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Anaerobic digestion of abattoir wastes for biogas production: optimization via performance evaluation comparison

Oladare Johnson Odejobi1, Ebenezer Leke Odekanle2, Ayorinde Bamimore3, Olayomi Abiodun Falowo4* and Funso Akeredolu1

Abstract: Anaerobic digestion of abattoir wastes under mesophilic conditions was carried out to investigate how different modeling tools affect biogas yield in order to be subsequently used for process optimization. Response Surface Methodology (RSM) and Artificial Neural Networks (ANNs) were employed to optimize the process, and to assess the individual and interactive effect of incubation time, temperature, and pH on biogas yield. The digester used in this study produced an average biogas yield of 0.00103 m³/kg VS daily from cow-dung. Gas chromatographic analysis of the produced biogas showed the methane content to be 66.8%. The conditions for optimum biogas yield as predicted by RSM were incubation time of 28.98 days, temperature and pH of 30.16°C, and 7.43, respectively. For ANNs, the incubation time, temperature, and pH for optimum biogas yield were 26.76 days, 30.94°C, and 7.27, respectively. With these conditions, biogas yield by RSM was Olayomi Abiodun Falowo m³/kg VS while that of ANNs was Olayomi Abiodun Falowo m³/kg VS. Model validation by experimental tests showed that ANN is better in terms of prediction and accuracy than the RSM, though, the two techniques complemented each other in interpreting the interactive effects of the input variables on the biogas production.

Subjects: Environmental Sciences; Environmental Management; Environment & Resources; Chemical Engineering

Keywords: Abattoir; Anaerobic digestion; Biogas; methane; Optimization

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1. Introduction
The recent upsurge in meat production in developing countries has resulted in the generation of a high quantity of animal wastes which consequently become a menace in many urban centers in developing nations (Venturin et al., 2018). Abattoir waste is one of such wastes that has created environmental challenges by its continuous generation, hence a call for a strategic approach that leads to its conversion into useful products (Chukwu et al., 2011; Zou et al., 2016). Generally, abattoirs are designated and approved premises where animals are slaughtered, prepared, and dressed for human consumption. However, the indifference and absence of government’s regulations on the operations of abattoirs in most developing countries have led to their poorly designed and unhygienic environment (Oruonye, 2015). In a typical abattoir in Nigeria, apart from liquid effluents that are washed directly into nearby streams, heaps of large quantity of solid wastes which comprise of bones, cow dung, and rumen contents are generated and left indifferently without regard to sound waste management practices. Thus, these wastes pose a health threat to humans and other terrestrial and aquatic lives (Khayum et al., 2018). Nonetheless, abattoir wastes can be converted into useful and harmless products through anaerobic digestion (Yao et al., 2018). This approach strategically solved pollution challenges while providing useable products at inexpensive cost. Through anaerobic fermentation process, organic matters are biodegraded by the action of microorganisms in the absence of oxygen to produce biogas (primarily methane and carbon dioxide) as well as stabilized sediment previously reported to be suitable for use as organic fertilizer (Chen et al., 2010). The outcome of this process serves a dual purpose by providing a solution to the environmental pollution problem and also alleviating energy crises being faced in developing nations. Generally, biogas produced through utilization of the abattoir wastes is thought to be one of the solutions to greenhouse gases generated from mineral fuels. This biofuel will contribute in no small measure to the transformation of energy sources from being fossil fuel dependent on into renewable energy, and will reduce both direct discharge of wastes to the nearby streams and indifferent abandonment of the wastes to pollute the environment (Oruonye, 2015).

Several researchers have carried out anaerobic digestion of substrates on a laboratory scale, which could be scaled up to a much larger design, using different waste materials (Dahunsi et al., 2017; Ilori et al., 2007). Anozie (Anozie et al., 2005) investigated biogas production from agricultural wastes using a batch pilot-scale digester. Uzodinma and Ofoefule (Uzodinma & Ofoefule, 2009) reported a high yield of biogas from a blend of field grass and wastes from animal. There are other documented studies on the use of several other wastes for the production of biogas (Alfa et al., 2014; Croce et al., 2016; Khayum et al., 2018) through anaerobic digestion technology.

To avoid guesswork in choosing important process factors influencing the biogas amount in a digester, modeling of anaerobic process is essential to establish optimal process values, thereby saving both operating time and production cost for the operators. The pH of the slurry as well as incubation time affect the activity of the microbial habitat which invariably influenced biogas generation from the waste. Recently, tools, such as Adaptive neuro-fuzzy inference system genetic (ANFIS), Artificial neural network (ANN), Response surface methodology (RSM), and Genetic algorithms (GA), are applied to model several biological processes (Betiku et al., 2015; Falowo et al., 2019; Ishola et al., 2017). RSM, an optimizing tool used a mathematical and statistical technique to analyze the effects of several dependent variables on the process response. Its applications in industrial science, biological and clinical sciences, social science, physical and engineering sciences have been reported by previous works (Betiku et al., 2015; Kumar et al., 2009; Weska et al., 2007). ANN models are purely data-based and it is suitable for modeling experimental data due to its capability to model a complex non-linear relationship (Maran et al., 2013; Rafigh et al., 2014). Besides, RSM and ANN have been utilized to optimize, and analyze the interactive effects of autonomous agents in numerous biochemical, bioenvironmental, and chemical processes (Betiku et al., 2015; Ishola et al., 2017), but only a few studies have documented its use in the modeling and analysis of anaerobic digestion processes (Amani et al., 2011; Tedesco et al., 2014).
For optimum performance of anaerobic digestion, suitable process parameters, namely, incubation temperature, pH, retention time, organic loading rate, and carbon-to-nitrogen ratio must be established to create a conducive environment for microbial growth. Biogas from cow dung through anaerobic digestion had been modeled using python and RSM (Iweka et al., 2021), the methane production has been enhanced by the addition of silica gel (Salam et al., 2015), and nanoparticles, such as nanoferries (Sliem et al., 2021) and Cobalt (Abdelwahab et al., 2021). Digestate which remains at the end of anaerobic process is rich in nutrient content, and was apply in place of mineral fertilizer to boost crop productivity (Tilviikiene et al., 2020). From techno-assessment analysis of anaerobic process from brewer’s spent wastes, return on investment for bioenergy generation and fertilizer production was huge (Sganzerla et al., 2021). However, the dearth of information on the optimization of process factors influencing anaerobic digestion of cow dung waste for maximum biogas generation using optimization tools inspired this study. This present study comparatively used ANN and RSM to optimized biogas production from cow dung. The study carried out an experimental investigation of biogas generation from the abattoir waste and its optimization using RSM and ANN to estimate the effects of pH, temperature, and incubation time on biogas yield. This is to establish optimum value for the process parameters combined in this study for maximum biogas yield. The choice of both RSM and ANN is borne out of the desire to assess the true interaction of the parameters in a non-linear form apart from the RSM second-order polynomial model. Apart from creating global awareness on renewable and sustainable biofuels, the results obtained in this study will provide a useful databank for the optimum combination of these factors based on technical, economic, and environmental aspects for maximum biogas yield.

2. Materials and methods

2.1. Substrate collection and preparation
Fresh cow dung, abattoir wastewater, and rumen contents of adequate microbial floral acting as inoculum were collected from the agricultural farm of Obafemi Awolowo University (OAU), Ile-Ife, Nigeria, and mixed in the ratio of 1:3:2 to form slurry. This ratio ensure adequate solubilization for digestion. After thorough mixing and dilution, the pH, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) concentrations in the substrate were determined according to standard procedures (AOAC, Official Methods of Analysis, 2000; APHA, 2012). Before anaerobic digestion of the slurry, substrate characterization was done. Hence, samples of the substrate were analyzed to quantify their important physicochemical properties. The characterization was performed pre-digestion and after anaerobic process at Hydrobiology and Animal Science laboratories OAU, Ile-Ife using recommended methods (Nwosu et al., 2011). The American Public Health Association (APHA, 2012) standard method for the examination of water and wastewater was used to determine the Chemical Oxygen Demand (COD) of the samples. Determination of total solids (TS) and volatile solids (VS) were done using the SFS 3008 protocol of the Finnish Standard Association (Finnish Standard Association, 1990). For TS, samples were dried at 105°C until a constant weight was achieved, while for VS, known weights of the dried samples were ignited at 575 ± 25°C to constant weight. The performance of the digester was recorded as initially modeled, daily measurements of the digester COD, VS, and TS according to the standard methods (AOAC, Official Methods of Analysis, 2000) were done. Gas chromatography fitted with a flame ionization detector (Clarus 580, PerkinElmer, USA) was utilized to determine the volatile fatty acids. The digester pH and incubation temperature were recorded as long as the biogas production continues using pH meter and thermometer.

2.2. The experimental procedure
In this study, automated anaerobic digester (Edibon PDANC 0007/144, Spain) with the attachment of two 10 liters reactors (control and optimization reactors) was used to digest the abattoir waste. The digester capacity was mainly 20 L reactor vessel unit. The digester consists of an automated temperature regulation mechanism, a loading opening, a sample withdrawal outlet, and an automated agitation device that is fully air-tight. At the back of this reactor are an attached water tank and water collector for maintenance of digester performance. Then, substrate slurry
was fed into the reactors up to three-quarters of the reactor volume, thereby, leaving sufficient space for the biogas collection. A batch digestion process was used for this study. The digester was operated for a retention period of 30 days under a mesophilic condition (28–36°C), while water displacement method was used to quantify the volume of biogas produced which was recorded at the 24-hour interval. The biogas produced in the digester displaced water in the water tank into the water collector. Generally, the amount of water displaced from the water tank is equivalent to the volume of biogas produced (Zuru et al., 2004).

2.3. Determination of biogas composition

The composition of produced biogas from abattoir waste was analyzed using the gas chromatography 5890 series II model. The column used was Haysep Q with a length of 10 ft.x 1/8 inch having a detector temperature of 150°C. The detector was a thermal conductivity detector (TCD). The carrier gas used for this analysis was helium gas with 25psi.

2.4. Modeling and optimization of biogas production

To model biogas production using anaerobic process, a five-level-three factor Central Composite Design (CCD) of RSM was employed to assess the effects of the process factors and their combination on biogas production. The choice of CCD was not only because it is an efficient statistical design used generally to decrease the number of experiments while maintaining statistical significance (Chaibaksh et al., 2009) also because of its success during anaerobic digestion experimental design (Dahunsi et al., 2017). For this study, incubation temperature (30–35°C), pH (6.5–7.5), and incubation time (20–30 days) were investigated based on the findings earlier reported (Mckenna & Sherlock, 2015; Zonta et al., 2013), while the volume of the biogas yield was the response. Depending on the exact pH of the medium, pH was regulated by mixing either a few pellets of sodium hydroxide or drops of hydrochloric acid with the substrate while the temperature was controlled by temperature regulator on the digester. The coded levels of the independent variables (temperature, pH and time) are presented in Table 1.

The experimental results obtained were subjected to statistical analysis to explore the fitness of the model generated by the Design-Expert 10.0 software (stat-Ease Inc., Minneapolis, USA). The coefficient of the quadratic model of the process response was fit using multiple regressions to correlate the response variable to all the three selected independent variables. The fitted quadratic response model is described by Equation (1).

\[ Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ij} x_i^2 + \sum_{i<j}^{k} b_{ij} x_i x_j + e. \]  

where \( Y \) is the process response; \( b_0 \) is intercept value; \( b_i \) (\( i = 1, 2, k \)) is the first-order model coefficient; \( b_{ij} \) is the coefficient of the interaction effect of \( X_i X_j \); \( b_{ij} \) is the quadratic coefficients of \( X_i \) while \( e \) is the random error.

ANN structure was implemented for the analysis of the experimental results obtained. The Neural network toolbox of MATLAB (The Mathworks, Inc., ver. 15a) was used for developing the ANN model. As shown in Figure 1a feed-forward multi-layered perceptron (MLP) neural network model with 3 inputs, 10 hidden layers, and 1 output layer was chosen for the building of the model with temperature, pH, and solid retention time representing inputs while the biogas yield was denoted by the output layer. The different hidden neurons were evaluated to obtain the best that suits the model. This was done by testing a different number of neurons until the highest value of the coefficient of determination \((R^2)\) was identified. The topology of the network was based on the number of hidden neurons chosen. To select the optimal number of the hidden neuron, investigatory approach was employed by using hidden neurons from 2 to 10.

Each hidden neuron is further trained several times and appraised to produce the highest value of \( R^2 \). The complete ANN model was established using tangent-sigmoid transfer function at the
Table 1: Independent factors for CCD in anaerobic digestion process

| Factors           | Units | Coded factor levels Axial (-α) | -1 | 0  | +1 | Axial (+α) |
|-------------------|-------|--------------------------------|----|----|----|------------|
| Incubation time (X₁) (day) | 16.591 | 20 | 25 | 30 | 33.409 |
| Temperature (X₂) (°C) | 28.2955 | 30 | 32.5 | 35 | 36.7045 |
| pH (X₃)           | 6.1591 | 6.5 | 7 | 7.5 | 7.8409 |

input layer and a linear transfer function at the output layer. The activation function for the hidden layer is chosen as:

\[ f_{h}(x) = \frac{2}{(1 + e^{-2x})} - 1. \]  

(2)

3. Results and discussion

3.1. Substrate and digestate characterization

The results of the physicochemical analysis of the substrate pre-digestion and post-digestion are presented in Table 2. The pH increased from 6.4 before the digestion to 7.5 at the end of the digestion (incubation) time of 30 days (Table 2). Although, the pH of the substrate decreased initially when organic material was first loaded into the digester and this is due to high volatile fatty acid formation. However, the pH of the substrate increased when methane-producing bacteria consumed the acids due to alkalinity being produced. The pH range obtained in this study is in agreement with the reported range of values for the efficient operation of anaerobic (Ogejo et al., 2009; Owamah et al., 2014). The temperature of the substrate within the digester was mesophilic throughout the retention time. Anaerobic digestion requires a stable temperature range for efficient microbial activities (Owamah et al., 2014). The values of other physicochemical properties observed after the digestion period were much lower than the pre-digestion concentration (Figure 2). This reduction is due to the adequate utilization of the substrate, which in turn could be attributed to a high degree of digestibility of the substrate. The concentrations of total suspended solids, total dissolved solids and biological oxygen demand, which reduced from 595 ± 5.2 mg TSS/l, 760 ± 7 mg TDS/l, and 500 ± 6 mg BOD/l, respectively, before digestion to 384 ± 2.3 mg TSS/l, 476 ± 3.6 mg TDS/l, and 130 ± 3 mg BOD/l, respectively, after digestion are still above the World Health Organization standards (Egwuonwu et al., 2012).

The moisture content of the substrate before anaerobic digestion was higher than the pre-digestion value.

This accounts for the reverse value observed in the percentage ash content of the digestate as shown in the results. Moreover, an increase in total carbon could be due to an increase in available
energy sources for the microbial community which could increase microbial activities within the digestate and facilitate the biodegradability of the substrate. It should be noted that as this trend continues, there was a rapid consumption of nitrogen by methanogens to meet their protein needs; hence, there was a shortage of nitrogen for the support of microbial growth revealed by the reduction in nitrogen level and increase in carbon/nitrogen ratio. The carbon/nitrogen (C/N) ratio increased from 27.44 before anaerobic digestion to 45.36 after anaerobic digestion. This C/N ratio agrees with the observations from previous research, which have shown that for biogas generation, C/N ratio must be within 20.1 and 30.1 (Romano & Zhang, 2008; Viswanath et al., 1992).

The high carbon-to-nitrogen ratio observed after anaerobic digestion is thought to be one of the major factors responsible for the stoppage of the generation of the gas because of the insufficient nitrogen for microbial growth. COD concentration of the digested substrate was reduced remarkably by almost 85% at the end of the digestion period. COD reduction is due to quick and efficient breakdown and adequate utilization of organic matter. Of the three identified volatile fatty acids (acetic, propionic, and butyric acids), acetic acid was the dominant volatile fatty acid. High volatile fatty acids (VFA) concentrations were recorded at the start of the digestion process. This could be attributed to higher acidogenic and lower methanogenic activities within the system. It is thought that both propionic and butyric acids were rapidly converted to acetic acid (hence, acetic acid prominence) which was subsequently converted to methane and CO₂ (Montero et al., 2008).

### Table 2. The physicochemical properties of abattoir waste

| Parameters            | Concentrations          | WHO standard |
|-----------------------|-------------------------|--------------|
| **Symbols (units)**   | **Parameters**          | **Pre-digestion concentration** | **Post-digestion concentration** |
| pH (NTU)              | pH                      | 6.4 ± 0.1    | 7.5 ± 0.1 |
| Tb (NTU)              | Turbidity               | 605 ± 4.6    | 578 ± 4.3 |
| Cond (µs/cm)          | Conductivity            | 17.3 ± 0.07  | 10.5 ± 0.07 |
| TSS (mg/l)            | Total suspended solid   | 595 ± 5.2    | 384 ± 2.2 |
| TS (mg/l)             | Total solid             | 754.20 ± 9   | 159.11    |
| Ash content (%)       |                         | 3.20         | 1.20      |
| Moisture content (%)  |                         | 82.90        | 89.90     |
| Crude fibre (%)       |                         | 0.29         | 0.11      |
| TDS                   | Total Dissolved Solid   | 760 ± 7      | 476 ± 3.6 |
| COD (mg/l)            | Chemical Oxygen Demand  | 1905.45 ± 89 | 289.27   |
| VS (mg/l)             | Volatile Solid          | 604.01 ± 2   | 97.87     |
| Total carbon (g/kg)   |                         | 53.78        | 54.89     |
| Total nitrogen (g/kg) |                         | 1.96         | 1.21      |
| C/N                   | Carbon to nitrogen ratio| 27.44        | 45.36     |
| Acetic Acid (mg/l)    |                         | 4.30 ± 0.02  | 1.05 ± 0.01 |
| Propionic acid (mg/l) |                         | 0.86 ± 0.01  | 0.003 ± 0.01 |
| Butyric acid (mg/l)   |                         | 0.25 ± 0.01  | 0.002 ± 0.01 |
| TVFAs (mg/l)          | Total volatile fatty acids | 5.90 ± 1.10 | 2.01 ± 1.00 |
| BOD (mg/l)            | Biological Oxygen Demand| 500 ± 6     | 130 ± 3   | 30.00 |
Continuous generation of biogas (methane and CO₂) leads to continuous consumption of acetate in the digester, hence a decrease in VFAs after digestion. The VAFs decreased from 5.90 mg/l at the start of the digestion to 2.01 mg/l post digestion. The values recorded in this study were lower than the reported values by Ajay (Ajay et al., 2012).

3.2. Biogas production
The first five days of the set-up produced no biogas. The anaerobic microorganisms were perhaps undergoing phase changes. Conversion of the substrate used into methane and carbon dioxide at this point passed through four synergistic processes, which include hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Biogas production commenced on the 6th day and increased steadily until the 15th day of the process, thereafter, a continuous decline in biogas amount was observed and remained until the end of the experiments (Figure 3). Several reasons are thought to be responsible for the delay in the generation of biogas until the sixth day. The delay could be linked to the presence of lignin in the undigested rumen content in the substrate which has been reported to slow down anaerobic digestion (Anozie et al., 2005). It could also be attributed to the period when the microorganisms acclimatize themselves to a new environment which is popularly referred to as a lag phase in a biological process. Also, the first stage of anaerobic digestion is the hydrolytic stage during which there is acid formation. During this period, biogas-producing microorganisms are unable to catabolize the organic acid produced by hydrolytic bacteria; hence, their growth is hindered (Zhang et al., 2013). The consequence is the accumulation of acid in the fermenting medium which makes the pH value to be slightly acidic. As there is an increase in the methanogenic bacteria, the ability of acid metabolism became strong and the pH gradually increased until the fermenting medium becomes alkaline when gas production commenced and continued to increase in the reactor until the action of biogas-producing organisms began to decline possibly because of the reduction in the available nutrients (death phase). This resulted in a progressive decrease in the volume of biogas produced.

The gas chromatographic analysis of biogas produced in this study revealed that the average gas composition was 66.8% methane. At a temperature range of 30–35°C, pH range of 6.4–7.5, and an incubation time of 30 days, the cumulative biogas volume of 0.0309 m³/kg VS was produced and remained constant after 26th day (Figure 4).

3.3. Optimization of Biogas yield
The results of the test of significance and the analysis of variance (ANOVA) for the second-order model for the regression coefficients are shown in Table 3. A large F-value of 13.60 and a low
corresponding p-value of 0.0002 obtained in this study was a confirmation that some of the model terms are significant and their effects on the biogas yield were very strong (p < 0.05). It also implies that in comparison to pure error, “lack of fit” is not significant. This signifies a good model and, thus suggesting that the model is fit to be used to theoretically produce biogas (Dahunsi et al., 2016). The correlation coefficient (R), determination of coefficient (R²), and adjusted R² for the RSM mathematical model were 0.9615, 0.9245, and 0.8566, respectively. The R² value, which is approximately 1.0, shows that there is a good correlation between the actual and predicted volumes of biogas by RSM and ANN.

For RSM, high R² value of 92.45% implies that the model can explain the variation observed in the model response, and it shows a good fit of the model in describing this process. Likewise, it also implies that the model regression equation was a true representation of the process and can be employed for the interpolation of the experimental domain. Also, at a 95% confidence interval, the terms in the model were tested using ANOVA. The values of “Prob > F” values which is less than 0.05 indicate terms in the model are significant. This implies that for this study, incubation time (X₁), pH (X₃) and the square of incubation time (X₁²) were significant terms in the model. Adequate precision (which measures the signal-to-noise ratio) of 13.048 recorded in this optimization study signifies high fitting and suitability of the model. This is an indication that the model can be used to navigate design space of the optimization of biogas produced since the value is greater than 4.
The regression model equation which described the interaction between the chosen independent factors (incubation time, temperature, and pH) and biogas yield (Y) in terms of actual values is described by Equation 3.

\[
Y = -0.711 + 0.00305X_1 + 0.00402X_2 + 0.174X_3 - 0.0000582X_1X_2 + 0.000356X_1X_3
- 0.000002X_2X_3 - 0.0000649X_1^2 - 0.0000392X_2^2 - 0.0126X_3^2.
\] (3)

Table 3. Test of significance and analysis of variance (ANOVA) for all regression coefficients

| Source | df | Sum of square | Mean square | F-value | P-value (Prob. >F) |
|--------|----|---------------|-------------|---------|-------------------|
| X_1    | 1  | 5.454E-005    | 5.454E-005  | 17.66   | 0.0018            |
| X_2    | 1  | 1.215E-008    | 1.215E-008  | 3.933E-003 | 0.9512           |
| X_3    | 1  | 1.453E-004    | 1.453E-004  | 47.04   | < 0.0001         |
| X_1X_2 | 1  | 4.234E-006    | 4.234E-006  | 1.37    | 0.2688           |
| X_1X_3 | 1  | 6.337E-006    | 6.337E-006  | 2.05    | 0.1826           |
| X_2X_3 | 1  | 5.000E-011    | 5.000E-011  | 1.619E-005 | 0.9969           |
| X_1^2  | 1  | 3.799E-005    | 3.799E-005  | 12.30   | 0.0057           |
| X_2^2  | 1  | 8.632E-007    | 8.632E-007  | 0.28    | 0.6086           |
| X_3^2  | 1  | 1.424E-004    | 1.424E-004  | 46.11   | < 0.0001         |
| Model  |    | 3.784E-004    | 9           | 4.205E-005 | 0.0002           |
| Residual| 5  | 3.089E-005    | 10          |         |                   |
| Lack of fit |    | 5             |             |         |                   |
| R      |    | 0.9615        |             |         |                   |
| R^2    |    | 0.9245        |             |         |                   |
| Adjusted R^2 |   | 0.8566        |             |         |                   |
| Adequate Precision | 13.048 |             |             |         |                   |

Further increase in these parameters resulted in a decrease in the biogas yield. Figure 5b showed the interaction between pH and incubation time. There is a strong synergy between pH and incubation time in a direct proportionality. At a pH of 7.5 and 30 days incubation time, the maximum biogas yield was 0.030 m^3/kg VS as shown in Figure 5c (i). Reduced values of the pH and incubation time resulted in reduced biogas yield (Figure 5b) (ii). As indicated in Figure 5c, the maximum biogas yield that was predicted at a pH of 7.0 and temperature of 35°C was 0.028 m^3/kg VS as shown in Figure 5c (i). This observation was also indicated on the contour plot shown in Figure 5c (ii). For ANN, the correlation coefficient (R) and the determination of coefficient (R^2) obtained for ANN were 0.99982 and 0.99964, respectively. This value of R (0.99982) shows that there is a strong agreement between the actual and predicted volumes of biogas by ANNs as also depicted in Figure 5b above which is evident in the overlapping of some of the actual and predicted values.
Table 4. Actual and predicted biogas yields

| Run | Independent Variables | Actual Yield (m$^3$/kg VS) | RSM predicted yield (m$^3$/kg VS) | ANN Predicted yield (m$^3$/kg VS) |
|-----|-----------------------|-----------------------------|-----------------------------------|----------------------------------|
|     | Retention Time, $X_1$ (day) | Temp, $X_2$ ($^\circ$C) | pH, $X_3$ |                            |                                |                                |
| 1   | 25                    | 32.5                        | 7   | 0.029                      | 0.028                          | 0.028                          |
| 2   | 30                    | 30                          | 6.5 | 0.023                      | 0.022                          | 0.023                          |
| 3   | 25                    | 32.5                        | 7   | 0.028                      | 0.028                          | 0.028                          |
| 4   | 20                    | 35                          | 6.5 | 0.022                      | 0.020                          | 0.022                          |
| 5   | 25                    | 32.5                        | 6.15| 0.012                      | 0.014                          | 0.011                          |
| 6   | 20                    | 35                          | 7.5 | 0.025                      | 0.024                          | 0.025                          |
| 7   | 30                    | 35                          | 6.5 | 0.023                      | 0.020                          | 0.023                          |
| 8   | 25                    | 32.5                        | 7   | 0.028                      | 0.028                          | 0.028                          |
| 9   | 25                    | 32.5                        | 7   | 0.029                      | 0.028                          | 0.028                          |
| 10  | 30                    | 35                          | 7.5 | 0.029                      | 0.029                          | 0.029                          |
| 11  | 20                    | 30                          | 7.5 | 0.022                      | 0.023                          | 0.022                          |
| 12  | 25                    | 32.5                        | 7   | 0.029                      | 0.028                          | 0.028                          |
| 13  | 33.4                  | 32.5                        | 7   | 0.026                      | 0.027                          | 0.026                          |
| 14  | 25                    | 32.5                        | 7   | 0.028                      | 0.028                          | 0.028                          |
| 15  | 25                    | 36.7                        | 7   | 0.025                      | 0.028                          | 0.025                          |
| 16  | 25                    | 32.5                        | 7.84| 0.026                      | 0.025                          | 0.026                          |
| 17  | 16.60                 | 32.5                        | 7   | 0.019                      | 0.020                          | 0.020                          |
| 18  | 30                    | 30                          | 7.5 | 0.030                      | 0.030                          | 0.030                          |
| 19  | 25                    | 28.30                       | 7   | 0.028                      | 0.028                          | 0.028                          |
| 20  | 20                    | 30                          | 6.5 | 0.019                      | 0.018                          | 0.019                          |

Also, $R^2$ value of 0.99964 shows a good fit of the model since the value is approximately 1. Generally, for a good correlation between experimental and predicted values, the value of the correlation coefficient should be close to unity and this has been shown in these models. The models have high values of $R$ and $R^2$ which are close to unity, hence a good fit for the model. The optimal conditions as predicted by ANNs were as follows: $X_1 = 26.76$ days, $X_2 = 30.94^\circ$C and $X_3 = 7.27$, while the predicted biogas yield was 0.0322 m$^3$/kg VS.

3.4. Performance evaluation of the predictive capability of the models

RSM and ANN's predictive abilities were critically evaluated to determine the comparative efficacy and accuracy of the two models. The mean square error (MSE), which measures the proximity of a fitted line to the data points, the root mean square error (RMSE), the correlation coefficient ($R$), the square of correlation coefficient ($R^2$), and the volume of biogas predicted by the two models were employed to compare the RSM and ANN. The $R$ and $R^2$ values for RSM (0.9615 and 0.9245 respectively) were lower than the values of $R$ and $R^2$ for ANN (0.99982 and 0.99964 respectively). It is thus obvious that $R$ and $R^2$ values for ANN are closer to unity than corresponding values for RSM. This indicates better performance of ANN. From the calculation of MSE, it is noticed that MSE for the process for RSM (0.00437) is higher than that of ANN (7.4167 $\times 10^{-5}$), while RMSE for RSM (0.066) is equally higher than that of ANN (8.6 $\times 10^{-5}$). The lower these statistical indicators are, the better the performance of the model, hence ANNs show better performance. The most desirable RSM predicted biogas value is 0.030 m$^3$/kg VS, while that of ANN is 0.0322 m$^3$/kg VS. Apart from this, the most probable disadvantage of the RSM is that the experimental data are fitted to a polynomial model at the second level. Not all systems with curvature are compatible with a second-order polynomial model. On the other hand, ANN is unrestricted in terms of the order of the model and thus more dynamic in simulating the true behavior of non-linear dataset (Mohd et al., 2013). Based on these indicators, ANN was found to be more accurate.
Figure 5. a (i) Surface plot showing the effect of temperature and time on biogas production. (ii): 2-D contour plot of temperature and incubation time on biogas yield. b (i): Surface plot showing effect of pH and Incubation time on biogas production. b (ii): 2-D contour plot of pH and incubation time on biogas yield c (i): Surface plot showing effect of pH and temperature on biogas yield. c (ii): 2-D contour plots of pH and temperature on biogas yield

(i): Surface plot showing effect of temperature and time on biogas production

(ii): 2-D contour plot of temperature and incubation time on biogas yield
(i): Surface plot showing effect of pH and Incubation time on biogas production

(ii): 2-D contour plot of pH and incubation time on biogas yield
(i): Surface plot showing effect of pH and temperature on biogas yield

(ii): 2-D contour plots of pH and temperature on biogas yield
Table 5. Model predicted and validation results

| Conditions                      | Model  | Actual (Validation) |
|--------------------------------|--------|---------------------|
|                                | RSM    | ANN                | RSM    | ANN    |
| Incubation time (day)          | 28.98  | 26.76              | 28.68  | 27.71  |
| Temperature (°C)               | 30.16  | 30.94              | 31.22  | 30.84  |
| pH                             | 7.43   | 7.27               | 7.61   | 7.34   |
| Biogas yield (m³/kg VS)        | 0.0300 | 0.0322             | 0.0314 | 0.0316 |

and efficient than the RSM for the optimization of biogas generation from abattoir wastes. Previous researches have also documented similar observations (Dahunsi et al., 2017; Mohd et al., 2013). Using the same digester, verification of the model predictions was done by validating the predicted optimal conditions through three independent experiments following the same method described above. The mean results from the validation as well as RSM and ANN's predictions were presented in Table 5. The validation results show a higher yield (0.0314 m³/kg VS of biogas) from the digester with the parameters obtained from RSM. Also, the study revealed that the maximum cumulative biogas yield with the incubation time of 26 days from the experimental setup is 0.0309 m³/kg VS, the models (RSM and ANN) predicted maximum biogas yields of 0.030 m³/kg VS and 0.032 m³/kg VS with incubation time of 28 and 26 days, respectively, while the validation experiment gave maximum biogas yield of 0.0314 and 0.0306 m³/kg VS in 28 and 27 days solid retention time, respectively. The values are very close and thus, the digester could be said to be efficient.

4. Conclusion
This study showed that statistical techniques like RSM and ANN are good tools to optimize biogas production from abattoir waste. The incubation time and pH of the anaerobic digestion process were the most significant variables affecting biogas production using RSM. Biogas yield from the abattoir waste was 72.70% influenced by the incubation time and 26.70% influenced by the pH of the digestion medium. High quantity (66.8%) of methane gas was obtained from the biogas produced from abattoir wastes digestion. This biofuel could be harnessed as a renewable energy source for a developing country like Nigeria. The two optimization methodologies explored in this study predicted a close value for biogas production. ANN seemed better in terms of prediction accuracy than RSM using applicable parameters such as RMSE and R².

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