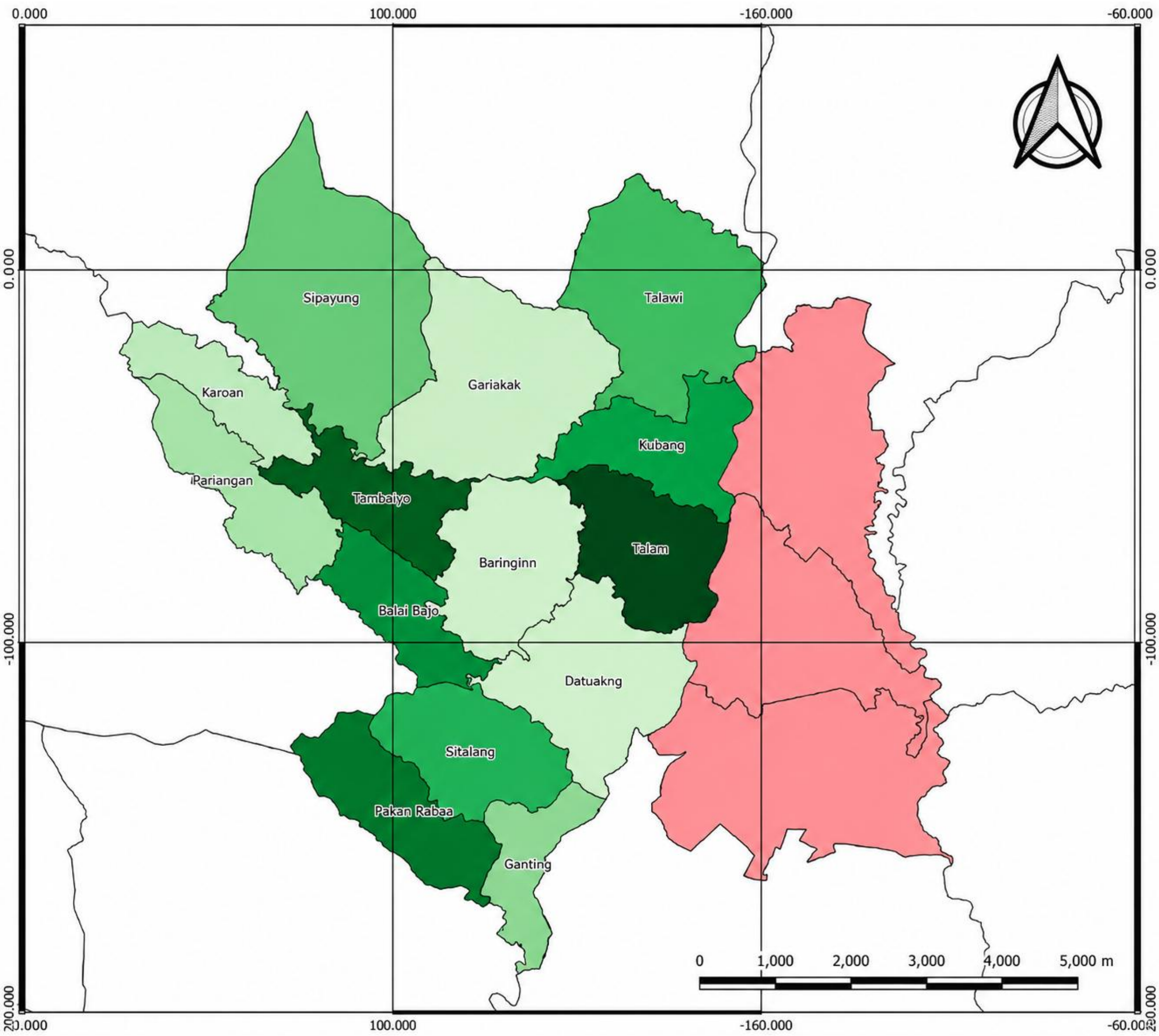

Figure 1. Study area location

TS content was calculated using the following equation:

$$TS(\%) = \frac{W_{\text{dry}} - W_{\text{dish}}}{W_{\text{wet}} - W_{\text{dish}}} \times 100$$

where, W_{dry} represents the weight of the oven-dried sample with the dish/crucible (mg), W_{wet} represents the weight of the wet sample with the dish/crucible (mg), and W_{dish} represents the weight of the empty dish/crucible (mg).

Given that all substrate combinations showed C/N ratios significantly higher than the desired optimal range of 20–30 for anaerobic digestion [18], an extra amount of urea was added in a batch at 32.79 g to amend the nitrogen deficiency and promote better conditions for methanogenic microorganisms. The amount of urea was calculated to reduce the initial high C/N ratio of the substrate mixture toward the recommended anaerobic digestion range of 20–30. However, the final adjusted C/N ratio after urea addition was not directly re-measured for each experimental run. Therefore, the urea addition should be interpreted as a nitrogen adjustment step based on substrate characterization and mass calculation, rather than a directly verified final C/N ratio. Future studies should measure the post-adjustment C/N ratio for each treatment condition to provide a clearer relationship between nutrient balance and gas productivity.

Rice husk was mechanically pretreated by grinding using a grinder prior to digestion. This physical pretreatment method was chosen for its operability and reduced maintenance needs compared to chemical and biological methods. This grinding process decreased the particle size of rice husk, which increased the specific surface area of lignocellulosic material and subsequently improved its accessibility towards anaerobic microorganisms during the hydrolysis step [16]. However, the particle-size distribution of the ground rice husk was not measured in this study. Therefore, particle-size indicators such as D10, D50, and D90 could not be reported. This limitation is acknowledged because particle size can influence hydrolysis efficiency and gas productivity. Future studies should include sieve analysis or laser diffraction analysis to quantify the particle-size distribution of ground rice husk.

2.3 Experimental design and biogas production

Lab-scale batch digester configuration experiments were conducted for biogas production. The gas volume was determined using the water displacement method, in which the volume of displaced water corresponded to the total gas produced. This method was used only to quantify cumulative gas volume and daily gas production. It did not distinguish between CH_4, CO_2, and other trace gases in the biogas mixture. Therefore, the response variable in this study was expressed as total gas productivity rather than biomethane productivity. Biomethane potential or methane composition analysis would require additional methods, such as gas chromatography or CO_2 absorption using alkaline/caustic scrubbing, which were beyond the scope of the present laboratory-scale experiment.

Digester construction and temperature control. Each digester was constructed using a 1 L high-density polyethylene (HDPE) plastic bottle wrapped in thermal foil to reduce temperature fluctuations during fermentation. The digesters were operated under mesophilic conditions, and the temperature was monitored daily using a digital temperature sensor. The observed temperature during the experimental period was maintained within the range of 30–40 °C, with minor daily fluctuations caused by ambient laboratory conditions. Although active temperature control was not applied, the thermal foil helped minimize rapid temperature changes. Each bottle was equipped with three ports to allow substrate inlet, slurry outlet, and gas outlet, as well as a digital temperature sensor for monitoring and controlling mesophilic operating conditions (30–40 °C). Gas leakage was checked before fermentation by sealing each digester and observing pressure stability and visible bubble formation at the connection points. No visible leakage was observed before the experimental runs. However, small undetected leakage could not be completely excluded and is acknowledged as a possible source of experimental uncertainty. To improve reproducibility, future studies should use pressure-decay testing or gas-tight reactors equipped with standardized fittings. Before each run, a leak test was conducted on all digesters to ensure the integrity of the anaerobic system.

A central composite design (CCD) under response surface methodology (RSM) was applied using Design-Expert version 13.0 software. Two independent factors were analyzed: TS (%) and substrate mixture volume (mL). Biogas productivity, expressed in mL/g, was used as the dependent variable (response). The control variables were reactor volume, inoculum source, and experimental time (35 d). The value limits for each factor were set at TS of 7%–14% and substrate volume of 500–750 mL (50%–75% of the total reactor capacity of 1,000 mL). These limits represented the factorial design range, while the Central Composite Design also generated axial or star points outside this range to estimate curvature and improve the fitting of the quadratic response surface model. Therefore, several experimental runs included TS values of 5.55% and 15.45%, as well as substrate volumes of 448.22 mL and 801.78 mL. These axial points were not intended as the main operational range, but were included for statistical model development and curvature estimation. Using the RSM-CCD design, 13 experimental runs, including factorial points, axial points, and center points for model verification, were developed, as shown in Table 2. Therefore, model predictions outside the tested CCD design space should be interpreted carefully and require further validation.

Table 2. Response surface methodology with central composite design (RSM-CCD) experimental runs for biogas production optimization

Std	TS (%)	Mixture Volume (mL)	Actual Productivity (mL/g)
7	10.50	448.22	21
2	14.00	500.00	18
10	10.50	625.00	14
13	10.50	625.00	13
5	5.55	625.00	37
4	14.00	750.00	56
1	7.00	500.00	38
3	7.00	750.00	25
9	10.50	625.00	29
11	10.50	625.00	16
8	10.50	801.78	49
6	15.45	625.00	19
12	10.50	625.00	21

Note: TS: total solids; Std: Standard order (design order generated by software)

Each experimental condition was conducted as a single batch run, except for the center-point runs generated by the RSM-CCD design. These center-point repetitions were used to estimate pure error and support model verification. However, independent triplicate digesters were not prepared for every experimental condition. Therefore, standard deviations or confidence intervals could not be reported for all runs. This is acknowledged as a limitation of the study, and future work should include at least three independent replicate digesters for each condition to reduce random error and improve statistical reliability. Daily biogas volume data were recorded from the start of fermentation for 35 days. Furthermore, to maintain process stability, pH and temperature were checked daily. All pH measurements were conducted using a calibrated pH meter, and all temperature readings were taken using the digital sensor attached to each digester. The daily pH and temperature data for each operating condition were summarized as mean, standard deviation, minimum, and maximum values. These values were then reported in the Results section to describe the stability of the anaerobic digestion process under each TS and substrate mixture volume condition. To maintain environmental consistency across digesters, all reactors were placed in the same laboratory area and operated under similar ambient conditions. The same substrate sources, inoculum source, pretreatment procedure, and urea adjustment approach were applied for all experimental runs. Temperature and pH were monitored daily to ensure that the digestion process remained within suitable conditions for anaerobic microbial activity. However, the daily pH and temperature records were used only for operational monitoring and were not systematically archived for each experimental run. Therefore, treatment-specific pH and temperature statistics, including mean, standard deviation, minimum, and maximum values, could not be reported or included in the RSM model. This limitation is acknowledged because pH and temperature variability may influence microbial activity and gas production. Microbial activity was not directly measured using indicators such as volatile fatty acids, alkalinity, microbial abundance, or methane-forming activity. Therefore, changes in microbial performance were interpreted indirectly from the daily gas production pattern. Future studies should systematically record daily pH and temperature data for each treatment and report them as mean, range, and standard deviation to better evaluate process stability. Future studies should include daily pH and temperature profiles, influent quality monitoring, and microbial activity indicators to better explain the relationship between environmental stability and gas yield.

After a 10-day acclimatization phase, the substrate was used. In this phase, 15% (v/v) rumen fluid from Penggaron slaughterhouse was mixed with water at a ratio of 1:1 (w/w), and the substrate, at a total volume of 50%, was added as inoculum. This acclimatization period was intended to help the microbial community adapt to the digester environment before being exposed to the full experimental substrate. The remaining 50% of the substrate was added after acclimatization according to the variation assigned for each run.

The substrate mixture for each run was prepared by mixing rice husk, cow manure, urea, and rumen starter with an adequate amount of water to achieve the desired TS concentration. The TS content of each substrate, determined during characterization (Section 2.2), was used to calculate the corresponding composition of the mixture so that the final mixture reached the specific TS percentage for each experimental run.

3. RESULTS

3.1 Substrate characterization

The three study villages, Sukosari, Tonoboyo, and Kebonagung, provided rice husk and cow manure as substrates. The results of physicochemical characterization are shown in Table 3. The rice husk samples had high TS values (86.26–92.59%), low organic carbon content (1.20–3.15%), and wide C/N ratios (40.89–77.11). Substrates with high C/N ratios indicate nitrogen deficiency, which is typical of lignocellulosic materials and can inhibit methanogenic activity if not corrected [17]. However, the final C/N ratio after urea addition was not directly measured for each experimental run. Therefore, the reported C/N values should be interpreted as measured initial substrate characteristics, while the adjusted C/N condition was estimated from mass-based calculation. In addition, although rice husk was mechanically ground before digestion, the particle-size distribution of the ground rice husk was not measured. Thus, D10, D50, and D90 values could not be reported. These limitations may affect the detailed interpretation of the relationship between substrate pretreatment, nutrient balance, and total gas productivity.

The UPPO samples had lower TS values (21.05–22.38%) and higher organic carbon content (10.46–11.40%), with C/N ratios ranging from 52.47 to 68.58. The TS and C/N ratio of the substrate obtained from the Penggaron slaughterhouse in Semarang were 18.63% and 50.75, respectively, for the rumen. Meanwhile, cow manure obtained from the study area was reported to have the highest TS value (44.30%) as well as the highest C/N ratio (57.81).

Table 3. Physicochemical characterization of substrates

Substrate	TS (%)	C-Organic (%)	N-Total (%)	C/N Ratio
Cow manure (Penggaron slaughterhouse)	44.30	7.19	0.124	57.81
Rumen (Penggaron slaughterhouse)	18.63	16.86	0.332	50.75
Rice husk (RMU Bandongan)	92.59	3.15	0.050	62.71
Rice husk (RMU Sukosari)	86.26	1.53	0.029	52.25
Rice husk (RMU Kebonagung)	87.87	1.20	0.029	40.89
Rice husk (RMU Tonoboyo)	87.52	1.48	0.019	77.11
Cow manure (UPPO Sukosari)	21.05	11.40	0.217	52.47
Cow manure (UPPO Kebonagung)	21.86	10.46	0.177	58.98
Cow manure (UPPO Tonoboyo)	22.38	10.72	0.156	68.58

Note: TS: Total solids; RMU: Rice Mill Units; UPPO: Organic Fertilizer Processing Units

Since the C/N ratios of all substrate combinations were extremely high, far beyond the ideal operating range of 20–30 recommended for stable anaerobic digestion [18], 32.79 g of urea per batch was added as a supplementary nitrogen source to adjust the C/N ratio to an acceptable level. For C/N ratios above 30, nitrogen (N) deficiency has been reported, which can restrict microbial growth and cause volatile fatty acid accumulation during the methanogenesis stage [17].

Prior to digestion, rice husk was mechanically pretreated by grinding (size reduction). This physical pretreatment was chosen because of its ease of application at the community scale and its lower maintenance requirements compared with chemical or biological methods. Grinding disrupted the lignocellulosic structure, reduced particle size, and increased the specific surface area, thereby improving microbial accessibility and hydrolysis efficiency [16]. Rice husk is a lignocellulosic residue, and its inherent characteristics can severely impede biodegradation and reduce biogas production if no pretreatment is applied [19]. Although urea was added to improve the nutrient balance of the substrate mixture, the post-adjustment C/N ratio was not experimentally measured for each run. In addition, the particle size distribution of ground rice husk was not quantified. These limitations may affect the interpretation of the relationship between pretreatment, nutrient balance, and gas productivity. Therefore, future studies should include particle size distribution analysis and post-adjustment C/N ratio measurement to strengthen process reproducibility and optimization accuracy.

3.2 Biogas production optimization

3.2.1 Daily biogas production profile

Under mesophilic conditions (30–40 °C), biogas production was monitored daily during the 35-day fermentation period. Although pH and temperature were checked daily as part of operational monitoring, the treatment-specific daily records were not systematically archived. Therefore, pH and temperature mean, range, and standard deviation for each operating condition could not be presented in the Results section. This limitation should be considered when interpreting the variability of total gas productivity among the experimental runs. A 10-day acclimatization phase was carried out before substrate addition, using rumen from Penggaron Animal Slaughterhouse (RPH) Penggaron as inoculum (15% of the total mixture volume), diluted with water at a 1:1 ratio, with substrate added at 50% of the total mixture volume. This acclimatization phase resulted in an initial gas volume of 85 mL, indicating that the microbial community had adapted to the digester environment.

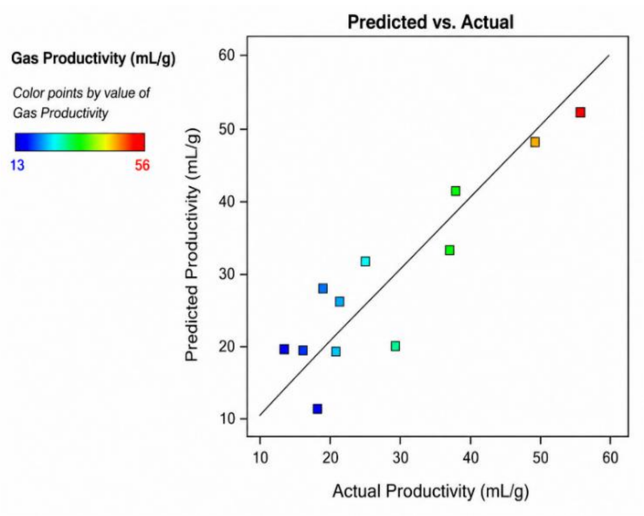

Figure 2. Comparison of actual and predicted biogas productivity values using Design Expert 13

After acclimatization, the fermentation process continued for 23 days with the remaining half (50%) of the substrate, added according to the experimental variations. At this stage, a further 343 mL of gas was produced, resulting in a total cumulative gas production of 428 mL under the optimum condition (Run 6: TS 14%, mixture volume 750 mL). On day 19, daily biogas production reached a maximum output of 56 mL, and then gradually declined as the available substrate in the system was naturally depleted. Figures 2 and 3 show the daily production profile and the actual versus predicted values, respectively.

The production pattern observed, namely a slow increase during the exponential phase, a clear peak on day 19, and a subsequent decline, is consistent with standard batch anaerobic digestion kinetics, in which substrate availability limits microbial activity and gas production [10]. The sharp peak during the active gas production phase (days 16–24) was likely associated with intensified anaerobic microbial activity, including acetogenesis and methanogenesis. However, because gas composition was not analyzed in this study, the relative contribution of CH_4 and CO_2 to the measured gas volume could not be confirmed. Therefore, this result should be interpreted as an increase in total gas production rather than verified methane production.

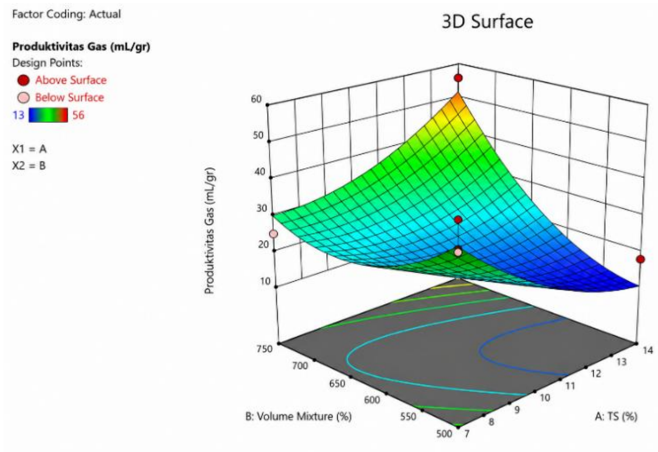

Figure 3. 3D surface plot of biogas productivity as a function of total solids (TS, %) and volume mixture (mL)

3.2.2 Response surface methodology with central composite design model and statistical analysis

Response surface methodology with central composite design (RSM-CCD), implemented using Design-Expert 13 software, was applied to the biogas productivity data obtained from the 13 experimental runs. The response data were best fitted by a quadratic model. The full analysis of variance (ANOVA) results is presented in Table 4.

Table 4. Analysis of variance (ANOVA) results for the quadratic model of biogas productivity

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value
Model	1873.99	5	374.80	6.88	0.0125*
A – TS	26.12	1	26.12	0.4798	0.5108
B – Volume Mixture	521.61	1	521.61	9.58	0.0174*
AB	650.25	1	650.25	11.94	0.0106*
A^2	201.91	1	201.91	3.71	0.0955
B^2	549.48	1	549.48	10.09	0.0156*
Residual	381.09	7	54.44	–	–
Lack of Fit	207.89	3	69.30	1.60	0.3224 (ns)
Pure Error	173.20	4	43.30	–	–
Cor Total	2255.08	12	–	–	–

Note: *Significant at $p < 0.05$; ns = not significant

The overall model was statistically significant (F-value = 6.88, p = 0.0125), indicating that the quadratic model adequately described the relationship between the independent variables and biogas productivity. Among the individual terms, mixture volume (B) was significant (p = 0.0174), whereas TS content (A) did not show a statistically significant independent effect (p = 0.5108). More importantly, the interaction term (AB) was significant (p = 0.0106), indicating that TS and mixture volume jointly affected biogas productivity. Likewise, the quadratic term of mixture volume (B^2) was significant (p = 0.0156), confirming a nonlinear relationship between mixture volume and biogas output.

The non-significant individual effect of TS (p = 0.5108) could be explained by the covariance between TS and mixture volume. This suggests that substrate concentration alone may not control biogas productivity when there is insufficient liquid medium to support microbial activity, which is a common behavior in L-AD systems, where both organic loading and liquid volume interact in regulating process efficiency [10].

The model produced an R^2 value of 0.8310 and an adjusted R^2 value of 0.7103, suggesting an adequate fit for a laboratory-scale trial. However, the discrepancy between the predicted R^2 (0.2244) and the adjusted R^2 (0.7103), which exceeded the recommended difference of 0.2, indicates that the model had limited predictive ability outside the experimental dataset. To further evaluate this condition, model diagnostic checks were conducted using residual-based analysis. The diagnostic plots included residuals versus fitted values, a normal Q–Q plot of residuals, and residuals versus leverage, as shown in Figure 4. These plots were used to assess residual distribution, normality, and the possible presence of outliers or influential observations.

The residuals versus fitted values plot showed that most residuals were distributed around the zero line, indicating that the quadratic model did not show a strong systematic error pattern. However, several experimental points had relatively larger residuals than the others. The normal Q–Q plot showed that the residuals generally followed the expected normal trend, although minor deviations were observed at the upper and lower tails. This suggests that the normality assumption was reasonably acceptable but affected by the small number of experimental runs. The residuals versus leverage plot showed that the factorial and axial design points had higher leverage than the center-point runs, which is expected in a CCD design (Figure 4). However, no observation exceeded the conventional high-leverage threshold. Influence diagnostics also showed that some runs had relatively larger influence on the model fit. In particular, the runs at TS 15.45% and 625 mL, TS 14% and 500 mL, TS 5.55% and 625 mL, and TS 7% and 750 mL showed higher Cook's distance values than the other observations. These points were not removed because they were part of the planned RSM-CCD design, and the lack-of-fit test was not significant. Nevertheless, their influence helps explain the low predicted R^2 value. The replicated center points also showed variation in actual productivity, indicating biological and operational variability among digesters. Therefore, the low predicted R^2 was likely caused by a combination of small sample size, experimental noise, biological variability, and influential design points.

A summary of the main diagnostic observations is presented in Table 5. These diagnostic results indicate that the model is suitable for preliminary design-space exploration and optimization within the tested range. However, its predictive ability outside the experimental dataset should be interpreted with caution. Future work should include additional replicate runs, validation experiments at the predicted optimum condition, and more detailed process monitoring to improve the reliability of the RSM prediction. Adequate precision (8.31) was greater than the minimum acceptable value of 4.0; therefore, the signal-to-noise ratio was considered sufficient for preliminary design space exploration [18]. A non-significant lack-of-fit f-value (1.60) with p = 0.3224 suggests that the model fitted the data without strong systematic error.

Table 5. Summary of model diagnostic observations

Std	TS (%)	Volume Mixture (mL)	Actual Productivity (mL/g)	Diagnostic Interpretation
6	15.45	625.00	19	Relatively large negative residual and stronger influence
2	14.00	500.00	18	Relatively large positive residual and stronger influence
5	5.55	625.00	37	Axial point with moderate influence
3	7.00	750.00	25	Factorial point with moderate influence
9	10.50	625.00	29	Center-point variation contributing to pure error

Note: Std: Standard order (design order generated by software); TS: Total solids

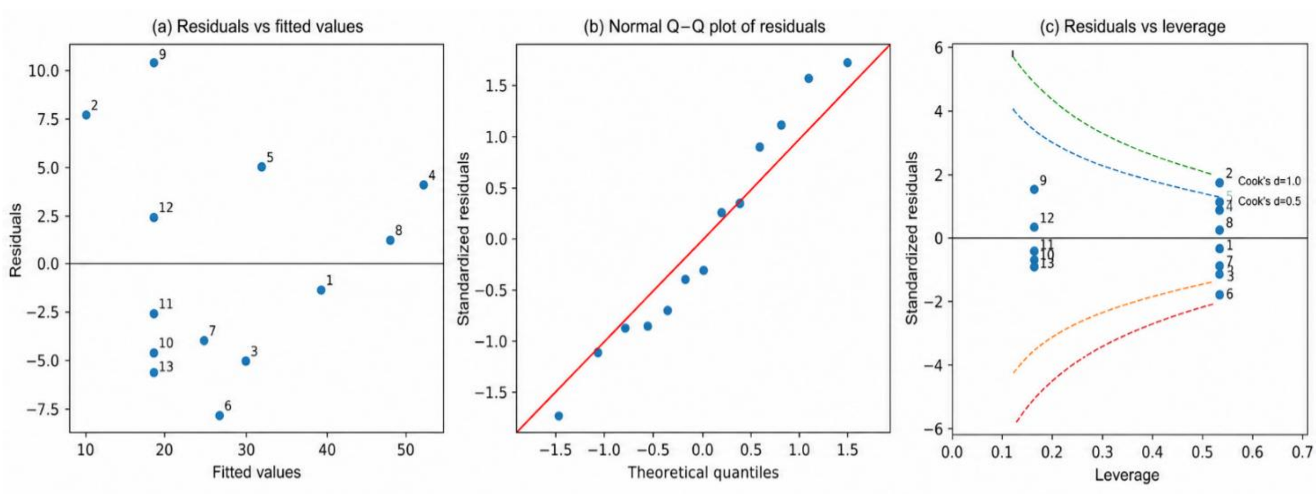

Figure 4. Diagnostic plots of the quadratic response surface methodology (RSM) model: (a) residuals versus fitted values, (b) normal Q–Q plot of residuals, and (c) residuals versus leverage

Table 6. Response surface methodology (RSM) optimization candidate solutions

No.	TS (%)	Volume Mixture (mL)	Predicted Productivity (mL/g)	Desirability
1*	14.000	750.000	51.893	0.904
2	14.000	748.834	51.533	0.896
3	14.000	747.244	51.046	0.885
4	14.000	747.074	50.994	0.884
5	14.000	740.361	48.969	0.836
6	7.000	500.000	39.357	0.613
7	7.000	500.986	39.181	0.609
8	7.082	500.000	38.763	0.599

Note: *: Selected solution; TS: total solids

Table 7. Mass balance summary at optimum condition (Total solids 14%, volume mixture 750 mL)

Input Component	Mass (g)	Output Component	Mass (g)
Rice husk	95.86	Total gas (428 mL)	0.495
Cow manure	138.96	Slurry solids	153.77
Urea	32.79	Slurry liquid	636.83
Rumen starter (15% v)	112.50	TOC in slurry	3.94 g C
Supplementary water	413.00	–	–
Total input	**793.11**	**Total output**	**≈ 791.10**

Note: TOC: Total organic carbon

3.2.3 Optimization and candidate solutions

Eight candidate solutions were generated through RSM optimization to maximize biogas productivity. The most favorable solution (desirability = 0.904) suggested TS = 14% and a mixture volume of 750 mL (75% of reactor capacity), with a predicted biogas productivity of 51.89 mL/g. The desirability value, which was close to one, confirms the strong validity and reliability of this operating condition, indicating that the projected results were within the expected range. A summary of these candidate solutions is presented in Table 6.

The experimental run corresponding to the optimal solution (Run 6: TS 14%, 750 mL) produced an actual biogas productivity of 56 mL/g, which was slightly higher than the model prediction (51.89 mL/g). This result supports the adequacy of the model in predicting biogas productivity under the selected operating conditions. These findings indicate that increasing TS within the L-AD optimal range (5–15%) can improve organic loading and consequently enhance total gas production, provided that inhibitory limits are not exceeded [10]. However, this study did not measure methane concentration; therefore, the optimized response represents total gas productivity and should not be interpreted as confirmed CH_4 or biomethane yield. The absence of CH_4 and CO_2 ratio data also limits the evaluation of biogas quality and energy potential. Since methane is the main combustible component of biogas, total gas volume alone is not sufficient to determine the actual energy recovery from the digestion process. Future experiments should include gas composition analysis to measure CH_4, CO_2, and trace gases, so that methane yield, calorific value, and energy potential can be more accurately assessed. The convergence of the top solutions around TS = 14% and mixture volume = 750 mL further strengthens the conclusion that this combination represents the optimum operating condition.

A limitation of this study is that gas production was measured only as total volume using the water displacement method. Although this method is simple and suitable for laboratory-scale screening, it cannot determine the composition of the gas mixture or distinguish CH_4 from CO_2. Therefore, the results should be interpreted as optimization of total gas productivity from rice husk and cow manure, not as direct biomethane potential. Future studies should include gas composition analysis using gas chromatography or CO_2 absorption methods, such as alkaline/caustic scrubbing, to determine CH_4 percentage, methane yield, energy potential, and CO_2-equivalent mitigation.

3.3 Mass balance analysis

The mass balance analysis, adjusted to the optimum condition (TS 14%, mixture volume 750 mL), was used to verify the conservation of material throughout the anaerobic digestion process. The total mass of the initial mixture was 793.11 g, consisting of rice husk (95.86 g, TS 92.59%), cow manure (138.96 g, TS 44.30%), urea (32.79 g) for C/N ratio adjustment, rumen starter (112.50 g, equivalent to 15% of the total mixture volume), and additional water (413.00 g) to achieve the target TS of 14%.

The C/N ratio of the mixture before fermentation was highly unbalanced (>35), which is unfavorable for methanogenic microorganisms because it indicates nitrogen deficiency relative to the high organic carbon content. This condition is mainly associated with the low protein content of rice husk and cow manure. Therefore, the addition of 32.79 g of urea was necessary to adjust the C/N ratio to the recommended range of 20–30. The fermentation cycle began with an 8-day acclimatization step, during which 85 mL of gas was produced. The subsequent 23-day active fermentation phase produced an additional 343 mL of gas, resulting in a total cumulative gas production of 428 mL. Assuming a biogas density of 1.158 kg/Nm³, the gas mass was calculated to be approximately 0.495 g.

Gas production peaked at 56 mL/day on day 19, representing the most active phase of methanogenesis during fermentation. Following this peak, a gradual decline in gas production was observed due to the decreasing availability of substrate. The complete mass balance data are presented in Table 7.

The final slurry (730 mL, 790.6 g) had a TS value of 19.45% and a volatile solids (VS) content of 82.28%, indicating that a large proportion of the remaining solids still contained biodegradable organic matter. The mass of the solid fraction was approximately 153.77 g, whereas the liquid fraction accounted for 636.83 g. The total organic carbon (TOC) concentration in the final slurry was 5,400 mg/L, equivalent to 3.94 g C in 730 mL of slurry.

The total carbon introduced from all input materials was calculated to be 13.09 g C, while the TOC remaining in the final slurry was 3.94 g C. This resulted in an apparent carbon deficit of approximately 9.15 g C, equivalent to about 69.9% of the input carbon. This relatively large deficit indicates that a substantial fraction of carbon was likely transformed into gaseous products, mainly CH_4 and CO_2, dissolved inorganic carbon, and microbial biomass. However, because the gas composition was not measured, the exact carbon fraction in the gas phase could not be calculated. The total gas mass was estimated at only 0.495 g based on total gas volume and assumed gas density, but this value cannot be directly converted into gas-phase carbon without knowing the CH_4 and CO_2 proportions. Therefore, the carbon balance should be interpreted as an approximate mass-based estimate rather than a complete elemental carbon balance. Future studies should include gas composition analysis and dissolved carbon measurement to close the carbon balance more accurately.

The total input mass was 793.11 g, while the total output mass was approximately 791.40 g, resulting in a mass difference of 1.71 g, which corresponds to a relative deviation of less than 0.22%. This difference is still within the acceptable range for laboratory-scale anaerobic digestion experiments and can be explained by four factors: (1) material replacement after the acclimatization phase, (2) water vapor loss through evaporation, (3) dissolved gas remaining in the liquid phase, and (4) measurement uncertainty associated with mass and volume readings. Overall, the mass balance supports the reliability of the experimental configuration and confirms the validity of the recorded data.

4. DISCUSSION

The results of this study show that rice husk and cow manure can be used as co-substrates for total gas production under L-AD conditions. The optimum condition was obtained at a TS of 14% and a substrate mixture volume of 750 mL, with a predicted productivity of 51.89 mL/g and an actual productivity of 56 mL/g. This result indicates that increasing TS within the L-AD range can improve organic loading and support gas formation, provided that sufficient liquid volume is available for microbial activity and mass transfer. This finding is consistent with previous studies reporting that L-AD performance is affected not only by solids concentration, but also by substrate moisture, mixing condition, and microbial accessibility [10, 18]. Compared with previous studies on lignocellulosic substrates, the use of rice husk requires pretreatment because its cellulose, hemicellulose, and lignin structure limit biodegradability. Mechanical grinding in this study was used to reduce particle size and improve substrate accessibility. Although chemical pretreatment, such as NaOH treatment, may produce stronger lignin disruption, mechanical pretreatment is simpler, cheaper, and more suitable for small-scale or community-based applications. Therefore, the present study provides a practical approach for using rice husk and cow manure as locally available substrates. From a practical application perspective, L-AD is suitable for small-scale digestion because the liquid phase supports mixing, microbial contact, and gas release. However, compared with SS-AD, L-AD may require more water, a larger reactor volume, and better slurry management. SS-AD may be more suitable for high-solid agricultural residues because it uses less water, but it can face limitations related to mass transfer, substrate compaction, and slower hydrolysis. Therefore, the selection between L-AD and SS-AD should consider substrate type, water availability, reactor design, and local operating capacity.

The statistical results showed that TS alone was not significant, while mixture volume and the interaction between TS and mixture volume were significant. This indicates that TS did not independently control total gas productivity. In L-AD, microbial activity depends on both organic matter availability and the liquid phase needed for hydrolysis, nutrient transport, and microbial contact. A higher TS value may increase organic loading, but without sufficient liquid volume, the digestion process can be limited by poor mass transfer and substrate accessibility. Therefore, the significant interaction between TS and mixture volume suggests that the balance between solids content and liquid availability was more important than TS alone. This result also indicates that optimizing TS without considering reactor filling volume may lead to incomplete interpretation of L-AD performance. For industrial or field-scale L-AD application, several technical challenges should be considered. First, mixing efficiency may decrease when reactor volume increases, especially when the substrate contains lignocellulosic material such as rice husk. Poor mixing can cause sedimentation, floating layers, uneven microbial contact, and unstable gas production. Second, temperature control becomes more difficult at a larger scale because heat distribution may not be uniform throughout the reactor. This can affect microbial activity and process stability. Third, feedstock heterogeneity may occur because rice husk and cow manure can vary in moisture content, TS, C/N ratio, and biodegradability depending on source and season. Therefore, industrial applications require feedstock homogenization, routine TS and C/N monitoring, controlled mixing, stable temperature regulation, and gas-tight reactor design. Optimization strategies may include pre-mixing tanks, gradual feeding, particle size control, co-substrate balancing, automated pH and temperature monitoring, and pilot-scale validation before full-scale implementation.

The RSM-CCD model was statistically significant and had adequate precision above the recommended value, indicating that the model was useful for preliminary design space exploration. However, the difference between adjusted R^2 and predicted R^2 was relatively large. This suggests that the model had limited predictive ability outside the experimental dataset. This condition may be related to experimental noise, biological variation among digesters, small sample size, temperature fluctuation, manual mixing, possible minor gas loss, and substrate heterogeneity. Therefore, the model should be interpreted as a preliminary optimization tool rather than a strong predictive model. Future studies should include more replicates, residual analysis, outlier diagnostics, and validation runs to improve model reliability.

Several limitations should also be considered. First, this study measured only total gas volume using the water displacement method. Therefore, the gas composition could not be distinguished between CH_4, CO_2, and other trace gases. As a result, the optimum condition represents total gas productivity, not confirmed methane or biomethane yield. Second, the experiment was conducted using 1 L laboratory-scale HDPE digesters without continuous stirring or active temperature control. These conditions may influence heat transfer, gas collection, mixing efficiency, and process stability during scale-up. Third, particle size distribution after grinding and the final C/N ratio after urea addition were not directly measured for each experimental run. In addition, although hybrid pretreatment methods such as mechanical–alkaline or mechanical–thermal pretreatment may improve rice husk biodegradability, their use may also increase chemical consumption, energy demand, operational cost, and process complexity. Thus, their feasibility should be assessed before field application. These limitations should be addressed in future research by including gas chromatography or caustic scrubbing for CH_4 analysis, pilot-scale validation, controlled stirring, improved gas-tight reactors, particle size analysis, post-adjustment C/N ratio measurement, and comparison with SS-AD or hybrid pretreatment systems to evaluate technical and economic feasibility. In addition, residual analysis and Cook's distance assessment were not fully performed, so the influence of possible outliers or high-leverage points could not be quantitatively confirmed. Another limitation is that the particle-size distribution of the ground rice husk was not quantified. Therefore, D10, D50, and D90 values could not be included in the analysis. Since particle size affects the surface area available for hydrolysis, this parameter should be measured in future studies. In addition, although the initial C/N ratios of the substrates were measured and reported, the final C/N ratio after urea addition was not directly verified for each treatment. Future studies should measure both the initial and post-adjustment C/N ratios to better explain the effect of nutrient balance on gas productivity.

5. CONCLUSIONS

This study aimed to optimize total gas production from a co-substrate mixture of rice husk and cow manure using L-AD combined with RSM-CCD. The results showed that the physicochemical characteristics of the substrates strongly influenced their suitability for anaerobic digestion. Rice husk exhibited high TS content and a wide C/N ratio, while cow manure and rumen content provided complementary characteristics as supporting substrates. However, the C/N ratios of all tested substrate combinations were far above the ideal range of 20–30 for stable anaerobic digestion, indicating the need for nitrogen supplementation through urea addition to create favorable conditions for methanogenic microorganisms. In addition, mechanical pretreatment of rice husk by grinding was considered important to increase the specific surface area and improve the hydrolysis of its lignocellulosic structure. During the 35-day fermentation period, biogas production followed a typical batch anaerobic digestion pattern. The acclimatization phase generated 85 mL of gas, while the active fermentation phase produced an additional 343 mL, resulting in a cumulative biogas volume of 428 mL under the optimum operating condition. The highest daily biogas production, 56 mL, was achieved on day 19, indicating the peak methanogenic phase of the digestion process. The RSM-CCD analysis produced a statistically significant quadratic model, demonstrating that the selected variables adequately explained the variation in biogas productivity. The results indicated that mixture volume had a significant effect on the response, while TS alone was not statistically significant, suggesting that substrate concentration did not act independently but interacted with liquid volume in influencing process performance. This finding is consistent with previous studies showing that process efficiency in L-AD systems is strongly affected by the interaction between solids concentration and liquid availability. The optimum operating condition was obtained at TS = 14% and mixture volume = 750 mL, with a desirability value of 0.904 and a predicted biogas productivity of 51.89 mL/g. The corresponding experimental run produced an actual total gas productivity of 56 mL/g, confirming the reliability of the model prediction. Furthermore, the mass balance analysis under optimum conditions showed only a minor deviation of 0.22%, indicating that the experimental system was valid and sufficiently robust for laboratory-scale application. The study confirms that the combination of rice husk and cow manure has promising potential for total gas production when supported by proper C/N ratio adjustment, mechanical pretreatment, and optimized operating conditions. In addition, most experimental conditions were conducted as single runs, except for the replicated center points; therefore, the variability of each treatment condition could not be fully quantified. However, because this study measured only total gas volume using the water displacement method, the gas composition could not be distinguished between CH_4, CO_2, and other trace gases. Therefore, the reported optimum condition should be interpreted as the optimization of total gas productivity rather than confirmed biomethane or methane yield. In addition, the use of 1 L HDPE digesters limits direct translation of the results to field or industrial scale because scale-up may affect mixing efficiency, heat transfer, gas collection, leakage control, slurry handling, and process stability. Compared with SS-AD, L-AD may be easier for microbial contact and gas release, but it generally requires more water and a larger reactor volume. Therefore, SS-AD or hybrid pretreatment systems, such as mechanical–alkaline or mechanical–thermal pretreatment, may be considered as alternative strategies for high-solid lignocellulosic residues, although their energy input, chemical use, cost, and operational complexity should be carefully evaluated. In addition, the lack of CH_4/CO_2 ratio data limits the assessment of biogas quality and energy potential. Therefore, total gas volume should be interpreted only as an initial performance indicator, not as a direct measure of usable energy output. Future studies are recommended to evaluate biogas composition, especially methane concentration, using gas chromatography or caustic scrubbing methods, investigate a broader range of TS levels, and scale up the process from laboratory to pilot scale to assess technical feasibility, energy recovery potential, economic viability, and GHG mitigation performance under field conditions.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT-3.0 to improve readability and language understanding. After utilizing this AI technology, the authors meticulously reviewed and amended the content as required, ensuring its accuracy and completeness. The authors assume complete accountability for the content of the publication.

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NOMENCLATURE

TS	Total solids, expressed as percentage (%)
W_{dry}	Weight of oven-dried sample + crucible (mg or g)
W_{dish}	Weight of empty crucible (mg or g)
W_{wet}	Weight of wet sample + crucible (mg or g)
OLR	Organic Loading Rate (kg VS/m³·day)
S	Inlet substrate concentration (kg VS/m³)
V	Reactor volume (m³)
HRT	Hydraulic Retention Time (day)
C/N	Carbon-to-nitrogen ratio (dimensionless)
VS	Volatile Solids, expressed as percentage (%)
L-AD	Liquid Anaerobic Digestion (dimensionless)
SS-AD	Solid-State Anaerobic Digestion (dimensionless)
RSM-CCD	Response Surface Methodology – Central Composite Design (dimensionless)

CH_4	Methane (dimensionless, as a compound)
CO_2	Carbon dioxide (dimensionless)
GHG	Greenhouse gas (dimensionless)
TOC	Total organic carbon (mg/L or g)