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Model Performance Evaluation of Continuous Stir Tank Bioreactor of Municipal Organic Solid Waste in Biogas Production

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ABSTRACT: This study presents the development, modelling, and performance evaluation of a continuous stirred tank bioreactor (CSTR) for biogas production from municipal organic solid waste (MSW). Mathematical models describing biogas yield were developed and statistically evaluated using analysis of variance (ANOVA) and response surface methodology implemented in MINITAB version 17.1. The influence of key operational parameters—pH, hydraulic retention time, and organic loading rate—on biogas yield was investigated. Results from the first-order model revealed that increasing slurry pH from 6.0 to 7.2 produced only a marginal improvement in biogas yield, as indicated by the relatively small inclination of the response line. In contrast, retention time and organic loading rate exerted pronounced effects on biogas production, with organic loading rate showing the strongest influence. The second-order (quadratic) model demonstrated superior predictive capability, evidenced by insignificant lack-of-fit, high coefficients of determination ($R^2 \approx 0.999$), and reduced error variance. Residual diagnostics confirmed uniform distribution of errors, with maximum error frequency of approximately 5.8 occurring within standardized residuals of 0.0–1.0, indicating minimal deviation between predicted and experimental values. Overall, the quadratic model was statistically adequate for representing biogas production behaviour in the CSTR, with improved accuracy and minimal outliers.

KEYWORDS: Performance modelling; Biogas; Continuous stirred tank bioreactor; Response surface methodology; Municipal solid waste; Anaerobic digestion

1. INTRODUCTION

A bioreactor is an artificially constructed or technologically engineered system designed to sustain a biologically active environment under controlled conditions [1]. Such systems provide an enclosed medium in which biochemical reactions occur through the action of microorganisms or biologically derived catalysts. The performance of bioreactors used for organic waste degradation is influenced by several interacting variables, including microbial population dynamics, substrate composition, operating temperature, pH, agitation intensity, and mixing patterns [2].

When effectively controlled, these parameters promote the anaerobic decomposition of organic matter, resulting in valuable products such as biogas and digestate suitable for use as organic fertilizer. However, poor bioreactor performance is frequently associated with inadequate slurry circulation and insufficient mixing, which may lead to localized nutrient depletion, temperature gradients, and incomplete degradation. These shortcomings often originate from insufficient consideration of hydrodynamic behaviour during reactor design and scale-up.

Previous studies have shown that bioreactor operational parameters can be determined experimentally, albeit at the expense of extended development time and increased cost [3]. Fluid flow and mixing behaviour have been identified as major constraints in large-scale bioreactor performance. To address these challenges, computational tools such as computational fluid dynamics (CFD) have been employed to predict circulation characteristics and optimize reactor design. Ogunbiyi [4] demonstrated that fluid circulation and mixing significantly influence overall bioreactor efficiency, particularly in systems where axial mixing is limited.

Given that effective circulation is a critical requirement for enhanced biogas production, this study focuses on the modelling and performance evaluation of a continuous stirred tank bioreactor for anaerobic digestion of municipal solid waste. Emphasis is placed on identifying the dominant operational factors affecting biogas yield and evaluating the adequacy of first- and second-order predictive models

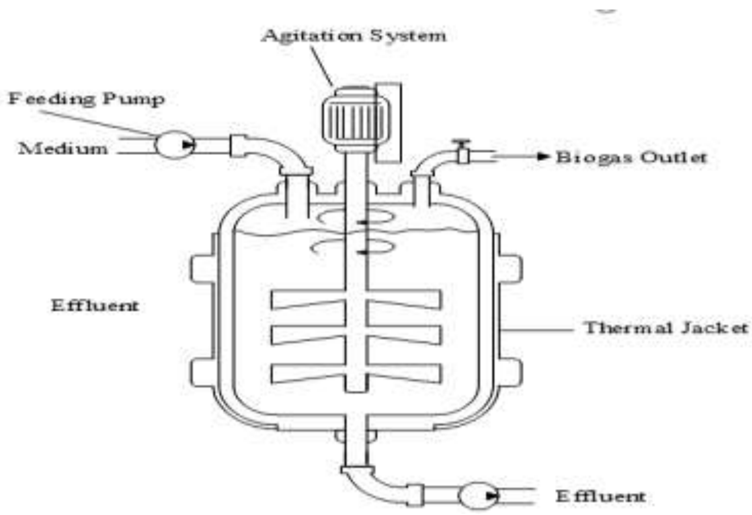

Figure. 1. Schematic Representation Of A Continuous Stirred Tank Reactor (CSTR) Used For Anaerobic Digestion.

1.1. Biogas Production In Continuous Stirred Tank Reactors

A continuous stirred tank reactor (CSTR) is a closed vessel, typically cylindrical in shape, equipped with a mechanical agitator to ensure uniform mixing of contents, as illustrated in Fig. 1 [5]. Depending on impeller configuration, axial, radial, or mixed flow patterns can be generated [6,7]. Adequate agitation enhances mass and heat transfer, prevents stratification, and ensures uniform distribution of microorganisms and substrates.

Insufficient agitation leads to poor transfer rates, localized inhibitory conditions, and reduced reactor productivity. To improve mixing efficiency, CSTRs are often fitted with baffles [8]. Several studies have reported successful application of CSTRs in wastewater and organic waste treatment. For example, dairy wastewater treatment achieved chemical oxygen demand (COD) removal efficiencies of up to 60%, with methane content ranging between 22.5% and 76.9%. Similarly, treatment of palm oil mill effluent (POME) using CSTRs yielded COD reductions of approximately 90% and methane content of about 64% at a hydraulic retention time of six days [9].

1.2. Biogas Formation Process

Biogas is produced through the anaerobic digestion of organic matter, a biological process occurring in the absence of oxygen. Anaerobic digestion involves a sequence of microbial-mediated reactions, culminating in methanogenesis, where intermediate products are converted into methane and carbon dioxide [10–13]. Modern anaerobic digestion technologies are widely applied for municipal solid waste stabilization and renewable energy generation [14].

The biochemical conversion of carbohydrates, proteins, and lipids during anaerobic digestion can be represented by the following simplified reactions:

Carbohydrate, nitrogenous compound, and fat breakdown can be simply represented using chemical formulations as follows:

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2C_2H_4O_2 + 2CO_2 + 4H_2 \quad (1)$$

From the acetic acid and hydrogen products of the above reaction, methane would be produced.

$$2C_2H_4O_2 \longrightarrow 2CH_4 + 2CO_2 \quad (2)$$

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \quad (3)$$

When these expressions are combined, the generalized equation for the anaerobic digestion process is obtained as follows

$$\text{organic matter} + \text{combine water} \xrightarrow[\text{microbes}]{\text{anaerobic}} \text{new cell} + \text{energy for cell} + CH_4 + CO_2 + \text{other end products}$$

1.3. Factors Affecting Bioreactor Performance

Bioreactor performance is governed by several critical parameters:

pH

Optimal methanogenic activity occurs within a pH range of 6.8–7.2. Values below 4.0 or above 9.5 inhibit microbial growth and reduce methane production [15,16].

Temperature

Anaerobic digestion proceeds effectively under mesophilic (30–50 °C) or thermophilic (50–60 °C) conditions. Temperature fluctuations can disrupt microbial communities and cause reactor instability [17,18].

Carbon-to-Nitrogen Ratio

An appropriate C:N ratio is essential for microbial metabolism. Excess carbon limits microbial growth, while nitrogen deficiency impairs cell synthesis.

Organic Loading Rate

Organic loading rate (OLR) reflects the quantity of degradable organic matter supplied per unit reactor volume. Excessive OLR can reduce COD removal efficiency and cause process inhibition [3].

Mixing and Flow Regime

Effective mixing ensures contact between microorganisms and substrate, minimizes mass transfer resistance, prevents accumulation of inhibitory intermediates, and maintains uniform reactor conditions [6].

1.4. Waste Characterization Waste Definition

Generally, waste is considered useless material that is unwanted and consequently discarded. This clarifies that waste represents "anything or anyone rejected as useless, worthless, or beyond what is necessary". Consequently, municipal solid waste (MSW) is characterized as "all waste gathered by private and public authorities from domestic, commercial, and certain industrial (non-hazardous) sources" [14] and [10]. MSW comprises small and moderately proportioned solid waste items from residences, businesses, and institutions.

Municipal Solid Waste Management

Like numerous other cities in Nigeria [19], substantial refuse volumes are generated daily in Port Harcourt and are also inappropriately discarded by inhabitants.

Energy Potential of Municipal Solid Waste

[7] References the 1997 assessment report of the Urban Development Bank of Nigeria Plc, and indicates that the estimated average per capita waste generation for the nation is 0.45kg/day, and for the Port Harcourt metropolis is 0.33kg/day.

2. MATERIALS AND METHODS

2.1. Sample Collection and Waste Characterization

Municipal solid waste samples were collected from waste bins at ten randomly selected locations within Enugu East metropolis, Nigeria. Laboratory analyses and reactor experiments were conducted at the Energy Research Centre, University of Nigeria, Nsukka.

Collected waste samples were weighed in their as-discarded state and subsequently compacted to constant volume. Samples were sorted into organic and inorganic fractions, and densities were determined. The compaction ratio was calculated as:

Results were utilized in determining 'as-discarded' and 'as-compacted' densities, and the "ratio of 'as-compacted' density (ρ_c) to 'as-discarded' density (ρ_d)) represents the compaction ratio (r) [5], which was employed in digester design for the waste.

$$r = \frac{\rho_c}{\rho_d} \quad (4)$$

Proximate Analysis

Moisture content, volatile matter, crude fibre, and lipid contents were determined using standard analytical procedures [20]. Density and specific gravity were measured using a specific gravity bottle, while energy content was estimated using [14]:

Reactor Experimentation

Five batch anaerobic digesters of 5 L capacity were operated under controlled conditions. The experimental setup is shown schematically in Fig. 2

Moisture Content Determination

Approximately 5.00g of the sample was weighed and dried in an oven at a regulated temperature of 105°C. The drying sample was continuously reweighed every 10-minute interval until a constant weight was achieved. The crucible and contents were retrieved and cooled in desiccators. Weight difference was recorded and moisture content calculated using equation 5:

$$\%\,Moisture = \frac{wt\ of\ wet\ sample - wt\ of\ oven\ dry\ sample}{wt\ of\ oven\ dry\ shell\ sample} \times \frac{100}{1} \quad (5)$$

Volatile Matter Content

Volatile matter content was obtained by heating 10g of moisture-free sample in a muffle furnace at 900°C for one hour. Heating absent air at elevated temperature removes volatile matter exclusively; its percentage was obtained using equation 6:

$$\%\,Volatile\ matter = \frac{weight\ loss}{weight\ of\ moisture\ free\ sample} \times \frac{100}{1} \quad (6)$$

Crude Fiber Content

Crude fiber content was determined using equation 7 with results shown in Table 1:

$$\%\,Crude\ fiber = \frac{Loss\ in\ weight\ on\ ignition\ (W_2 - W_1) - (W_3 - W_1)}{Weight\ of\ shell\ sample} \times \frac{100}{1} \quad (7)$$

Lipid Content

The sample underwent exhaustive lipid extraction for 3 hours by heating the flask on an electric hot plate at 50°C. After 3 hours, the extractant (petroleum ether) was distilled off while the flask and contents were cooled in a desiccator before reweighing [20].

Lipid percentage was calculated using equation 8:

$$\% \, Lipid (or \, fat) = \frac{weight \, of \, lipid}{weight \, of \, sample} \times \frac{100}{1} \qquad (8)$$

Density and Specific Gravity

A clean, empty specific gravity bottle was weighed on an electronic balance, and mass (W_1) was noted. It was then filled with a sample at the required temperature, and mass (W_2) and volume were noted. Sample mass (W_s) was the difference between W_2 and W_1 Density, ρ, was calculated using:

$$Density = \frac{Mass}{Volume} \qquad (9)$$

The bottle was washed, dried, and filled with an equal volume of water at the required temperature, and the mass (W_3) was noted. The mass of water (W_w) was the difference between W_3 and W_1. The specific gravity of the sample was determined using the equation:

$$Specific \, gravity = \frac{Weight \, of \, sample}{Weight \, of \, equal \, volume \, of \, water} \qquad (10)$$

That is:

$$Specific \, gravity = \frac{W_2 - W_1}{W_3 - W_1} \qquad (11)$$

The energy content of the waste was determined using the equation for the estimation of the energy content of MSW, presented by [14] as:

$$EC = 0.051[F + 3.6(CP)] + 0.352(PLR) \qquad (12)$$

Where: Ec - Energy content of MSW, MJ/kg

F - % of food by weight

CP - % of cardboard and paper by weight

PLR - % of plastic and rubber by weight

Reactor Experimentation was done at **the Energy** Research Center, Nsukka laboratory. Five batch-wise anaerobic digesters, each of 5 liters in volume, were set up for this experiment. The schematic of the experimental design layout is shown in Fig. 2 below:

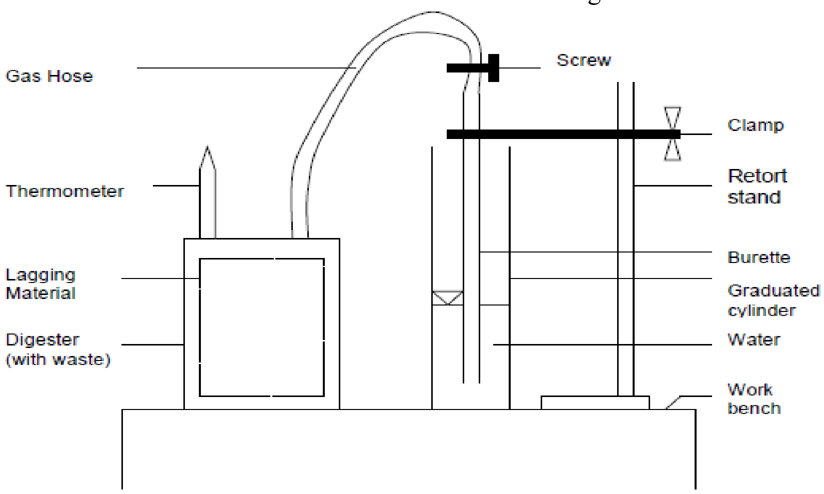

Figure 2. Schematic Layout of the Experimental Anaerobic Digestion Reactor Setup. [21]

2.2. Models for the CSTR Design

The development of models for the CSTR is strictly based on the requirements of a high rate, low solids processing, where there is "continuous mixing and continuous or intermittent sludge feeding and sludge withdrawal, and the contents are in a homogenous state [22]. [21] states that the volume of a CSTR is defined as the product of the rate of flow of the medium and the hydraulic retention time.

This is stated mathematically as:

$$V_c = Q\theta_h \qquad (10)$$

Where;

Vc = overall volume of the continuous digester, m^3

Q = influent sludge flow rate, m^3/day

θ_h = hydraulic retention time, days.

But hydraulic retention time, θ_h is equal to;

$$\theta_h = \frac{\alpha}{(1-\alpha)} \left[\frac{K_s + [S]}{K[X]} \right]$$

Substituting equation 13 into equation 14, we have;

$$V_c = Q \left\{ \frac{\alpha}{(1-\alpha)} \left[\frac{K_s + [S]}{K[X]} \right] \right\}$$

The diameter and height of the tank is related to the tank volume by the following equation;

$$V_c = A_c \times H_c \quad (13)$$

Where

A_c = cross-sectional area of the tank, and

H_c = height of the tank.

Therefore, adapting the established relation between the digester tank diameter and height as being in the ratio of 2:1 according to [21], so that $D_c = 2H_c$ or $H_c = {D_c}/{2}$ Then equation 16 becomes

$$H_c = \sqrt[3]{\frac{2}{\pi}\left[Q\left\{\frac{\alpha}{(1-\alpha)}\left[\frac{K_s+[S]}{K[X]}\right]\right\}\right]} \quad (14)$$

Models for Methane and Biogas Production

The amount of methane (CH_4) produced in the anaerobic decomposition of organic materials is usually determined using the stoichiometric equation developed by Buswell, and has been used over time, which [21] as well is also used as presented below:

$$C_nH_aO_b + \left[n - \frac{a}{4} - \frac{b}{2}\right]H_2O \rightarrow \left[\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right]CO_2 + \left[\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right]CH_4 \quad (15)$$

According to Avogadro's postulation:

1 mole of $CH_4 \equiv 0.0224\ m^3$

Therefore, 18.519 moles $CH_4 \equiv 18.519 \times 0.0224 = 0.415\ m^3$

This shows that 1 kg of the organic matter would yield 0.415 m^3 of CH_4, which accounts for the difference in concentration of the MSW as it is digested from So to Se. Therefore, the unit of CH_4 generated will be represented as:

Kg/m^3 of CH_4 produced = 0.415 (So–Se) (16)

Then, with a conversion factor (M = 1.43) of COD to VS, the equation becomes

$C_m = 0.415M\ (S_o - S_e)$ (17)

Where C_m - actual concentration of dissolved methane gas

3. RESULTS AND DISCUSSION

3.1. Performance model development and analysis

There are many factors that affect the yield of biogas from a particular substrate, such as pH, retention time (days), and organic loading rate (kg), etc. In order to determine the main factors influencing the yield of biogas from municipal solid waste (MSW), experimental design methods are used. It is created by mathematical models and used to ascertain the effect of factors, as well as the determine the yield of biogas from MSW. One of the experimental design methods is factorial design, in which all combinations of the examined factors can be investigated. A designed experiment will enhance the determination of suitable conditions for achieving process optimization.

The relationship between the performance parameters (responses); yield of biogas from MSW, and its operational parameters (factors), as well as the determination of the optimal settings of the parameters, was investigated using response surface analysis for a first-order model. This involved the design and development of the response surface experiment, model fitting, selection, and optimization of the best response surface functions for the responses. The response variables investigated were yield of biogas, pH, retention time (days), and organic loading rate (kg), coded asx_1, $x_{2,}$, *and* $x_{3,}$ are the factors studied?

The yield of biogas from municipal solid waste (MSW) was the response in (ml). The empirical linear relationships between each of the operational and performance parameters of the anaerobic digestion of MSW developed and analysed are shown in Equation 19 below.

$$Y(ml) = -22.2 + 2.18x1 + 0.509x2 + 5.01x3 - 0.0083x1 * x2 - 0.383x1 * x3 - 0.0813x2 * x3 \quad (19)$$

The adequacy of the linear models to fit the measured operational parameter was carried out using the residual plots, analysis of variance (ANOVA), lack of fit test, and coefficient of determination. Analysis of variance (ANOVA) and lack of fit test for each of the models are shown in Table 1 below, while the error standard deviation and the coefficient of determination are shown in Table 2 below.

Table 1: Analysis Of Variance (ANOVA) For the First-Order (Linear) Model Describing Biogas Yield from Municipal Solid Waste.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	18.5756	3.0959	2.91	0.050
Linear	3	16.0019	5.3340	5.20	0.016
Interactions	3	2.5737	0.8579	0.81	0.512
Error	13	13.8224	1.0633		
Lack-of-Fit	8	12.9740	1.6218	9.56	0.012
Pure Error	5	0.8483	0.1697		
Total	19	32.3980			

From Table 1, it can be observed from the analysis of variance of the linear model for the biogas yield that there exists a significant lack of fit, and also the p-values for the model and interactions show that it is not statistically significant. These are indications that the model does not fit the data properly, and hence second order should be considered.

The empirical linear relationships between each of the operational and biogas yields from MSW were developed and analyzed. However, the main effects plots, model adequacy measures, and residual diagnostic plots displayed along with the fitted linear models were used to evaluate the functions, though they adequately approximated true responses, but the models exhibit a significant lack of fit with poor coefficient of determination and error standard deviation in Table 3. In addition, the residual plots for the biogas yield from MSW show a high level of skewness and outliers, which are undesirable and an indicator that the models do not fit the data properly; hence, second-order models were considered

Table 2. Coefficient Of Determination and Standard Error for the First-Order Biogas Yield Model

Responses	S	R-sq	R-sq(adj)
Biogas yield (ml)	1.03114	57.34	37.64

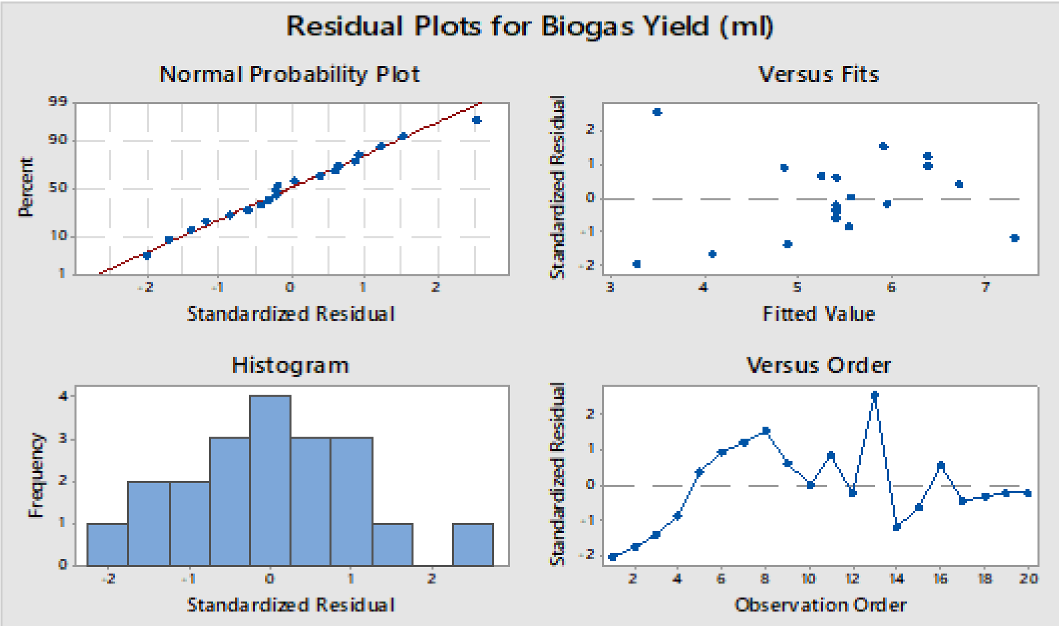

Figure 3. Residual Plots for The First-Order Model of Biogas Yield from Municipal Solid Waste.

3.2. Main Factor Effects

The main effects plots are used for comparing the magnitude of the data means of responses. The points in the plots represent the data means of the response variables at various levels of each factor, with a reference line drawn as the grand means of the response data. The main effects plot comprises a plot line and the magnitude of the effect of each factor on the response is measured by the angle of inclination of the plot line to the centre line, where positive inclination means positive effect and negative inclination means negative effect, while lines horizontally parallel to the x- axis means the factor has no or insignificant effect on the response.

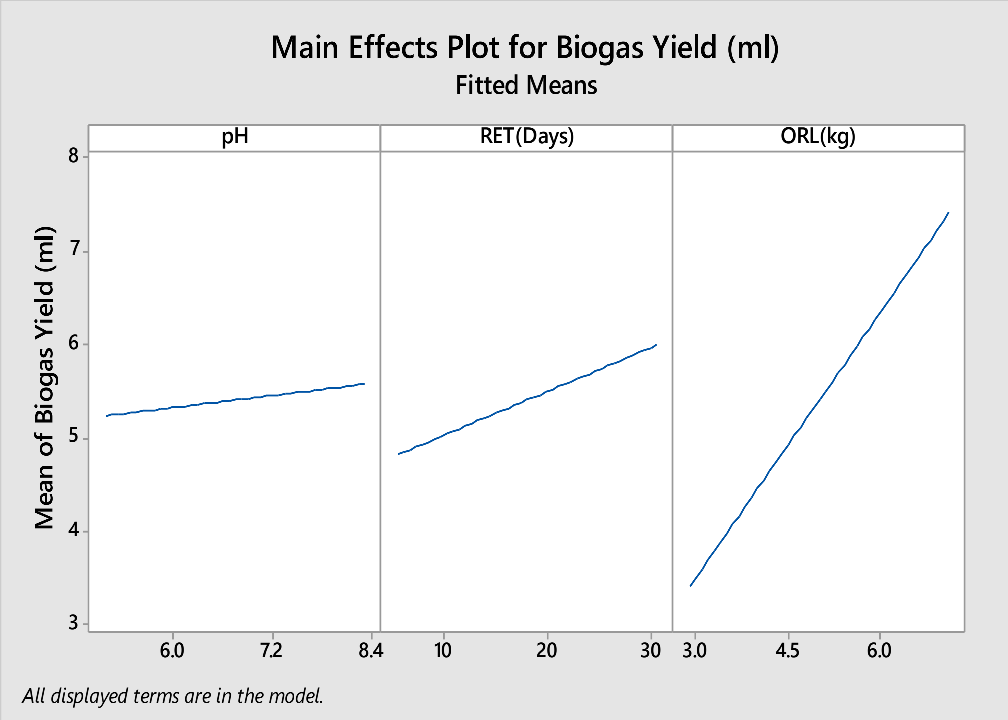

Figure 4. Main Effects Plots Showing the Influence of Ph, Retention Time, And Organic Loading Rate on Biogas Yield.

Hence, it can be seen from the main effects plot, Fig. 4, for biogas yield that x_1 (pH), x_2 (RET)and x_3 (OLR) have a positive inclination, that is, an increase in the pH, RET and OLR will result in an increase in the biogas yield. However, it can be observed from Fig. 4 that increasing the pH. The slurry from 6.0 to 7.2 has a slight increase in the biogas yield, as suggested by the small angle of inclination of the change in pH line, whereas increasing the retention time and organic loading rate of the substrate have large effects on the biogas yield as depicted by their angle of inclination, with that of OLR having the highest effects.

Interaction Factor effects

The features of these plots are the possession of factor pairs representing the levels of the factorial design - high (+1) and low (-1) – of each factor, and if the lines are parallel to each other, the interaction between the factors is said to be statistically insignificant on the response. Also, if the lines intersect, the interaction between the factors is said to be statistically significant to the response. Positive and negative interaction effects occur when the intersection is on the high level of the factors and the low levels of the factors, respectively.

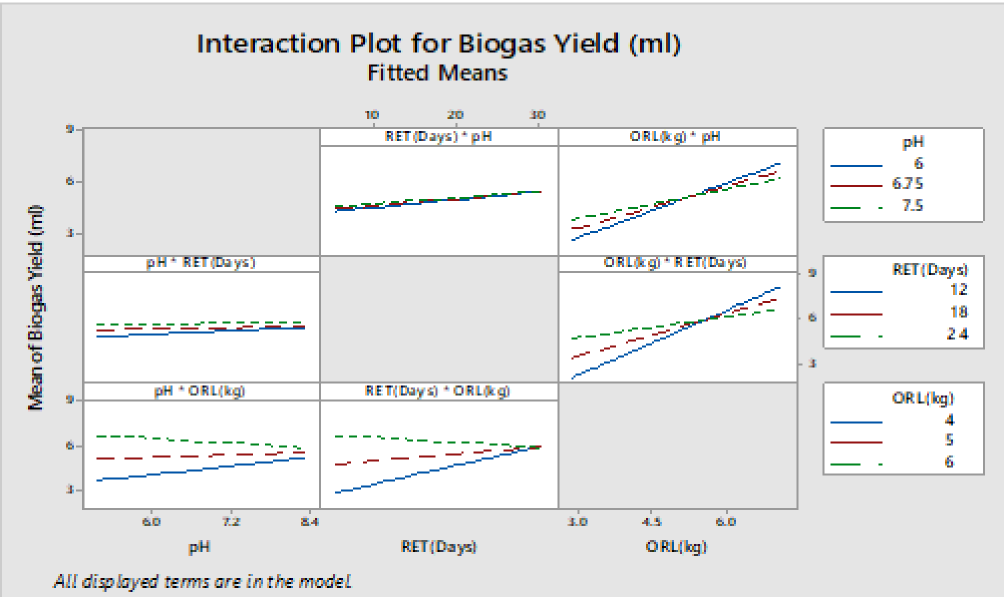

Figure 5. Interaction Plots Illustrating Combined Effects of Operational Parameters on Biogas Yield

Inspection of Fig. 5 shows a low level of interaction between factors and corresponding response (biogas yield), and hence, the linear model was augmented to a second-order (or quadratic) model to analyse the effects of the factor interactions on the corresponding responses.

Second Order Model

The central composite design of response surface methodology in MINITAB version 17.1 was used to develop a second-order model for biogas yield from anaerobic digestion of MSW. The factors considered are; x_1 (pH), x_2 (RET)and x_3 (OLR),while the responses are the biogas yield. A total of 20 runs were generated using the MINITAB version 17.1, comprising eight (8) factorial points, two (2) axial points, and ten (10) centre points. The second-order models developed for the biogas yield are shown in equation 20. The uncoded/actual response functions of the factors affecting the yield of biogas from MSW investigated are as follows:

$$\textbf{Biogas Yield(ml)} = -60.2 + 7.4x1 + 0.61x2 + 13.91x3 - 0.368x1^2 - 0.0060x2^2 - 1.117x3^2 - 0.051x1x2 - 0.30x1x3 - 0.012x2x3$$

The analysis of variance for the models of the biogas yield performance parameters from MSW are shown in Tables 3

Table 3. Analysis Of Variance (ANOVA) For the Second-Order (Quadratic) Response Surface Model of Biogas Yield.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	18.5716	2.0635	21029.38	0.000
Linear	3	15.9977	5.3326	54344.72	0.000
x_1	1	0.1053	0.1053	1073.12	0.000
x_2	1	1.2656	1.2656	12898.09	0.000
x_3	1	14.6268	14.6268	149062.93	0.000
Square	3	0.0001	0.0000	0.32	0.011
x_1^2	1	0.0000	0.0000	0.48	0.002
x_2^2	1	0.0000	0.0000	0.40	0.041
x_3^2	1	0.0000	0.0000	0.48	0.002
2-Way Interaction	3	2.5738	0.8579	8743.10	0.000
x_1x_2	1	0.0113	0.0113	114.65	0.000
x_1x_3	1	0.6612	0.6612	6738.85	0.000
x_2x_3	1	1.9013	1.9013	19375.80	0.000
Error	10	0.0010	0.0001		
Lack-of-Fit	5	0.0000	0.0000	0.01	1.000
Pure Error	5	0.0010	0.0002		
Total	19	18.5726			

From the analysis of variance for the quadratic model of the biogas yield from MSW in Table 3, it was observed that the p-values of the model, linear term, interactive term and quadratic term for all the factors affecting the yield of biogas investigated were significant since they are less than 0.05 and there was an insignificant lack of fit which suggest that the quadratic term improved the model. The coefficient of determination and error standard deviation of the quadratic model are shown in Table 4. A comparative evaluation of the coefficient of determination and error standard deviation of the linear and quadratic models (Table 3) indicates that the quadratic models were adequate. This is because the values of 'R^2'and 'adj ' adj-R2' increased while the value of 'S' reduced in the model, and hence it can be deduced that the interaction and square terms improved the adequacy of the models.

Table 4. Model Adequacy Statistics for the Quadratic Biogas Yield Model, Including Standard Error and Coefficients of Determination.

Responses	S	R-sq	R-sq(adj)	R-sq(pred)
Biogas Yield (ml)	0.0099058	0.999	0.9988	0.9999

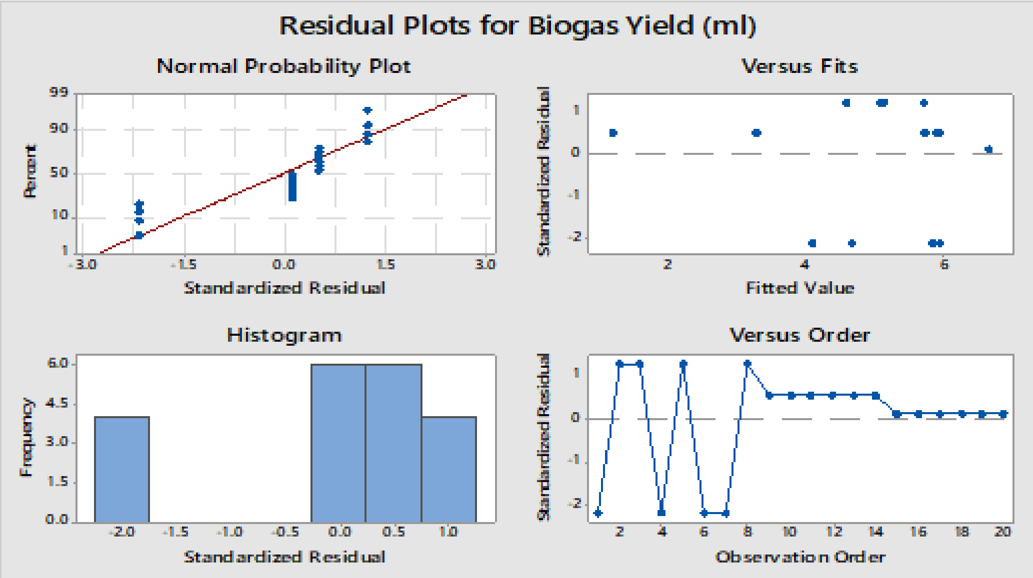

Figure 6. Residual Diagnostic Plots for The Second-Order (Quadratic) Biogas Yield Model. The Model

Adequacy for the biogas yield is confirmed as shown in Fig. 6 using the residual plots, which comprise the normal probability plot, histogram, residual versus fitted values, and residual versus observation. Shows that the residual data points were evenly distributed. Also, the histogram plot indicated that the highest error frequency of approximately 5.8 occurred at standardized residue points of (0.0 - 1.0), which showed that the model error deviation from experimental value is insignificant. Hence, the second-order model was adequate to statistically fit the data with few outliers and reduced error.

4. CONCLUSION

This study successfully developed and evaluated performance models for biogas production from municipal solid waste using a continuous stirred tank bioreactor. The first-order model revealed that pH had only a marginal influence on biogas yield within the investigated range, whereas retention time and organic loading rate significantly enhanced production, with OLR being the dominant factor. The second-order model provided a statistically robust representation of the process, characterized by high predictive accuracy, insignificant lack-of-fit, and minimal error variance. Overall, the quadratic model is recommended for reliable prediction and optimization of biogas yield in CSTR-based anaerobic digestion of municipal solid waste.

Abbreviations

CFD	Computational Fluid Dynamics
ANOVA	Analysis of Variance
CSTR	Continuous Stirred Tank Reactor
COD	Chemical Oxygen Demand
POME	Palm Oil Mill Effluent
OLR	Organic Loading Rate
C	Carbon
N	Nitrogen
MSW	Municipal Solid Waste
RET	Retention
PLR	Plastic and Rubber Weight
Cp	Cardboard and Papper weight
W	mass

Availability of data and materials

The datasets supporting the conclusions of this article are included in the article

Conflict of interests

The authors declare that they have no competing interests

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Authors' contributions

ICI wrote up the manuscript. **ICI and AN** conducted the laboratory experiments. **AN** and **ICI** supervised the laboratory experiment and structured, edited, read, and approved the final manuscript. The authors read and approved the final manuscript.

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