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OPTIMIZATION OF ANAEROBIC DIGESTION OF COW DUNG AND PLANT WASTES FOR ENHANCED BIOGAS YIELD USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

This study investigated the optimization of anaerobic digestion of cow dung co-digested with selected plant-based wastes for enhanced biogas production using Response Surface Methodology (RSM). The plant wastes utilized included unripe plantain peels, watermelon residues, unripe banana peels, cabbage waste, yam peels, breadfruit husk, potato peels, and melon husk. All analytical equipment used in the study were locally fabricated except the pH meter and thermometer, while analytical grade chemicals were used throughout the experiment. A Central Composite Design (CCD) consisting of 21 experimental runs was employed to evaluate the effects of feedstock quantity (X_1), pH (X_2), and retention time (X_3) on methane (CH_4), carbon dioxide (CO_2), and hydrogen sulphide (H_2S) production. The results showed that retention time had the greatest influence on biogas composition and methane yield. The highest methane volume of 0.00299 L was obtained at pH 6, feedstock quantity of 10, and retention time of 35 days. Regression analysis showed that X_3 significantly affected methane yield ($p = 0.0015$), while interaction effects involving X_1X_3 and X_2X_3 were also significant. However, the overall methane model was not statistically significant at the 95% confidence level ($p = 0.1116$), with an R^2 value of 0.6408. For CO_2 production, the regression model was significant ($p = 0.0046$) with an R^2 value of 0.8222, while X_3 showed the strongest positive effect. Similarly, the H_2S model was significant ($p = 0.0170$) with an R^2 value of 0.7664, and both X_1 and X_3 significantly. Therefore the study established that co-digestion of cow dung with mixed plant wastes can enhance methane generation under optimized conditions, particularly at extended retention times.

Keywords: Biogas, Cow dung, Plant wastes. Response surface methodology.

1. Introduction

Methane generation associated with petroleum-based activities has contributed significantly to the depletion of the world's already declining natural resources. The combustion of fossil fuels, for instance, is linked to environmental problems such as ozone layer depletion, while oil spills and poor waste management practices further degrade ecosystems. However, the adverse effects arising from fossil fuel use can be

reduced through the adoption of cleaner and more sustainable energy systems. Biomaterials, particularly agricultural residues, serve as viable sources of renewable energy and can meet future energy demands (Shehu et al., 2012). Nevertheless, when these materials are not properly managed, they can release harmful gases that pose risks to both the environment and human health. Therefore, it is essential to implement controlled processes in biogas production to minimize such impacts. Anaerobic digestion is a biogas production technology in which biodegradable materials are enclosed within an oxygen-free system and decomposed by microorganisms to yield energy (Alexopoulos, 2012). The process typically generates biogas containing about 60% methane and 40% carbon dioxide, which can be utilized for heat and electricity generation or as a vehicle fuel. The transformation of these materials into methane and carbon dioxide occurs through a sequence of microbial stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, complex biomaterials are broken down into simpler compounds. These compounds are then metabolized by acidogenic bacteria to produce volatile fatty acids, ethanol, carbon dioxide, and hydrogen. Subsequently, acetogenic bacteria convert these products into acetic acid, carbon dioxide, and hydrogen, while methanogenic microorganisms utilize hydrogen and acetate to generate methane and carbon dioxide. The residual digestate produced after the process can be applied as an organic fertilizer (Ampese, 2022). Overall, anaerobic digestion offers several benefits over other methods, including cost efficiency, environmental friendliness, availability of feedstock, renewability, and the added advantage of producing organic fertilizer (Cater *et al.*, 2015). Previous studies have extensively investigated biogas production through anaerobic digestion of various biomaterials (Aboudi *et al.*, 2015; Moutaz *et al.*, 2019; Daniel *et al.*, 2023; Mofijur *et al.*, 2020b), among others. Several parameters are known to influence the efficiency of the anaerobic digestion process, including pH, temperature, feedstock composition, carbon-to-nitrogen (C/N) ratio, hydraulic retention time (HRT), mixing conditions, presence of inhibitory substances, and organic loading rate (Tamara et al., 2021; Banerjee *et al*., 2022; Gautham *et al.*, 2022). To obtain optimal biogas yield, these factors must be carefully controlled, integrated, and continuously monitored. One effective approach for achieving this optimization is the use of response surface methodology (RSM). Response surface methodology is a statistical technique employed to develop predictive models and optimize the relationship between process variables and system performance (Rastega et al., 2011). This approach was first introduced by Box and Wilson (Box, 1951). Process parameters such as temperature, mixing rate, substrate characteristics, C/N ratio, organic loading rate, pH, and HRT serve as independent variables, while the biogas yield or system response represents the dependent variable. Response surface methodology is widely adopted for examining the relationship between multiple factors and a response, particularly because theoretical models describing such interactions are often highly complex. It is used to identify the optimum conditions for anaerobic digestion by determining the most suitable operational parameters that enhance biogas yield while minimizing experimental errors. In most cases, the response surface is illustrated graphically, either through contour plots or three-dimensional representations (Montgomery, 2017; Alinkunju *et al.*, 2017; Cochran and Cox, 1957). Numerous studies have applied RSM-based designs to optimize various independent variables using different modeling approaches (Bashir, 2015; Bradley, 2009). In this study, the Box–Wilson experimental design matrix was employed to evaluate three independent process variables: feedstock composition (food wastes and cow dung) represented as quantity (X_1), pH (X_2), and retention time (X_3). These variables were selected because achieving optimal anaerobic digestion performance depends on maintaining conditions that support microbial stability and activity. In particular, feedstock composition is critical, as excessive loading can rapidly lead to digester failure. Appropriate analytical approaches are essential for determining operating

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conditions that ensure effective control of anaerobic digesters and their interactions. Consequently, this research focused on identifying the optimal conditions required to maximize biogas production efficiency from plant wastes and cow dung using a response surface optimization approach.

2. Methodology

2.1 Materials

This study utilized various plant-based wastes, including unripe plantain peels,watermelon residues, unripe banana peels, cabbage waste, yam peels, breadfruit husk, potato peels and melon husk, in addition to cow dung. All analytical equipment employed was locally fabricated, with the exception of the pH meter (HANNA, HI98107) and the thermometer (HANNA, HI98107). Analytical grade chemicals were used.

2.1.1 The study area

Akwa Ibom state is in the South-South region of Nigeria that coordinates between latitude and longitude of 5° 2' 20.2668" N and 7° 54' 34.0920" E. Abak as one of the city in Akwa Ibom State lies on the South West of Akwa Ibom State and its latitude and longitude lie between 4° 58' 56.50" N and 7° 47' 21.19" E while Akpan Andem, Uyo the capital of Akwa Ibom State lies at coordinates of 5°01′07″N latitude and 7°55′31″E longitude.

2.1.2 Sample collection and treatment

The waste materials were collected from different locations (Abak and Akpan Andem, Uyo) in Akwa Ibom state. The cow dung was collected from Abak main market slaughter along Ikot Okoro road, Abak, Akwa Ibom State and stored in a covered twenty-litre bucket while the plant wastes were collected from Akpan Andem market, Uyo, Akwa Ibom State. The plant samples were pulverized using a wooden mortar and pestle, then uniformly blended to ensure proper homogeneity, and subsequently stored in a black polyethylene bag. The combined plant and animal wastes were maintained in a sealed condition for a period of 25 days to facilitate the growth and activity of methanogenic bacteria, which are responsible for methane generation.The moisture content, total solid and total volatile solids of the wet plant samples were determined along with that of cow dung that serves as inoculum.

2.2 Determination of the moisture content of the feedstock (plant wastes and cow dung)

The moisture content of the feedstock was determined using the gravimetric method. The crucible was thoroughly washed, dried in an oven at 100 °C for approximately 35 minutes, and then allowed to cool. The empty, dried crucible was weighed before use (W_1). A wet sample of each feedstock was placed in the crucible and dried in an oven at 100 °C for about 4 hours until a constant weight was achieved (W_2). It was then cooled and reweighed using a weighing balance (W_3). The moisture content was calculated using Equation 1 below, as described by Bradley (2010):

$$MC = [(W_2 - W_1) - (W_3 - W_1)] / (W_2 - W_1) \times 100\% \quad \text{.....(1)}$$

2.3 Determination of total solid (TS)

After the moisture content had been removed, the residue remaining in the crucible for each feedstock sample was further dried in an oven at 100 °C. The percentage of total solids was then calculated following the method described by (Orhorhoro et al., 2017), using Equation 2:

$$TS = (W_3 - W_1) / (W_2 - W_1) \times 100\% \quad (2)$$

2.4 Determination of total dissolved solids (TDS)

The determination of total dissolved solids was carried out following the procedure reported by (Orhorhoro *et al.*, 2017). The residue obtained after total solids analysis for each feedstock sample was subjected to ignition in a muffle furnace at 550 °C for 1 hour until a grayish-white ash was formed. After ignition, both the crucible and its contents were allowed to cool in the kiln for approximately 6 hours. The cooled sample was then weighed and recorded as W_4. The percentage of total dissolved solids was calculated using Equation 3:

$$TDS = (W_3 - W_4) / (W_3 - W_1) \times 100\% \quad (3)$$

where W represents the combined weight of the crucible and residue after ignition, and %TDS denotes the percentage of total dissolved solids.

2.5 Fabrication of bio-digester

Five portable bio-digesters were constructed using 20-litre plastic buckets measuring approximately 36 cm in height and 30 cm in diameter. Other materials used included motorcycle tubes, bicycle valves, clips, and polyvinyl chloride (PVC) pipes of about 0.7 cm in diameter, following the method described by Mrosso et al. (2023). An opening was created on the lid of each digester to allow connection of the PVC pipe to the motorcycle tube, which functioned as the gas collection unit. The PVC pipe was fitted over the valve outlet and securely fastened with a clip to ensure an airtight seal. The opposite end of the pipe was then connected to the motorcycle tube serving as the gas collector. Additionally, a pressure outlet was installed on the body of the bucket using a PVC pipe. Temperature within the biodigester was continuously monitored using a thermometer.

2.5 Experimental design

In this study, response surface methodology (RSM) was applied to develop a predictive model for identifying the optimum conditions for biogas production from plant wastes and cow dung. The Box–Wilson experimental design matrix (Table 2) was adopted to investigate three process variables—feedstock quantity, pH, and retention time—across five coded levels: −1.682, −1, 0, +1, and +1.682 (Cochran and Cox, 1957). These three independent variables were chosen due to their critical influence on biogas yield. Each variable was examined at five evenly spaced coded levels (−1.682, −1, 0, +1, +1.682), as presented in Table 1. The range between the minimum and maximum levels for each parameter was sufficiently broad to allow clear observation of their individual effects on the response.

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Table 1: Process variables used in the central composite design K=3

Independent variables		Variable levels				
	Process code	-1.682	-1	0	+1	+1.682
Quantity of Feedstock (kg)	X_1	6	8	10	12	14
pH	X_2	2	4	6	8	10
Retention time (days)	X_3	7	14	21	28	35

Experimental runs	Coded Independent Processes Variables			Real Independent Process Variables		
	X_1	X_2	X_3	X_1	X_2	X_3
1	-1	-1	-1	8	4	14
2	-1	-1	1	8	4	28
3	-1	1	-1	8	8	14
4	-1	1	1	8	8	28
5	1	-1	-1	12	4	14
6	1	-1	1	12	4	28
7	1	1	-1	12	8	14
8	1	1	1	12	8	28
9	1.682	0	0	14	6	21
10	-1.682	0	0	6	6	21
11	0	1.682	0	10	10	21
12	0	-1.682	0	10	2	21
13	0	0	1.682	10	6	35
14	0	0	-1.682	10	6	7
15	0	0	0	10	6	21
16	0	0	0	10	6	21
17	0	0	0	10	6	21
18	0	0	0	10	6	21
19	0	0	0	10	6	21
20	0	0	0	10	6	21
21	0	0	0	10	6	21

Table 2: Box Wilson experimental design matrix for coded and real independent process variables

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15 experimental runs were generated by the design but replication at 15th run into six times generated a total of 21 experimental runs, as shown in Table 2. Each row corresponds to an experimental run conducted at different levels of the independent variables. The effects of these process variables on the responses were examined and statistically analyzed. For Real Independent Process Variables; X_1= Quantity of feedstock, X_2 = pH, X_3 = Retention time.

2.6 Experimental set-up for biogas production

Different quantities of feedstock (6, 8, 10, 12, and 14 kg volatile solids) consisting of plant wastes and cow dung in a 1:1 ratio were prepared and mixed with distilled water at a ratio of 1:2 (kg volatile solids per litre). From each mixture, a fixed volume of 10 litres was introduced into separate reactor buckets for each experimental run. The setup involved variations in feedstock quantity (6–14 kg VS), retention time (7, 14, 21, 28, and 35 days), and pH levels (2, 4, 6, 8, and 10). The pH of the slurry was adjusted using 0.1 M HCl and 0.1 M NaOH. Hydrochloric acid (0.1 M) was used to achieve lower pH values, while 0.1 M sodium hydroxide was applied for higher pH levels. These reagents were selected due to their mild acidic and basic properties, enabling effective control of slurry pH, which initially ranged between 3 and 4.5. This approach is consistent with methods reported by (Asyikin *et al.*, 2023). Prior to feeding the digesters, the rubber hose connected to the gas outlet was temporarily disconnected to keep the outlet open, thereby preventing the development of negative pressure within the system. The prepared slurry was then introduced into each digester, and the pH was maintained according to the design matrix for each run using the appropriate reagent (0.1 M HCl or 0.1 M NaOH). Temperature within the digesters was continuously monitored using a thermometer, and each unit was tightly sealed to ensure anaerobic conditions by preventing air and gas leakage. The slurry occupied approximately half of the digester volume, leaving sufficient headspace for gas accumulation. The digestion process was allowed to proceed according to the specified retention time for each experimental run, and the produced biogas was collected through the gas collection system.

2.7 Compositional Analysis of Biogas

The composition of the biogas produced in each digester was determined following the method described by (Bagudo *et al.*, 2011) with difference in the use of gas constant (R). Hydrogen sulfide (H_2S), carbon dioxide (CO_2), and methane (CH_4) were quantified from the total biogas by connecting the gas collector to a series of transparent containers designed for gas separation and absorption. First, the biogas was passed through a container containing silica gel to remove moisture. The outlet of this container was then connected to another container filled with a lead acetate solution in 3 M ethanoic acid to absorb H_2S gas, forming black precipitates of lead sulfide as represented in Equation 4:

$$H_2S + Pb(COOCH_3)_2 \rightarrow PbS + 2CH_3COOH \quad (4)$$

After H_2S removal, the remaining gas was directed into a container containing 10% potassium hydroxide (KOH) solution, which absorbed CO_2 according to Equation 5:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O \quad (5)$$

The methane remaining after the absorption of H_2S and CO_2 was collected by downward displacement of water. The average masses of H_2S and CO_2 absorbed in each setup were measured and then converted to volumes using the Van der Waals equation:

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$$(P + an^2 / V^2)(V - nb) = RT \quad (6)$$

where P is the gas pressure, V is the gas volume, n is the number of moles, R is the universal gas constant ($0.0821 L.atm.mol^{-1}.K^{-1}$), and a and b are gas-specific constants (Atkin and Paula, 2002).

The volume of each absorbed gas was calculated by determining the difference in weight of the absorbent before and after absorption. This procedure was applied consistently across all 21 experimental runs.

3. Results and Discussion

3.1 Effect of Independent Variables on the Dependent Variables (Volume of H_2S, CO_2, and CH_4)

Response surface methodology (RSM) focuses on the maximum and minimum yields obtained in an experiment. In this study, the highest biogas yields were recorded in the 13th run, with volumes of 0.00299 L CH_4, 0.00099 L CO_2, and 0.00040 L H_2S at a feedstock quantity of 10 kg/l, pH 6, and a retention time of 35 days. The lowest yields were observed in the 14th run, with 0.00061 L CH_4, 0.00031 L CO_2, and 0.00010 L H_2S at the same feedstock quantity and pH but a shorter retention time of 7 days, as presented in Table 3.The reactor temperature ranged between 27 °C and 33 °C, which falls within the mesophilic range. Although methanogens are generally reported to perform optimally at neutral pH (6.8–7.5), the highest biogas yield in this study was observed at pH 6. This may be attributed to enhanced hydrolysis and acidogenesis of the substrate, microbial adaptation, and the buffering capacity of cow dung, which maintained microbial activity under mildly acidic conditions (Ali *et al.*, 2019; Achinas *et al.*, 2019). The highest biogas production at pH 6 can be attributed to the optimal activity of methanogenic microorganisms, which perform best near a pH of 6.5–8.2, while their activity declines at higher or lower pH values (Rao and Singh, 2024). The moisture content of the feedstocks was 53.00 ± 0.01% for plant waste and 47.00 ± 0.01% for cow dung. Total solids were 99.12 ± 0.06% for plant waste and 95.78 ± 0.05% for cow dung, while total dissolved solids were 97.00 ± 0.05% and 92.18 ± 0.03%, respectively. Adequate moisture content promotes biochemical conversion and supports microbial activity. Overall, the results indicate that the volume of biogas generated is primarily influenced by the feedstock quantity and retention time.

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Table 3: **Response values showing coded and real independent process variables used in the biogas production.**

Experimental runs	Coded Independent Processes Variables			Real Independent Process Variables			Volume of CH_4 in biogas (litre)	Volume of CO_2 in biogas (litre)	Volume of H_2S in biogas (litre)
	X_1	X_2	X_3	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	-1	-1	-1	8	4	14	0.00125	0.00064	0.00020
2	-1	-1	1	8	4	28	0.00166	0.00059	0.00030
3	-1	1	-1	8	8	14	0.00131	0.00067	0.00020
4	-1	1	1	8	8	28	0.00159	0.00071	0.00030
5	1	-1	-1	12	4	14	0.00123	0.00055	0.00020
6	1	-1	1	12	4	28	0.00151	0.00077	0.00020
7	1	1	-1	12	8	14	0.00120	0.00061	0.00020
8	1	1	1	12	8	28	0.00155	0.00075	0.00039
9	1.682	0	0	14	6	21	0.00153	0.00068	0.00030
10	-1.682	0	0	6	6	21	0.00150	0.00077	0.00020
11	0	1.682	0	10	10	21	0.00148	0.00068	0.00030
12	0	-1.682	0	10	2	21	0.00149	0.00061	0.00020
13	0	0	1.682	10	6	35	0.00299	0.00099	0.00040
14	0	0	-1.682	10	6	7	0.00061	0.00031	0.00010
15	0	0	0	10	6	21	0.00159	0.00056	0.00030
16	0	0	0	10	6	21	0.00159	0.00056	0.00030
17	0	0	0	10	6	21	0.00159	0.00056	0.00030
18	0	0	0	10	6	21	0.00159	0.00056	0.00030
19	0	0	0	10	6	21	0.00159	0.00056	0.00030
20	0	0	0	10	6	21	0.00159	0.00056	0.00030
21	0	0	0	10	6	21	0.00159	0.00056	0.00030

For Real Independent Process Variables; X_1= quantity of feedstock composition, X_2 = pH, X_3 = Retention time.For dependent variables: Y_1=Volume of H_2S in biogas (L),Y_2=Volume of CO_2 in biogas (L),Y_3=Volume of CH_4 in biogas (L).

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Table 4. Estimated regression coefficient and ANOVA for volume of H_2S in biogas.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	686.15	9	76.24	4.01	0.0170
X_1	21.33	1	21.33	1.12	0.0312
X_2	86.66	1	86.66	4.56	0.0521
X_3	430.06	1	430.06	22.62	0.0006
X_1^2	69.04	1	69.04	3.63	0.0831
X_2^2	69.04	1	69.04	3.63	0.0831
X_3^2	74.21	1	74.21	3.90	0.0738
X_1X_2	9.355E-003	1	9.355E-003	4.921E-004	0.9827
X_1X_3	5.41	1	5.41	0.28	0.6044
X_2X_3	16.79	1	16.79	0.88	0.3675
Residual	209.09	11	19.01		
Lack of Fit	209.09	5	41.82		
Pure Error	0.000	6	0.000		
Cor Total	895.24	20			

R-Squared	0.7664
Adj R-Squared	0.5753
Pred R-Squared	0.9455

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	29.49	1	1.62	25.92	33.06	
X_1	2.46	1	2.32	-2.65	7.58	1.14
X_2	4.96	1	2.32	-0.15	10.08	1.14
X_3	10.91	1	2.29	5.86	15.95	1.11
X_1^2	-6.50	1	3.41	-14.01	1.01	1.06
X_2^2	-6.50	1	3.41	-14.01	1.01	1.06
X_3^2	-6.73	1	3.40	-14.22	0.77	1.07
X_1X_2	0.15	1	6.96	-15.15	15.46	1.27
X_1X_3	-3.62	1	6.79	-18.56	11.32	1.23
X_2X_3	6.38	1	6.79	-8.56	21.32	1.23

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3. 2 Interpretation of Regression Coefficients and ANOVA for Volume of H_2S in Biogas

The analysis of variance (ANOVA) results for the regression model developed for the prediction of hydrogen sulphide (H_2S) volume in biogas revealed that the model was statistically significant. The model F-value obtained was 4.01 with a probability value (Prob > F) of 0.0170 (Table 4). Since the p-value was less than 0.05, the regression model was considered significant at the 95% confidence level. This indicates that the selected process variables collectively had significant effects on the volume of H_2S produced during biogas generation. The significance of the individual linear terms was evaluated using the probability values (Prob > F). For the effect of X_1; the linear term X_1 had a p-value of 0.0312, which is less than 0.05. Therefore, X_1 significantly influenced the volume of H_2S in biogas production. The positive regression coefficient of +2.46 indicates that increasing X_1 resulted in an increase in H_2S volume. For X_2 effect, the linear term X_2 had a p-value of 0.0521, which is slightly greater than 0.05. Therefore, X_2 was not statistically significant at the 95% confidence level, although it showed a moderate effect on the response. The positive coefficient of +4.96 suggests that increasing X_2 tended to increase the H_2S volume. For X_3 effect, the linear term X_3 had a p-value of 0.0006, which is far below 0.05. This indicates that X_3 had a highly significant effect on the volume of H_2S. The positive correlation between retention time and H_2S volume can be explained by the need to sustain microbial activity during longer digestion periods, as hydraulic retention times below 10–25 days may lead to microbial washout, reducing gas production (Maamri and Amrani, 2014). The coefficient estimate of +10.91 shows that X_3 had the strongest positive influence on H_2S generation among all the studied variables. For the significance of quadratic terms; the quadratic terms X_1^2, X_2^2, and X_3^2 had p-values of 0.0831, 0.0831, and 0.0738 respectively. Since all the p-values were greater than 0.05, the quadratic effects were not statistically significant. Although the coefficients were negative, suggesting curvature in the response surface, the effects were insufficient to be considered statistically important. For the significance of interaction terms; the interaction terms X_1X_2, X_1X_3, and X_2X_3 had p-values of 0.9827, 0.6044, and 0.3675 respectively. These values are greater than 0.05, indicating that the interaction effects between the variables were not significant. Therefore, the combined interactions among the independent variables did not significantly affect H_2S production. The coefficient of determination (R^2) obtained for the model was 0.7664. This indicates that 76.64% of the variation in H_2S volume was explained by the regression model, while the remaining 23.36% could be attributed to experimental errors or factors not included in the model. Accordingly, a linear model was selected for this optimization study and this is in agreement with Wang et al. (2012), who reported that an R^2 value of 0.75 is sufficient for predictive purposes. The adjusted R^2 value was 0.5753, while the predicted R^2 value was 0.9455. The high predicted R^2 value suggests that the model possesses good predictive capability. The lack-of-fit test could not be reliably interpreted because the pure error value obtained was zero. This situation usually occurs when replicated experimental runs produce identical response values. After removing the statistically non-significant terms, only the significant variables X_1 and X_3 were retained in the final predictive model. Therefore, the reduced regression equation for predicting the volume of H_2S in biogas is given as:

$$Y = 29.49 + 2.46X_1 + 10.91X_3 \quad (7)$$

where: Y = Predicted volume of H_2S in biogas and X_1 and X_3 = coded independent variables. Therefore, the regression analysis showed that the developed model adequately described the variation in the volume of H_2S produced during biogas generation. The overall model was statistically significant with a p-value of 0.0170. Among the studied variables, X_3 exerted the highest influence on H_2S production, followed by X_1.

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The quadratic and interaction effects were statistically insignificant and were therefore removed from the final predictive model. The reduced regression equation obtained can be effectively used for predicting H_2S volume within the experimental range studied.

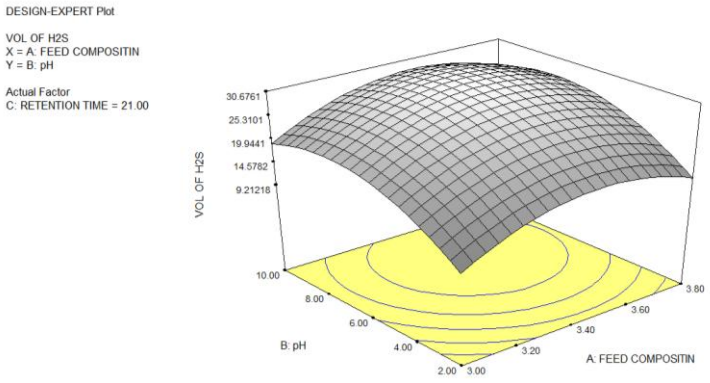


Figure. 1: Response surface methodology (RSM) plot for the interactive effect of the quantity of feedstock composition and pH on the volume of H_2S in biogas.

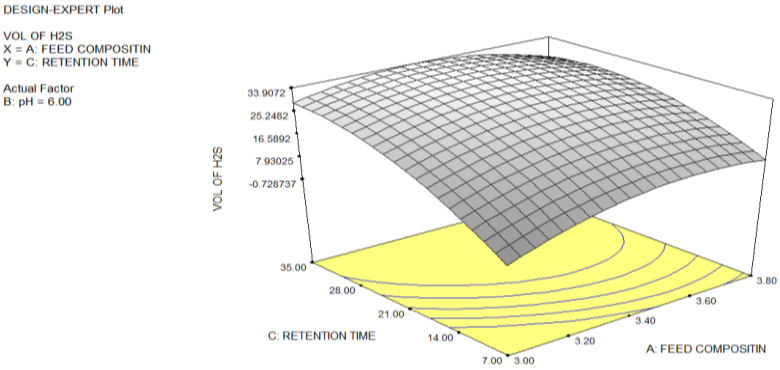


Figure 2: Response surface methodology (RSM) plot for the interactive effect of the quantity of feedstock composition and retention time on the volume of H_2S in biogas.

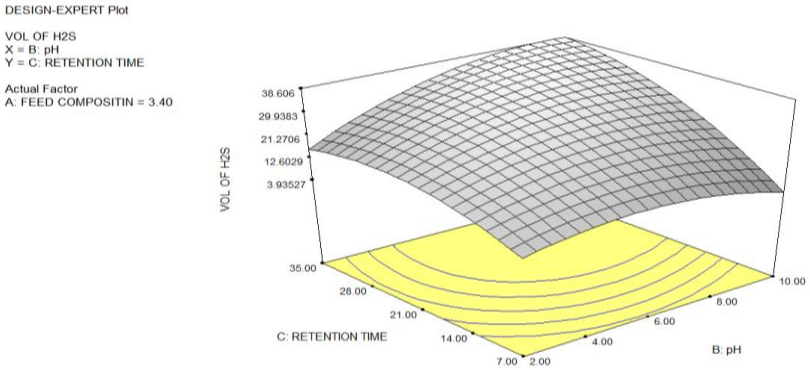


Fig.3: Response surface methodology (RSM) plots for the interactive effect of pH and retention time on the volume of H_2S in biogas.

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Table 5: Estimated regression coefficient and ANOVA for volume of CO_2 in biogas obtained.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	2.810E+005	9	31222.27	5.65	0.0046
X_1	800.83	1	800.83	0.14	0.7107
X_2	17290.53	1	17290.53	3.13	0.1046
X_3	1.944E+005	1	1.944E+005	35.19	< 0.0001
X_1^2	41648.84	1	41648.84	7.54	0.0190
X_2^2	10377.76	1	10377.76	1.88	0.1979
X_3^2	11358.15	1	11358.15	2.06	0.1795
X_1X_2	11399.86	1	11399.86	2.06	0.0178
X_1X_3	5222.84	1	5222.84	0.95	0.0352
X_2X_3	1877.76	1	1877.76	0.34	0.0547
Residual	60780.50	11	5525.50		
Lack of Fit	60780.50	5	12156.10		
Pure Error	0.000	6	0.000		
Cor Total	3.418E+005	20			

R-Squared	0.8222
Adj R-Squared	0.6767

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	558.51	1	27.64	497.67	619.34	
X_1	15.08	1	39.62	-72.12	102.28	1.14
X_2	70.08	1	39.62	-17.12	157.28	1.14
X_3	231.87	1	39.09	145.83	317.90	1.11
X_1^2	159.74	1	58.18	31.68	287.79	1.06
X_2^2	79.74	1	58.18	-48.32	207.79	1.06
X_3^2	83.20	1	58.03	-44.53	210.94	1.07
X_1X_2	-170.33	1	118.58	-431.33	90.67	1.27
X_1X_3	112.53	1	115.74	-142.22	367.27	1.23
X_2X_3	-67.47	1	115.74	-322.22	187.27	1.23

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3.3 Interpretation of Regression Coefficients and ANOVA for Volume of CO_2 in Biogas

The analysis of variance (ANOVA) results for the regression model developed for the prediction of carbon dioxide (CO_2) volume in biogas indicate that the model is statistically significant. The model F-value is 5.65 with a probability value (Prob > F) of 0.0046 (Table 5). Since the p-value is less than 0.05, the model is significant at the 95% confidence level. This implies that the independent variables collectively have a significant effect on CO_2 production in biogas. X_1 has a p-value of 0.7107, which is greater than 0.05. Therefore, X_1 is not statistically significant in influencing CO_2 production. Effect of X_2 has a p-value of 0.1046, which is also greater than 0.05. This indicates that X_2 does not significantly affect CO_2 production at the 95% confidence level. X_3 has a p-value of less than 0.0001, indicating a highly significant effect on CO_2 production. The positive coefficient (231.87) shows that X_3 has the strongest positive influence on CO_2 volume among all variables. The quadratic terms show mixed significance: ${X_1}^2$ has a p-value of 0.0190 and is statistically significant, ${X_2}^2$ has a p-value of 0.1979 and is not significant while ${X_3}^2$ has a p-value of 0.1795 and is not significant. This indicates that only X_1 exhibits a curvature (non-linear) effect on CO_2 production. The interaction terms show the following results: X_1X_2 has a p-value of 0.0178 and is significant. X_1X_3 has a p-value of 0.0352 and is significant while X_2X_3 has a p-value of 0.0547 and is marginally not significant. This indicates that interactions involving X_1 play an important role in CO_2 production. The coefficient of determination (R^2) is 0.8222, indicating that 82.22% of the variation in CO_2 volume is explained by the model. The adjusted R^2 value is 0.6767, indicating acceptable model reliability after adjusting for the number of predictors. The lack-of-fit test could not be reliably interpreted because the pure error value is zero. This typically occurs when replicate experimental values are identical. After removing statistically non-significant terms, the final reduced model includes only significant variables.

The final predictive equation is:

$$Y = 558.51 + 231.87X^3 + 159.74X^{12} - 170.33X^1X^2 + 112.53X^1X^3 \quad (8)$$

Where: Y = Volume of CO_2 in biogas; X_1, X_2 and X_3 = coded independent variables

Therefore the regression analysis shows that CO_2 production in biogas is significantly influenced by the model variables, particularly X_3, which has the strongest effect. The quadratic effect of X_1 and interaction effects involving X_1 also significantly influence CO_2 production. The reduced model provides a reliable predictive equation with strong explanatory power (R^2 = 0.8222) and can be used for optimization within the experimental range. The increase in CO_2 volume observed at longer retention times can be attributed to sustained microbial activity, as adequate hydraulic retention time (typically not less than 10–25 days) is necessary to prevent the washout of microorganisms involved in the digestion process (Maamri and Amrani, 2014). Additionally, the positive relationship between feedstock quantity (10 kg) and CO_2 production may be linked to higher levels of dissolved solids, as reported by Somashekar et al. (2014).

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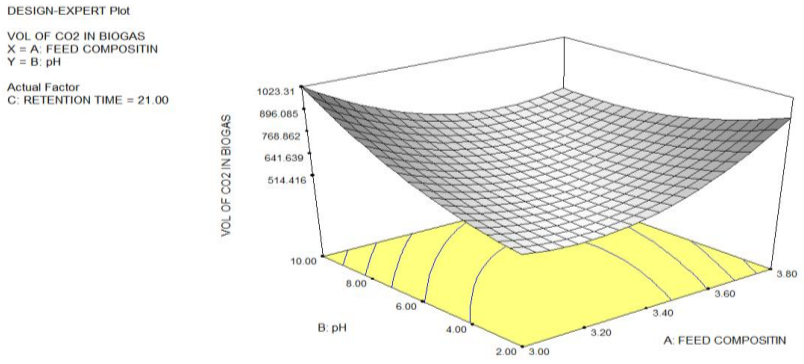


Figure 4: Response surface methodology (RSM) plot for the interactive effect of the quantity of feedstock composition and pH on the volume of CO_2 in biogas.

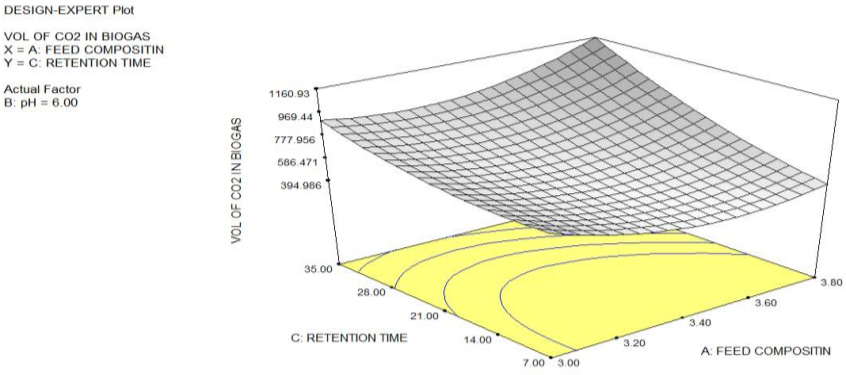


Figure 5: Response surface methodology (RSM) plot for the interactive effect of the quantity of feedstock composition and retention time on the volume of CO_2 in biogas.

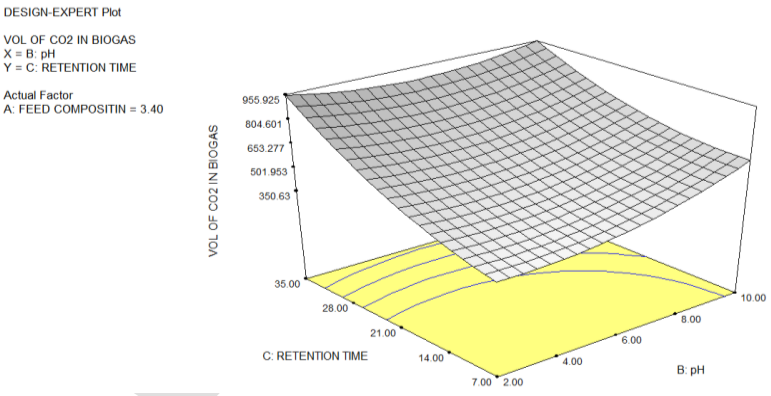


Figure 6: Response surface methodology (RSM) plots for the interactive effect of pH and retention time on the volume of CO_2 in biogas.

Table 6: Estimated regression coefficient and ANOVA for volume of methane in biogas obtained.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	2.150E+006	9	2.389E+005	2.18	0.1116
X_1	9505.75	1	9505.75	0.087	0.7738
X_2	23649.92	1	23649.92	0.22	0.6513
X_3	1.911E+006	1	1.911E+006	17.44	0.0015
X_1^2	55439.53	1	55439.53	0.51	0.4917
X_2^2	74957.93	1	74957.93	0.68	0.4257
X_3^2	11553.30	1	11553.30	0.11	0.7515
X_1X_2	42235.43	1	42235.43	0.39	0.0547
X_1X_3	4313.70	1	4313.70	0.039	0.0463
X_2X_3	4313.70	1	4313.70	0.039	0.0463
Residual	1.205E+006	11	1.095E+005		
Lack of Fit	1.205E+006	5	2.410E+005		
Pure Error	0.000	6	0.000		
Cor Total	3.355E+006	20			
R-Squared	0.6408				
Adj R-Squared	0.3469				
Pred R-Squared	-2.5250				

Factor	Coefficient Estimate	Standard DF	95% CI Error	95% CI Low	High	VIF
Intercept	1553.00	1	123.06	-336.30	440.23	
X_1	51.96	1	176.40	-306.30	470.23	1.14
X_2	81.96	1	176.40	343.85	1110.01	1.14
X_3	726.93	1	174.05	-754.48	385.90	1.11
X_1^2	-184.29	1	259.06	-784.48	355.90	1.06
X_2^2	-214.29	1	259.06	-484.82	652.66	1.06
X_3^2	83.92	1	258.40	-1490.00	834.29	1.07
X_1X_2	-327.85	1	528.01	-1032.03	1236.56	1.27
X_1X_3	102.27	1	515.36	-1032.03	1236.56	1.23
X_2X_3	102.27	1	515.36		515.36	1.23

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3.4 Interpretation of Regression Coefficients and ANOVA for Volume of Methane (CH_4) in Biogas

The analysis of variance (ANOVA) results for the regression model developed for the prediction of methane (CH_4) volume in biogas indicate that the overall model is not statistically significant. The model F-value is 2.18 with a probability value (Prob > F) of 0.1116 (Table 6). Since the p-value is greater than 0.05, the model is not significant at the 95% confidence level. This implies that the combined effect of the independent variables does not adequately explain the variation in methane production. X_1 has a p-value of 0.7738, which is greater than 0.05. Therefore, X_1 does not significantly influence methane production. X_2 has a p-value of 0.6513, which is also greater than 0.05. This indicates that X_2 does not significantly affect methane volume. X_3 has a p-value of 0.0015, which is less than 0.05. This shows that X_3 has a statistically significant and strong positive effect on methane production. The coefficient of +726.93 confirms that X_3 is the dominant factor influencing methane volume. The quadratic terms ${X_1}^2, {X_2}^2$, and ${X_3}^2$ have p-values of 0.4917, 0.4257, and 0.7515 respectively. Since all p-values are greater than 0.05, none of the quadratic terms are statistically significant. This indicates that methane production does not show meaningful curvature effects within the studied range. The interaction terms show mixed results: X_1X_2 has a p-value of 0.0547 (marginally not significant), X_1X_3 has a p-value of 0.0463 (significant) while X_2X_3 has a p-value of 0.0463 (significant). This indicates that methane production is influenced mainly by interaction effects involving X_3. The coefficient of determination (R^2) is 0.6408, indicating that 64.08% of the variation in methane volume is explained by the model. The adjusted R^2 value is 0.3469, which is relatively low, indicating weak model reliability after adjustment. The predicted R^2 value is -2.5250, which indicates very poor predictive performance and possible model overfitting or inadequate structure. The lack-of-fit test could not be properly evaluated because the pure error is zero. This typically occurs when replicate experimental values are identical. After removing statistically non-significant terms, the reduced model includes only significant effects. The final predictive equation is:

$$Y = 1553.00 + 726.93X_3 + 102.27X_1X_3 + 102.27X_2X_3 \quad (9)$$

Where: Y = Volume of methane (CH_4) in biogas; X_1, X_2, X_3 = coded independent variables

Therefore regression analysis shows that methane production is not adequately explained by the overall model at the 95% confidence level (p = 0.1116). However, X_3 has a strong and significant influence on methane production, both directly and through interaction effects with X_1 and X_2. Despite these significant individual effects, the model exhibits poor predictive performance (negative predicted R^2), indicating that it should be used with caution for prediction purposes within the studied experimental range.

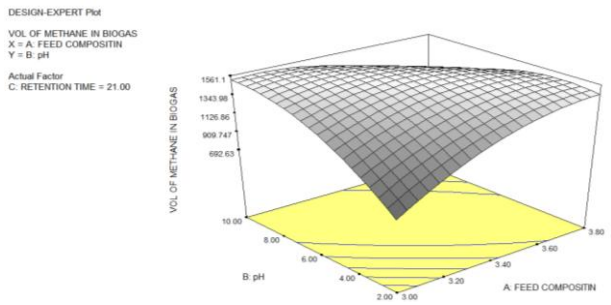


Figure 7: Response surface methodology (RSM) plot for the interactive effect of the quantity of feedstock composition and pH on the volume of CH_4 in biogas.

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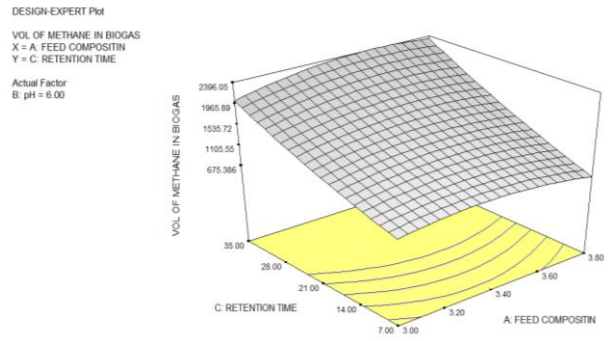


Figure 8: Response surface methodology (RSM) plot for the interactive effect of the quantity of feedstock composition and retention time on the volume of CH_4 in biogas.

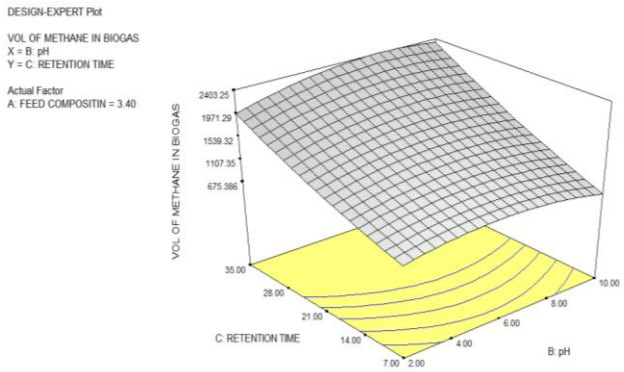


Figure 9: Response surface methodology (RSM) plots for the interactive effect of pH and retention time on the volume of CH_4 in biogas.

4. Conclusion

This study successfully optimized the anaerobic digestion of cow dung co-digested with mixed plant-based wastes, including unripe plantain peels, watermelon residues, unripe banana peels, cabbage waste, yam peels, breadfruit husk, potato peels, and melon husk, for enhanced biogas production using Response Surface Methodology (RSM). The application of the Central Composite Design (CCD) enabled the evaluation of the effects of feedstock quantity (X_1), pH (X_2), and retention time (X_3) on methane (CH_4), carbon dioxide (CO_2), and hydrogen sulphide (H_2S) production during anaerobic biodigestion. The experimental results demonstrated that retention time (X_3) was the most influential operational parameter affecting the anaerobic digestion process. Methane production increased considerably with increasing retention time, indicating that longer digestion periods enhanced microbial degradation and methanogenic activity. The highest methane yield obtained was 0.00299 L at pH of 6, feedstock quantity of 10, and retention time of 35 days. This confirms that co-digestion of cow dung with the selected plant wastes can improve methane generation under optimized operating conditions. Although the overall methane regression model was not statistically significant at the 95% confidence level ($p = 0.1116$), retention time showed a highly significant positive effect on methane yield. Interaction effects involving retention time (X_1X_3 and X_2X_3) were also significant, indicating that methane production depends strongly on the

combined influence of digestion time with other operational conditions. The results therefore suggest that methane yield was improved primarily through optimization of digestion time rather than through strong individual effects of pH or substrate loading alone. The regression analysis for CO_2 and H_2S production revealed statistically significant models with good explanatory power. Retention time exerted the strongest positive influence on both gases, while pH also significantly influenced H_2S generation. The increase in H_2S and CO_2 at longer retention times indicates sustained microbial metabolism during digestion. However, since elevated H_2S concentrations may reduce biogas quality and contribute to corrosion, appropriate gas purification measures may be necessary before utilization of the produced biogas. Based on the optimization results, the recommended operational conditions for enhanced methane production are: pH of approximately 6, feedstock quantity of 10 and retention time of 35 days. Under these conditions, the anaerobic digestion system achieved the highest methane yield within the experimental range studied. The practical implication of this work is that locally available agricultural and food-processing wastes can be effectively converted into renewable bioenergy through anaerobic co-digestion with cow dung. This provides an environmentally sustainable approach for managing biodegradable wastes while simultaneously generating useful energy. The study also demonstrates the usefulness of Response Surface Methodology as a reliable optimization tool for improving biodigestion performance and reducing experimental cost and time. Furthermore, the use of largely locally fabricated equipment highlights the feasibility of developing low-cost biogas technologies suitable for rural and resource-limited communities.

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