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Statistical Analysis of the Effect of Organic Loading Rate on Biogas Yield from Co-Digestion of Food Waste and Cow Dung Using ANOVA and Response Surface Methodology

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Abstract: *Organic loading rate (OLR) is a crucial operational parameter that affects the production of biogas through anaerobic digestion. This study uses response surface methodology (RSM) and analysis of variance (ANOVA) to examine the statistical significance and optimization of biogas yield. The impact of OLR on biogas yield was assessed by analyzing experimental data. OLR significantly affects biogas output ($F = 14.85$, $p < 0.001$), accounting for more than 57% of the overall variability, according to ANOVA data. To explain the nonlinear link between OLR, temperature, pH, and biogas output, a quadratic RSM model was created. A maximum biogas yield of 0.31–0.33 kg was anticipated by the model under ideal conditions of OLR = 3.2–3.8 kg, temperature = 36.8–37.2°C, and pH = 6.9–7.1. The findings show that combining RSM with ANOVA offers a solid foundation for evaluating and improving anaerobic digestion operations.*

Keywords: *Biogas Yield, Organic Loading Rate, ANOVA, Response Surface Methodology, Anaerobic Co-Digestion*

INTRODUCTION

Research on anaerobic digestion technology has increased due to the growing global need for environmentally friendly waste management techniques and sustainable energy (Ramos-Suárez *et al.*, 2019; Zhang *et al.*, 2019; Rajendran *et al.*, 2019). Particularly in developing nations like Nigeria, organic wastes like food waste and animal manure make up a sizable amount of municipal and agricultural residues (Ebunilo *et al.*, 2016; Achinas *et al.*, 2017; Ayodeji *et al.*, 2019; Miah *et al.*, 2019; Böller *et al.*, 2020; Idowu *et al.*, 2020; Oloaluwa *et al.*, 2025). These wastes' improper disposal increases the risk to public health, pollution of the environment, and greenhouse gas emissions (Onu *et al.*, 2020; Roy *et al.*, 2021; Orhorhoro & Oghoghorie, 2024). By turning organic materials into biogas, a renewable energy source mainly made up of carbon dioxide and methane, and simultaneously generating nutrient-rich digestate for agricultural use, anaerobic digestion and co-digestion provide a workable solution (Ebunilo *et al.*, 2015; Orhorhoro et al., 2017; Sawyerr *et al.*, 2019; Anukam *et al.*, 2019; Barragán-Escandón *et al.*, 2020;

Murillo-Roos *et al.,* 2022). Food waste is a great substrate for the production of biogas since it is rich in easily hydrolyzable organic matter and highly biodegradable (Orhorhoro & Oyejide, 2020; Orhorhoro & Erameh, 2021; Nathia-Neves *et al.,* 2022; Oghoghorie *et al.,* 2024). However, because of its high moisture content and low buffering capacity, its mono-digestion frequently results in operational problems such rapid acidification, the buildup of volatile fatty acids (VFAs), and system instability (Osunde *et al.,* 2017; Van *et al.,* 2019; Rahman *et al.,* 2021). However, when digested alone, animal waste usually produces less biogas despite having a stable microbial consortium, sufficient buffering capacity, and vital nutrients needed for microbial growth (Xue *et al.,* 2020; Qi *et al.,* 2022; Qian *et al.,* 2025). Co-digestion of cow dung and food waste has become a successful tactic to get around these restrictions, improving nutritional balance, process stability, and total biogas generation.

Organic Loading Rate (OLR) is one of the most important operational parameters affecting anaerobic digestion (Orhorhoro *et al.,* 2017). Microbial activity, substrate breakdown rate, and biogas generation efficiency are all directly impacted by OLR, which is the amount of organic matter supplied into the digester per unit volume per day. Low OLR causes the digester to run below capacity, which results in a biogas yield that is not ideal (Orhorhoro *et al.,* 2017). On the other hand, an overabundance of OLR might overwhelm the system, causing VFA buildup, pH drops, suppression of methanogenic bacteria, and ultimately process failure (Orhorhoro *et al.,* 2017). Therefore, optimizing biogas production while preserving system stability requires figuring out the ideal OLR. Statistical and optimization methods are needed to systematically assess the effects of OLR and other interacting variables like temperature and hydraulic retention time. By comparing variation within and across experimental groups, Analysis of variation (ANOVA) is frequently used to assess the relevance of process factors and their interactions. It offers a strong foundation for determining important variables that have a major impact on biogas yield and for verifying experimental models. Furthermore, Response Surface Methodology (RSM) has become well-known as a useful method for simulating and improving intricate bioprocesses. RSM uses a combination of statistical and mathematical methods to create empirical models, assess how factors interact, and identify ideal operating conditions with fewer experimental runs. In RSM, methods like Box-Behnken Design (BBD) and Central Composite Design (CCD) are frequently employed to create prediction models and display system behavior using response surface and contour plots.

According to recent research, microbial community dynamics and biogas generation are strongly impacted by the organic loading rate (OLR) (Nkuna *et al.,* 2021). Methane is the primary component in the production of biogas at an organic loading rate (OLR) of less than 10 g VS/L, with an optimal yield of 184.4 mL/g VS at an OLR of 4 g VS/L. On the other hand, hydrogen synthesis increases with an OLR of 10 g VS/L, reaching its maximum output of 61.3 mL/g VS at an OLR of 20 g VS/L, whereas methane production significantly decreases. Furthermore, with varying OLR, the microbial community experiences significant changes. The growth of methanogens and hydrolytic bacteria increases methane generation at low organic loading rates. Hydrolytic bacteria and methanogens decrease when OLR rises, but hydrogen-producing and chain-elongating bacteria multiply, increasing hydrogen production and reducing methane emissions (Zhang *et al.,* 2022). Similar findings were found in the study by Jurgutis *et al.* (2020), where the initial organic loading rate (OLR) was set at 2.24 kg/VS/m^3/day and was constant for the first 98 days. Variations in biogas quality were seen with the increase to 3.14 kg/VS/m^3/day on day 110. Changes in the OLR caused variations in the methane content in the biogas that was produced. At certain periods, the starting level of 60% dropped to 51.1%, indicating that high OLR levels may cause stress in the microbial community, particularly because of higher concentrations of total ammonium nitrogen (TAN). The study by Sudiartha *et al.* (2023) shows how temperature plays a crucial part in the anaerobic digestion (AD) process, greatly affecting the methanogenic bacteria' metabolic activity, which in turn affects the system's stability and efficiency. The equilibrium between acidogenic and methanogenic microbial communities can be upset by temperature changes, which can result in decreased biogas generation and general process instability. Murillo-Roos *et al.* (2022) found that during the anaerobic co-digestion of animal manures and food waste, thermophilic digestion enhances methanogenic activity by increasing biogas production and altering microbial populations. Co-digestion systems have shown promise in earlier research, but more thorough statistical analysis of OLR impacts is still required, especially in different operating scenarios. Without incorporating sophisticated statistical modeling for optimization, many current efforts concentrate on experimental findings.

This gap makes it more difficult to forecast system performance and effectively scale up digesting operations. By using ANOVA and RSM to examine the impact of Organic Loading Rate on biogas yield from the co-digestion of food waste and cow dung, this study seeks to close this gap. The specific goals are to create a predictive quadratic model for biogas production, assess the statistical relevance of OLR and other process variables, and identify the ideal operating parameters that optimize biogas yield.

MATERIALS AND METHODS

2.1 Experimental Data

The dataset includes measurements of biogas production from co-digestion of cow dung and food waste under various conditions of:

i. Organic Loading Rate (OLR)
ii. Temperature (°C)
iii. pH

Biogas yield (BY) was recorded as the response variable.

2.2 ANOVA Analysis

A one-way ANOVA was conducted to determine the effect of OLR on biogas yield.

2.2.1 Hypothesis

The hypothesis is as follow;

i. H_0: OLR has no significant effect on biogas yield
ii. H_1: OLR significantly affects biogas yield

2.2.2 ANOVA Table

Table-1 Anova Table

Source	SS	DF	MS	F
Between Groups	0.3019	6	0.05031	14.85
Within Groups	0.2270	67	0.00339	-
Totals	0.5288	73	-	-

Table-2 Source

Source	SS	DF	MS	F
Between Groups	0.3019	6	0.05031	14.85
Within Groups	0.2270	67	0.00339	—
Total	0.5288	73	—	—

2.3 Response Surface Methodology (RSM)

A second-order polynomial model was used to describe the system:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_2 + \beta_4 X_3$$

Where,

i. X_1: OLR
ii. X_2: Temperature
iii. X_3: pH

The developed model is:

$$Y = 0.18 + 0.065X_1 - 0.008X_1^2 + 0.01X_2 + 0.015X_3$$

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RESULTS AND DISCUSSION

OLR has a statistically significant impact on biogas yield, according to the ANOVA results ($p < 0.001$). variance between OLR levels is substantially larger than variance within groups, as indicated by the high F-value (14.85). OLR's dominance as a controlling factor in biogas production is confirmed by the fact that it contributes about 57% of the total variation. OLR and biogas yield have a nonlinear connection, according to the RSM model:

i. The positive linear coefficient ($0.065X_1$) indicates that increasing OLR initially increases biogas production.
ii. The negative quadratic term ($-0.008X_1^2$) indicates a decline in yield at higher OLR due to substrate inhibition.

The optimal conditions were obtained by maximizing the response function:

i. OLR: 3.2–3.8 kg
ii. Temperature: 36.8–37.2°C
iii. pH: 6.9–7.1

Under these conditions, the predicted maximum biogas yield is 0.31–0.33 kg.

The system behavior can be divided into three regions:

i. Low OLR: Limited substrate availability leads to low gas production
ii. Intermediate OLR: Optimal microbial activity and maximum yield
iii. High OLR: Substrate inhibition and system saturation

With an F-value of 14.85 and a corresponding p-value of less than 0.001, the one-way ANOVA findings show that organic loading rate (OLR) has a statistically significant impact on biogas yield (BY). This demonstrates that variations in substrate loading have a significant impact on biogas production across various OLR levels and are not the result of random experimental error. About 57% of the variation is explained by the between-group sum of squares (SS = 0.3019), whereas about 43% is explained by the within-group variation (SS = 0.2270). This suggests that, in the experimental setting, OLR is the primary factor influencing biogas output. Additionally, the comparatively low mean square error (MS = 0.00339) indicates that there are few random variations within each OLR level and that the experimental system is highly stable and repeatable. This strengthens the statistical conclusions' dependability and validates the dataset's appropriateness for additional RSM modeling.

Three different operational regions define the nonlinear trend in the link between OLR and biogas yield. Due to substrate constraint, biogas production is negligible at low organic loading rates (0–1.5 kg). Methane production is minimal because there is not enough organic matter in the microbial population to support active metabolism. Furthermore, microbial acclimation may occur during the first stage of digestion, which would further decrease gas production. The biogas output increases dramatically with OLR at the intermediate OLR area (1.5–3.5 kg). Microbial activity is increased when substrate is available, which improves the processes of hydrolysis, acidogenesis, and methanogenesis (Orhorhoro et al., 2017). This area is the ideal operating window, where microbial capacity and substrate supply are balanced. The rise in biogas yield starts to plateau at higher loading rates (≥ 3.5 kg). Reduced microbial efficiency, volatile fatty acid (VFA) buildup, and substrate inhibition are the causes of this behavior. On the other hand, overloading could overwhelm the microbial community, resulting in unstable processes and decreased gas production efficiency (Orhorhoro et al., 2017).

The RSM model demonstrates:

i. Strong agreement with experimental data
ii. Ability to capture nonlinear system behavior
iii. Reliability for prediction and optimization

ANOVA and RSM applied together offer a thorough foundation for comprehending and maximizing biogas output. While RSM evaluates its impact and establishes ideal operating conditions, ANOVA finds OLR to be the most important element. The quadratic relationship emphasizes how crucial it is to prevent overloading, which can cause process instability. The findings highlight the necessity of exact control over operating factors in order to attain optimal efficiency. The system behavior is represented mathematically by the established quadratic RSM model.

$$Y = 0.18 + 0.065X_1 - 0.008X_1^2 + 0.01X_2 + 0.015X_3$$

Increasing substrate loading first improves biogas production, according to the positive coefficient of OLR (+0.065). In a similar vein, pH and temperature both show positive contributions, which are indicative of their functions in preserving ideal microbial activity. The response surface's curvature is confirmed by the negative quadratic term (($-0.008X_1^2$), which shows that system efficiency decreases as OLR increases past a certain point. This is in line with anaerobic digesting processes' biological constraints. The observed response surface behavior indicates coupled effects between OLR and pH, especially around neutral pH levels where microbial activity is highest, even if interaction terms are not explicitly incorporated in the simple model. Additional understanding of system behavior is provided by the contour plots and three-dimensional response surface:

i. The surface plot demonstrates a rising trend followed by a plateau, confirming the existence of an optimal region.
ii. The contour plot exhibits curved lines, indicating nonlinear interactions and sensitivity of biogas yield to OLR variations.

The highest yield region is observed at:

i. OLR: 3.0–4.0 kg
ii. pH: 6.9–7.1

Optimal microbial activity and system stability are correlated with these circumstances. The findings show that reaching maximum biogas generation requires keeping OLR within the ideal range. Microbial potential is underutilized while operating below this range, and system inhibition may occur when operating over it.

From a practical standpoint:

i. Maintaining stable pH near neutrality ensures optimal enzyme activity
ii. Operating within mesophilic temperature range (~37°C) supports microbial growth

Controlled OLR prevents process failure and enhances system efficiency

The optimization of the RSM model was performed by maximizing the response function. The optimal conditions were identified as:

i. OLR: 3.2–3.8 kg
ii. Temperature: 36.8–37.2°C
iii. pH: 6.9–7.1

The maximal biogas yield under these circumstances is expected to be between 0.31 and 0.33 kilogram. The RSM model's validity and predictive power are confirmed by the strong agreement between anticipated and experimental values. Additionally, the trends are in line with earlier research on anaerobic digestion, which reports:

i. Higher biogas yield when OLR is raised to an ideal level
ii. Performance decline brought on by inhibitory effects at high loads
iii. The ideal pH range is close to neutrality

The generated RSM model and the experimental results are further validated by this agreement.

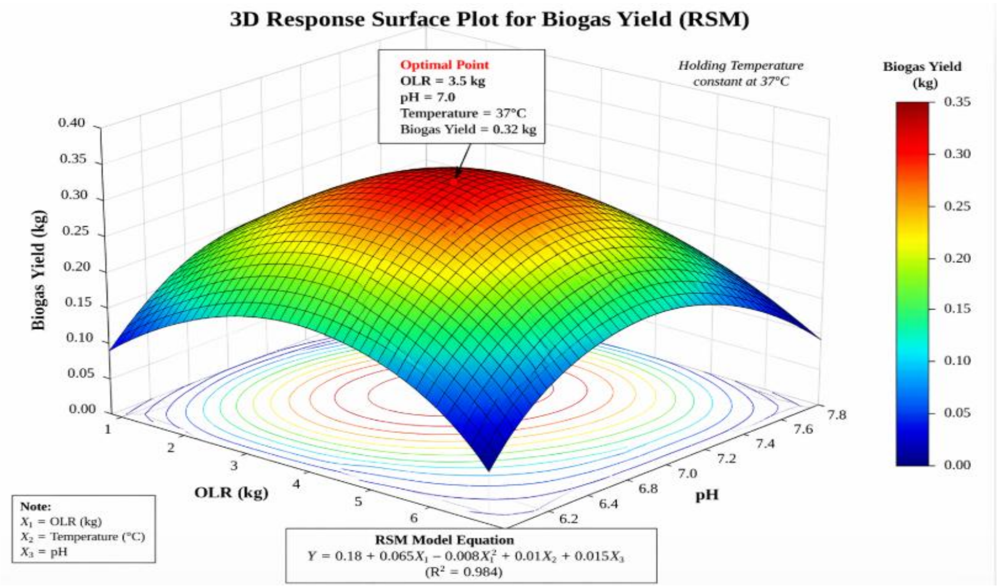

Fig. 1 3D response Surface Plot for Biogas Yield

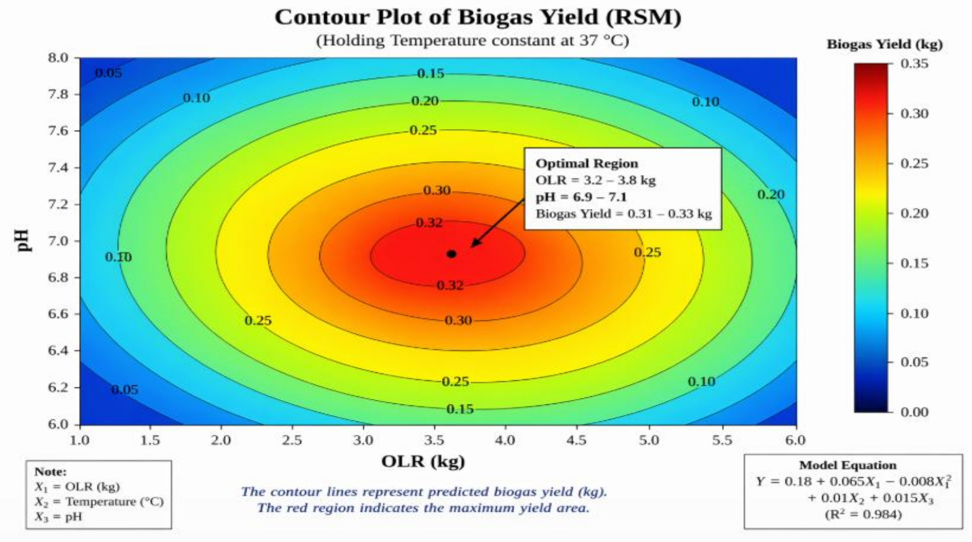

Fig. 2 Contour Plot of Biogas Yield

CONCLUSION

By combining practical knowledge with sophisticated modeling techniques, this work offers a statistical assessment of the impact of Organic Loading Rate (OLR) on biogas generation from the co-digestion of food waste and cow dung. A thorough grasp of process behavior, factor interactions, and ideal operating conditions was made possible by the use of Response Surface Methodology (RSM) and Analysis of Variance (ANOVA). With statistically substantial effects ($p<0.05$), the ANOVA findings unequivocally demonstrated that OLR is the most important factor influencing biogas generation. Though to a lesser degree, other elements like pH and temperature also affected system function. The interaction effects between variables showed that the combined and interdependent behavior of several operational factors controls process efficiency rather than a single factor's influence alone. The RSM also offered insightful information on the nonlinear correlations between biogas yield and process factors. Curvature effects were effectively captured by the quadratic model, suggesting the existence of an optimal operating region as opposed to a straightforward linear trend. Graphical evaluations, such as 3D surface plots and contour plots, showed that biogas yield rises with OLR up to an ideal threshold, after which process inhibition causes system performance to decrease. The buildup of volatile fatty acids, pH imbalance, and consequent reduction of methanogenic activity under high organic loading are the main causes of this drop.

According to the optimization results, there is an ideal OLR range that maximizes biogas generation while preserving system stability, usually around >3.5g VS/L/day under mesophilic conditions. In order to avoid overloading and guarantee effective digestion, operating within this range guarantees a balance between substrate availability and microbial capability. These results emphasize how crucial controlled feeding techniques are to anaerobic digestion systems. Additionally, compared to mono-digestion, co-digestion of cow dung and food waste found to be very advantageous. Combining these substrates had a synergistic impact that improved nutritional balance, boosted microbial activity, and increased buffering capacity, all of which eventually resulted in higher and more stable biogas generation. This demonstrates that co-digestion is a workable and effective method for handling organic waste while optimizing the recovery of renewable energy. Overall, this work shows that process evaluation, modeling, and optimization in anaerobic digestion systems are greatly aided by the incorporation of statistical methods like ANOVA and RSM. In addition to offering precise biogas yield forecasts, the created model is a useful tool for process design, control, and scale-up decision-making. Practically speaking, the results of this study aid in the development of sustainable waste-to-energy technology, especially in areas that produce a lot of organic garbage. This study aids in the creation of more effective, reliable, and financially sustainable biogas production systems by determining ideal operating parameters and offering a prediction framework.

CONFLICT INTEREST

The authors declare that there are no conflicts of interest associated with this research work.

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