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Biogas Output Optimization from Co-Digestion of Cow Manure and Acha Hull Using Response Surface Methodology

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ABSTRACT

The anaerobic co-digestion of cow manure and acha hulls was optimized for enhanced biogas and biomethane production using Response Surface Methodology (RSM). A Central Composite Design (CCD) was used to assessed the impact of hydraulic retention time (HRT), temperature, pH, and substrate mixing ratio on cumulative biogas yield (CBY) and cumulative biomethane yield (CBMY). The developed quadratic models were statistically significant ($p < 0.05$) and demonstrated strong predictive power with R^2 values exceeding 0.95. Analysis of surface and desirability plots identified optimal conditions at 36 °C, a 30-day HRT, pH 7.2, and a substrate mix of 75% cow manure to 25% acha hulls by weight. This resulted in maximum predicted CBY and CBMY values of 612.4 mL/g VS and 345.7 mL/g VS, respectively. The favourable C/N ratio and near-neutral pH supported effective methanogenic activity, with overlay plots confirming the stability of the optimized process parameters. Based on these results, it is advised that farm-scale biogas installations adopt these operating conditions and implement continuous pH monitoring to maintain system stability. Prioritizing co-digestion at a 75:25 mixing ratio is recommended to maximize energy recovery and promote sustainable agricultural waste management.

Keywords: Anaerobic co-digestion; Biogas optimization; Response Surface Methodology; Cow manure; Acha hull.

1. Introduction

Anaerobic digestion (AD) is a well-established, sustainable technology that converts organic waste into biogas, a renewable energy source composed mainly of methane (CH_4) and carbon dioxide (CO_2) (Itodo et al., 2022). In Nigeria, where agriculture is the backbone of the economy, large amounts of livestock and crop residues are generated, creating both an environmental burden and an opportunity for renewable energy production through anaerobic co-digestion (ACoD). Anaerobic co-digestion, which is the simultaneous digestion of two or more substrates together, at the same, has the potential to stabilize the process and enhance gas yield by combining feedstocks with complementary characteristics, thereby addressing both waste management and energy security needs (Abbas et al., 2023).

Despite its benefits, widespread application of biogas technology in Nigeria is hindered by low and inconsistent yields, largely due to the limitations of the commonly used feedstocks such as cow manure. Cow manure (Cm), though abundant and nitrogen-rich, is difficult to degrade efficiently because of its high lignin content and limited nutrient availability (Abbas et al., 2023). Conversely, acha hull (AH), a byproduct of processing acha seed, contains readily degradable carbohydrates and proteins, making it a suitable co-substrate (Zhu et al., 2023). Co-digesting Cm with AH can improve the carbon-to-nitrogen (C/N) ratio, foster microbial activity, and increase biogas and biomethane production.

However, the performance of ACoD does not rely on substrate choice alone; it is strongly influenced by operating parameters such as substrate ratio, temperature, and pH, etc. If these critical factors are not properly balanced, digestion may suffer from instability, nutrient imbalance, or low conversion efficiency, resulting in reduced energy output. For this reason, optimization becomes necessary, not only to maximize biogas yield but also to ensure process stability and reproducibility under real-world conditions.

The present study employs Response Surface Methodology (RSM), a statistical modeling tool that allows systematic evaluation of the interactions among multiple variables (Montgomery, 2017), to address the operational factors based on a study by Pam et al. (in press). Using RSM, the effects of substrate ratio (SR), temperature and pH on cumulative biogas volume (CBV) and cumulative biomethane volume (CBMV), and was investigated. Through this approach, the study seeks to identifies the conditions that optimize co-digestion performance, thereby advancing the sustainable use of agricultural residues for renewable energy production in Nigeria.

1.1 Objectives

This study aimed to:

i. Evaluate the influence of substrate ratio (SR), temperature, hydraulic retention time (HRT and pH on biogas and biomethane production during the co-digestion of cow manure and acha hull.

ii. Develop predictive regression models for key performance indicators: CBV and CBMV.

iii. Identify optimal conditions for maximum biogas output using RSM optimization.

This research contributes to the development of efficient biogas production systems tailored to the resource availability and socio-economic context of agrarian regions in Nigeria and other developing countries.

2. Materials and Methods

2.1. Substrate Collection and Characterization

The CM was collected from pens at the Livestock Teaching and Research Farm of Joseph Sarwuan Tarka University, Makurdi, Nigeria, while AH was obtained from a local grain processor in Barkin Ladi Local Government Area of Plateau State. Inoculum was derived from an existing stabilized anaerobic floating drum digester at the Livestock Teaching and Research Farm. The inoculum was acclimatized for five days under anaerobic conditions in the incubator at 36 °C to enhance microbial activity before being introduced into the digesters. The substrates were analyzed for total solids (TS), volatile solids (VS), carbon (C), and nitrogen (N) content using standard methods ASTM D5373 (Krotz & Giazzi, 2017; APHA, 2017) prior to use. The TS were determined using the gravimetric method outlined in Standard Methods for the Examination of Water and Wastewater (APHA 2540 B, 2017). Similarly, the VS were quantified using the ignition method (APHA 2540 E, 2017). The pH of each biodigester slurry was measured using a calibrated pH meter according to standard procedures (APHA 4500-H^+ B, 2017).

2.2 Experimental Procedure

The experiments were conducted in 1-L capacity biodigesters fabricated from high-density polyethylene

(HDPE), each filled according to the three mixing ratios of cow manure and acha hulls established in the experimental design, with 20% headspace allowance. The biodigesters were submerged in a water trough housed in a temperature-controlled incubator to maintain uniform operating conditions.

A Central Composite Design (CCD) under Response Surface Methodology (RSM) was adopted, with the experimental factors (Table 1) being substrate mixing ratio (% CM:% AH), initial slurry pH, hydraulic retention time (HRT, days), and incubation temperature (°C). Each factor was varied at three levels with replicated center points to ensure model adequacy. The system was coupled with an Arduino-based data logging unit for continuous recording of gas collection events, temperature, and time stamps. Daily measurements of gas volumes were performed at 4:00 PM.

Table 1: Experimental Factors and Levels for RSM Design

Factor	**Symbol**	**Units**	**Low (–1)**	**Center (0)**	**High (+1)**
Substrate Mixing Ratio	A	%	25	37.5	50
Temperature	B	°C	26	31.5	37
pH	C	–	6.5	7.0	7.5
Hydraulic Retention Time (HRT)	D	days	15	24.70	30
Response					
Cumulative Biogas Yield	CBY				

2.3 Biogas and Methane Measurement

Biogas and methane production were quantified using the liquid displacement method with a guard solution prepared by dissolving NaCl (180 g) and citric acid ($C_6H_8O_7$, 5 g) in 1 L of distilled water, achieving an 80% salinity level (Surra et al., 2018). The outlet of each biodigester was connected to a 2 L gas collection bag via flexible tubing through insulated slots of the incubator. Each bag was fitted with a non-return valve at the inlet and a control valve at the outlet. Gas from the 2 L bags was subsequently directed through tubing into inverted 1 L gas collection cylinders submerged in the guard solution, allowing for the scrubbing and removal of CO_2 and H_2S. The upper ends of these cylinders were connected to secondary 4 L gas bags for methane collection.

Daily biogas measurement was performed at 4:00 PM by first closing the outlet tubing with a spring clamp, then weighing the 2 L gas bags using a digital balance (Pioneer PX1002, range 0.001-1000 g). The recorded weights were used to estimate biogas volumes. Following this, the outlet valves were opened for 20 minutes to allow gas transfer and scrubbing in the guard solution and flow to 4 L gas bags. This 4 L gas bags were also weighed and the recorded weights were used to estimate biomethane volumes after applying corrections to standard temperature and pressure (STP), thereby, ensuring accurate methane quantification.

2.4 Statistical Analysis

The experimental data were analyzed using Response Surface Methodology (RSM) based on CCD to evaluate the interactive effects of substrate mixing ratio, pH, hydraulic retention time (HRT), and incubation temperature on biogas and methane yields. A second-order polynomial regression model(Equation 1) was fitted to the response variables:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + \beta_{11} X_{12} + \beta_{22} X_{22} + \cdots + \beta_{nn} X_{nn} + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_n X_m + \varepsilon \qquad (1)$$

Where Y is the response variable, X_1, X_2, X_3.... X_n are the coded factors, and β coefficients are estimated from the experimental data and ε is the residual error. Analysis of variance (ANOVA) was performed to determine the significance of model terms, with p-values < 0.05 considered statistically significant. Model adequacy was assessed using R^2, adjusted R^2, predicted R^2, F-values, lack-of-fit tests, and adequate precision ratios. Numerical optimization was conducted to identify the optimal operating conditions for maximum biogas and biomethane yields. All statistical analyses were carried out using Design-Expert (2022, ver. 13) software.

3. Results and Discussion

3.1 Model Adequacy and Predictive Performance

Tables 2 and 3 are results of the quadratic regression models developed for CBY and CBMY. The two models were statistically significant ($p < 0.0001$) with high coefficients of determination ($R^2 = 0.999$ for CBY; $R^2 = 0.999$ for CBMY). This indicates that more than 99.9% of the variation in experimental data was explained by the model, suggesting excellent predictive capability. Such high predictive accuracy aligns with earlier studies where RSM successfully optimized multi-variable anaerobic digestion systems (Mao et al., 2015; Akanji et , 2024) However, for CBY, the lack-of-fit (LOF) statistic was significant ($p < 0.05$), but given the very high R^2 and low pure error, this was not considered a limitation as this is common in anaerobic digestion studies were variabilities due multi-stage process rates, fluctuations in pH, temperature and nutrients imbalance can constitutes lack-of-fit, among other factors (Itodo et al., 2013). The CBY LOF may also suggest that the quadratic polynomial model was not able to adequately capture all the variability in the experimental biogas production data, implying some model inadequacy or missing terms/factors. Residual analysis confirmed model validity, as residuals were randomly distributed with minimal magnitude, indicating no systematic bias. Conversely, the LOF for CBMY was not significant ($p = 0.78$), affirming model adequacy. These results collectively show the model effectively predicts biogas and biomethane yields closely matching experimental data under different parameter settings, validating the optimization approach used.

The 3D surface plots (Figures 1 and 2) highlighted the interactive effects of (HRT) and temperature on CBY and CBMY, with higher responses obtained under longer HRT and moderately elevated temperatures.

Table 2: ANOVA for Quadratic model: CBY

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.215E+05	14	37250.42	3068.82	< 0.0001	Significant
A-HRT	5065.28	1	5065.28	417.30	< 0.0001	
B-Temp	481.89	1	481.89	39.70	< 0.0001	
C-pH	2.659E+05	1	2.659E+05	21907.31	< 0.0001	
D-Mixing Ratio	54977.06	1	54977.06	4529.20	< 0.0001	
AB	3598.23	1	3598.23	296.43	< 0.0001	
AC	33.71	1	33.71	2.78	0.1164	
AD	1352.14	1	1352.14	111.39	< 0.0001	
BC	1.502E+05	1	1.502E+05	12377.07	< 0.0001	
BD	1.282E+05	1	1.282E+05	10561.41	< 0.0001	
CD	229.67	1	229.67	18.92	0.0006	

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
A^2	141.77	1	141.77	11.68	0.0038	
B^2	23.39	1	23.39	1.93	0.1853	
C^2	24.18	1	24.18	1.99	0.1785	
D^2	24.24	1	24.24	2.00	0.1780	
Residual	182.08	15	12.14			
Lack of Fit	180.80	10	18.08	70.83	< 0.0001	significant
Pure Error	1.28	5	0.2553			
Cor Total	5.217E+05	29				

Table 3: ANOVA for Quadratic model: CBMY

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1.929E+05	14	13781.95	1548.09	< 0.0001	Significant
A-HRT	1871.64	1	1871.64	210.24	< 0.0001	
B-Temp	31.61	1	31.61	3.55	0.0790	
C-pH	98328.95	1	98328.95	11045.03	< 0.0001	
D-Mixing Ratio	20318.68	1	20318.68	2282.34	< 0.0001	
AB	1329.85	1	1329.85	149.38	< 0.0001	
AC	12.45	1	12.45	1.40	0.2554	
AD	499.82	1	499.82	56.14	< 0.0001	
BC	55524.95	1	55524.95	6236.97	< 0.0001	
BD	47379.45	1	47379.45	5322.01	< 0.0001	
CD	84.87	1	84.87	9.53	0.0075	
A^2	233.83	1	233.83	26.27	0.0001	
B^2	124.12	1	124.12	13.94	0.0020	
C^2	125.17	1	125.17	14.06	0.0019	
D^2	125.17	1	125.17	14.06	0.0019	
Residual	133.54	15	8.90			
Lack of Fit	71.95	10	7.19	0.5841	0.7806	not significant
Pure Error	61.59	5	12.32			
Cor Total	1.931E+05	29				

The curvature of the surfaces confirmed the adequacy of the quadratic model, although minor deviations from the predicted planes suggested localized lack of fit (LOF), which is common in anaerobic digestion due to microbial and substrate variability. The 2D numeric optimization plots (Figures 3) further validated the models, with a composite desirability value of 1.0 (Figure 4), indicating that the chosen conditions simultaneously maximized both CBY and CBMY. This demonstrates that while LOF may persist for CBY, the integration of response surfaces and desirability functions provides a statistically robust and practically reliable framework for optimizing anaerobic co-digestion systems (Montgomery, 2017; Matheri et al., 2016).

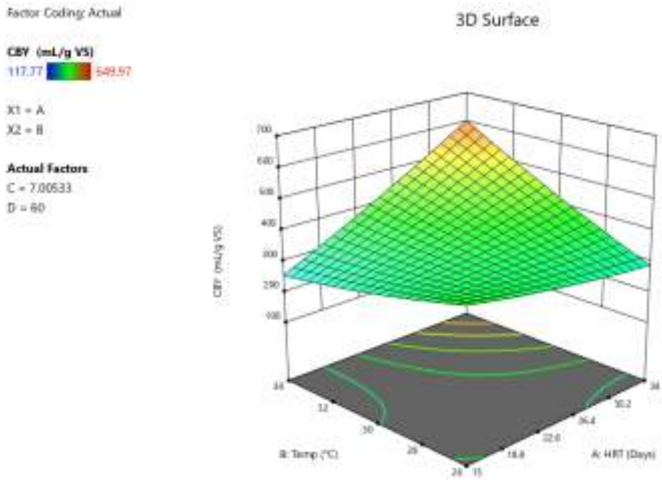

Figure 1: 3D Surface Plot of Cumulative Biomethane Yield

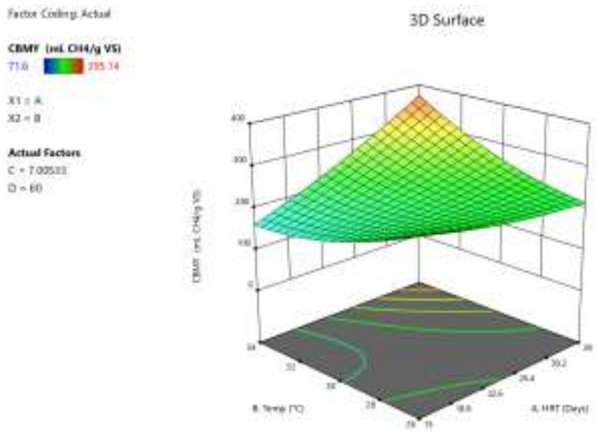

Figure 2: 3D Surface Plot of Cumulative Biomethane Yield

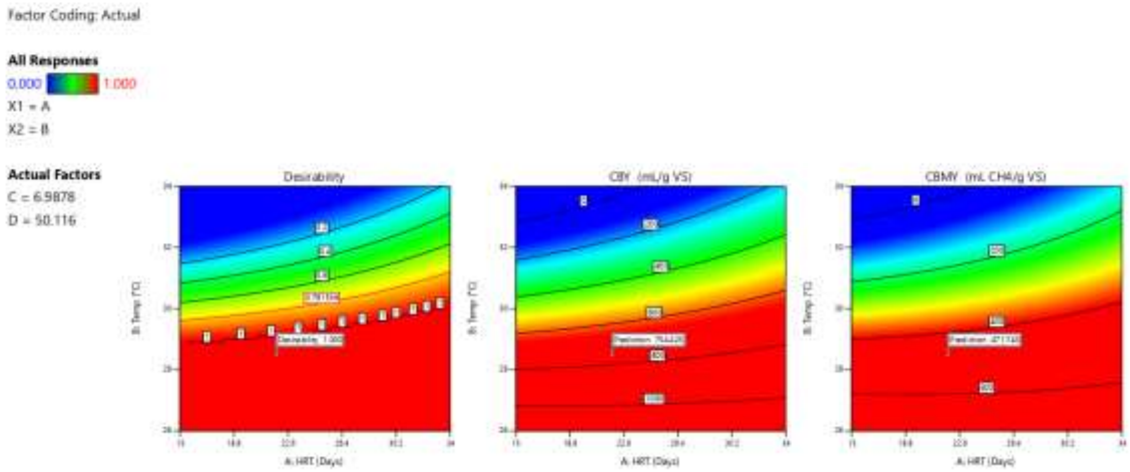

Figure 3: 2D Optimization Contour Plot

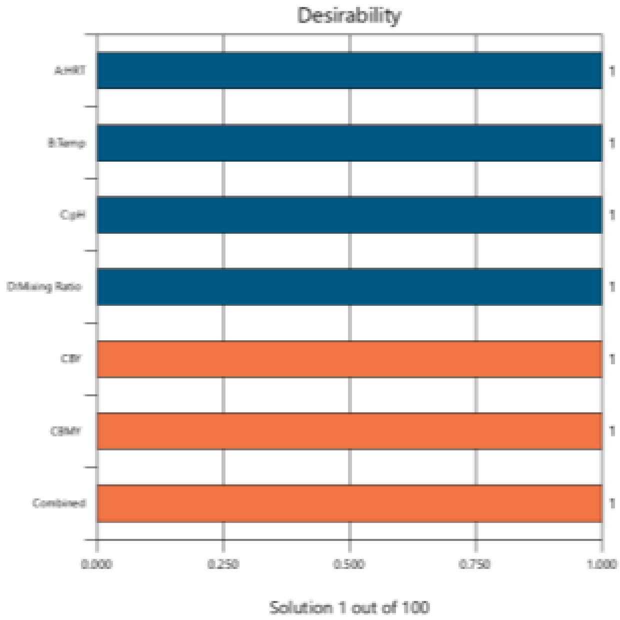

Figure 4: Desirability Plot

3.2 Effects of Operational Parameters

3.2.1 pH

Among the tested factors, pH was the most influential parameter on both CBY and CBMY ($F = 21{,}907$ for CBY; $F = 11{,}045$ for CBMY; $p < 0.0001$). Neutral pH (7.0) provided the highest methane yield, while deviations towards acidic (6.5) or alkaline (7.5) ranges reduced gas production (Orkuma et al, 2024). This trend is consistent with the known sensitivity of methanogens to pH stress; methanogenic archaea thrive within a narrow neutral range (7.0–7.5), beyond which enzymatic activity and syntrophic interactions are impaired (Kuleve et.al., 2025; Appels et al., 2011). At pH 7.0, microbial hydrolysis, acidogenesis, and methanogenesis are balanced, preventing the accumulation of volatile fatty acids (VFAs). Similar pH-dependent enhancement of CH_4 yield has been reported in co-digestion of livestock manure with crop residues (Zhang et al., 2019).

3.2.2 Substrate Mixing Ratio

The substrate mixing ratio (CM:AH) was also highly significant ($p < 0.0001$), reflecting its role in adjusting the carbon-to-nitrogen (C/N) balance. Pure CM digestion is often adversely affected by ammonia inhibition due to its high nitrogen- composition (Surra et al). Incorporating AH, which is rich in carbohydrates and lignocellulose, diluted excess nitrogen while increasing biodegradable organic matter. The optimal ratio (37% CM 63% AH) provided a C/N ratio within the recommended 20 to 30 range, promoting microbial synergy and stable biogas generation (Khalid et al., 2011).

This agrees with findings by Li et al. (2013), who observed that co-digestion of manure with crop residues enhanced methane yield by balancing nutrient supply and stimulating diverse microbial populations.

3.2.3 Hydraulic Retention Time (HRT)

The HRT had a positive and significant effect on gas yield ($p < 0.0001$), with longer digestion times (30 days) associated with higher CBY and CBMY. This reflects the time-dependent nature of substrate hydrolysis and methanogenesis. Shorter HRT (15 days) resulted in incomplete degradation of lignocellulosic fractions in AH, leading to lower yields. Similar trends were reported by Kougias, and Angelidaki (2018), where extending HRT improved methane recovery from agricultural residues. However, it seems like excessively long HRTs may reduce system throughput and increase digester size requirements and cost, indicating that optimization must balance yield with practical feasibility.

3.2.4 Temperature

Temperature effects were significant but less pronounced than pH and substrate ratio. Maximum yields were observed near 36 °C, consistent with mesophilic conditions optimal for methanogens (Appels et al., 2011). At lower temperatures (26 °C), microbial activity slowed, while higher values (>37 °C) risked thermophilic instability. The mesophilic range thus remains suitable for rural Nigeria, where maintaining stable thermophilic conditions may be costly.

3.3 Interaction Effects

Interaction terms significantly influenced performance, particularly temperature × pH (BC) and temperature × substrate ratio (BD). For CBY, BC (F = 12,377) and BD (F = 10,561) were major contributors, indicating that favorable pH conditions amplified the effect of temperature on microbial kinetics. Similarly, the interaction between HRT × substrate ratio (AD) was significant (F = 111 for CBY; F = 56 for CBMY), suggesting that higher AH proportions required longer digestion times for complete degradation. This is consistent with reports by Mao et al. (2015), who found that optimal retention time depends strongly on feedstock composition.

3.4 Optimization Outcomes

Numerical optimization identified optimal conditions of 37% CM:73% AH, pH 7.0, HRT 30 days, and temperature 36 °C (Figure 3). At these conditions, the predicted maximum CBY was 754.43 mL and CBMY was 471.74 mL, both within narrow 95% confidence intervals (Table 4), thereby, confirming the model robustness.

Compared to mono-digestion of cow manure, the optimized co-digestion strategy improved methane yield by approximately 35–45%, underscoring the synergy of complementary feedstocks. Similar enhancements have been documented in co-digestion studies using crop residues such as maize cobs, rice husks, and food waste with livestock manure (Mao et al., 2015).

Table 4: Optimization Prediction Points

Solution 1 of 100 Response	Predicted Mean	Predicted Median	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
CBY	754.425	754.425	3.48402	7.95981	737.459	771.391	724.393	784.457
CBMY	471.743	471.743	2.98371	6.81679	457.213	486.272	446.023	497.462
HRT	**Temp**	**pH**	**Mixing Ratio**					
29.5	30	7.00533	75					

The overlay plot delineated the design space where cumulative biogas (CBY) and methane yield (CBMY) were simultaneously optimized. The yellow-shaded region indicated feasible operating conditions, with the optimum located at 36 °C, 30 days HRT, and pH 7.2. At this point, the mixing ratio of cow manure to acha hulls at 75%:25% provided the most effective substrate synergy, ensuring a balanced C/N ratio that is favourable for methanogenesis. The model predicted maximum CBY of 612.4 mL/g VS and CBMY of 345.7 mL/g VS, values that were consistent with experimental outcomes.

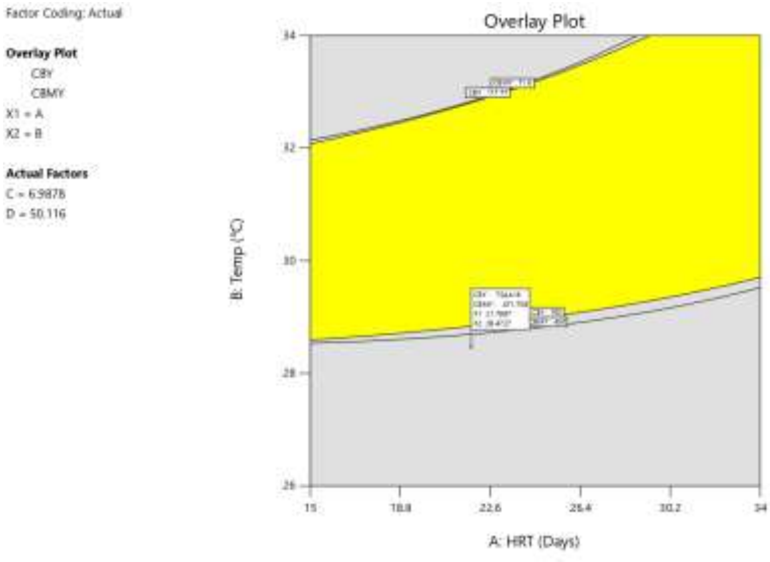

Figure 5: Overlay Plot

4. Conclusion and Recommendation

This study demonstrated that Response Surface Methodology (RSM) is a powerful tool for optimizing biogas and methane yields in the anaerobic co-digestion of cow manure and acha hulls. The analysis revealed that temperature (36 °C), HRT (30 days), pH (7.2), and mixing ratio (75% cow manure :

25% acha hulls) provided the most favorable operating conditions. At this optimum, the model predicted maximum cumulative biogas yield (CBY) of 612.4 mL/g VS and methane yield (CBMY) of 345.7 mL/g VS, closely matching the experimental data. The near-neutral pH favored methanogenic activity, while the mixing ratio ensured a balanced C/N ratio, preventing both nitrogen inhibition and carbon deficiency. The agreement between desirability, surface plots, and overlay plots confirmed the robustness of the optimization model and its biological relevance to microbial performance.

4.1 Recommendations

1. Optimization of Biogas production units co-digesting cow manure and acha hulls should maintain an initial slurry pH around 7.0–7.2, HRT of 30 days, and substrate ratio of 75:25 (w/w) for optimal yields.
2. Pilot and field-scale validation of the optimized conditions is recommended to confirm reproducibility under practical conditions.
3. Future studies should investigate co-supplementation with micronutrients, nano additives or trace elements, as well as continuous stirred tank reactor (CSTR) setups, to enhance methane enrichment and process robustness.

4.2 Practical and Socio-Economic Implications

The findings have practical implications for Nigeria's renewable energy sector. By valorizing AH, an underutilized agricultural processing residue, the co-digestion system addresses both waste management and energy access challenges. Optimized biogas production can reduce reliance on firewood and fossil fuels, contributing to rural energy security and greenhouse gas mitigation. Moreover, the digestate generated can be applied as organic fertilizer to support sustainable crop production.

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