

## Research Article

# Investigation and Optimization of Operational Conditions of Anaerobic Digestion Process for Enhanced Biogas Production Yield in a CSTR Using RSM

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In this study, pilot-scale experiments were performed on the anaerobic digestion (AD) in a semicontinuous process using a continuously stirred tank reactor to determine the effects of organic loading rate (OLR), temperature, and mixing levels on biogas production yield. Fresh cow manure was considered the input feedstock of this study due to its high production volume. Response surface methodology (RSM) was employed to design and analyze experiments to optimize OLR, temperature, and mixing intensity in biogas production. The central composite design was applied as a RSM optimization tool. Four cubic mathematical models were derived to predict the responses. The optimization study was carried out to identify the highest yields achievable when temperature and mixing factors are minimized. Temperature and OLR have been found to have a greater influence on biogas production compared to mixing intensity. The AD process is significantly influenced by temperature. Variations in temperature, specifically near 40 and 55°C, lead to an increase in the rate of biogas production. Therefore, the best temperatures for biogas production rate are near 40 and 55°C. The results of the performed optimizations suggest to adopt OLR values range of 1.6–2.5 kgVS<sub>Cow manure</sub>/m<sup>3</sup> · day, and 2.5–3.4 kgVS<sub>Cow manure</sub>/m<sup>3</sup> · day in the case of temperature ranges 20–40°C and 40–55°C, respectively.

## 1. Introduction

Anaerobic digestion (AD) is a biological process that converts decomposable materials into biogas in the absence of oxygen. Biogas is a high-calorific fuel that can be utilized for generating electricity and heat [1]. Biogas usually contains 50%–80% methane, 20%–40% carbon dioxide, and small amounts of nitrogen, hydrogen, and hydrogen sulfide [2].

The AD process consists of a series of complex microbial metabolisms to convert organic compounds into biogas, divided into four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These four stages exhibit different biochemical reactions with different substrates and microorganisms [2, 3, 4, 5]. The active microorganisms present in these phases include hydrolytic, acidogenic, acetogenic,

and methanogenic species. These microorganisms facilitate the breakdown of intricate organic substances into simpler compounds, culminating in the stabilization of organic residues and the generation of biogas [2, 6].

AD performance mainly depends on feed characteristics, feeding parameters (organic loading rate (OLR)), pH, temperature, oxidation–reduction potential, hydraulic retention time, solid retention time, and agitation characteristics [7]. According to previous research, AD performance is affected by essential parameters, such as temperature [8, 9, 10], the OLR [11, 12, 13, 14], and mixing regime [15, 16, 17].

In AD, the OLR should be paid much attention. Overloading organic matter stops digestion. Organic overloading causes acidification during AD, which is one of the main causes of process failures and reduced methane yield in anaerobic

digesters [18]. Typically, the loading rate of organic matter for complete mixing in AD digesters is between 1 and 5 kgCOD/m<sup>3</sup> · day [19, 20]. The maximum biomass concentration achievable within a continuously stirred tank reactor (CSTR) digester is determined by the critical requirement for efficient and thorough mixing to facilitate optimal biological processes [5].

Efficient and effective agitation significantly influences the performance of AD [21, 22]. The primary goals of mixing in AD reactors are uniform distribution of total solids and maximum direct contact between active biomass and raw sludge (nonstabilized sludge can be taken from wastewater treatment plants). Mixing serves the additional function of mitigating foam formation and temperature gradients within the reactor. Inadequate mixing can result in a buildup of nutrients and pollutants locally, as well as high or low local temperatures. This does not prevent the buildup of toxins in the sites above dangerous levels or promote balanced function throughout the digestive system. However, too much mixing breaks up the microbes [21, 22]. Approximately, 20% of the overall energy input into the reactors is attributed to the energy consumption associated with the mixing process. Industrial designers and operators in the present day are actively motivated to moderate the level of agitation intensity to lower expenses and mitigate environmental impacts, all while ensuring that biogas production remains unaffected.

The temperature within the reactor significantly influences the biogas production process. AD can be performed in psychrophilic (less than 30°C), mesophilic (30–40°C), thermophilic (50–60°C), and extreme thermophilic (55–82°C) temperature ranges [23].

AD process includes complex chain reactions requiring several assumptions to solve the governing physical, chemical, and biological equations. Therefore, reliable models can predict the factors considered in the mass balance but cannot estimate the related responses [24]. Response surface methodology (RSM) is a set of statistical and mathematical methods for modeling and analyzing problems in which the desired response is affected by various variables. The goal is to optimize the response [25]. The RSM technique has a significant application in process design, optimization, and improvement of existing designs. This method is more practical than other methods because it originates from a practical method that includes the interactions of variables and will finally show the overall effect of factors on the process [26]. Central composite design (CCD) is widely used in creating quadratic surface response models. The CCD method is one of the essential experimental designs used in process optimization studies [25, 27, 28, 29, 30].

In the study of Ghaleb et al. [31], the CCD-RSM has been used to optimize methane production from AD of oily-biological sludge and sugarcane bagasse. Under optimal conditions, results have shown the highest predicted methane yield of 63.52 ml<sub>methane</sub>/gVS<sub>removed</sub>. In another CCD-RSM study on AD of poultry manure [32], it was found that the process parameters considerably affect the production of biogas and chemical oxygen demand degradation. According to Yilmaz and Şahan [33], the CCD-RSM has a positive

effect on prediction of the biogas production from poultry manure. The findings of this study indicate a maximum cumulative biogas production of 8,965.87 ml. Furthermore, predictive analyses revealed that the biogas produced comprises 71.298% methane. Finally, according to the studies, it can be concluded that the RSM is a successful method for predicting optimal conditions of the AD process to gain maximum biogas production rate [34, 35, 36, 37].

Experiments utilizing RSM will determine the optimal AD conditions for achieving efficient biogas production. This approach aims to identify the ideal operational parameters with high efficacy while minimizing the number of experimental trials required, thus eliminating the need to explore all potential combinations exhaustively. In addition, the input levels for a specific response surface of various variables can also be determined. The polynomial function must contain quadratic terms based on Equation (1) and third-order terms based on Equation (2) to determine the critical point (minimum, maximum, or buffer):

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{1 < i < j}^n \beta_{ii} x_i^2 + \sum_{1 < i < j}^n \beta_{ij} x_i x_j + \varepsilon, \quad (1)$$

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{1 < i < j}^n \beta_{ij} x_i x_j + \sum_{i=1}^n \beta_{iii} x_i^3 + \sum_{1 < i < j}^n \beta_{ijj} x_i^2 x_j + \sum_{1 < i < j}^n \beta_{jjj} x_i x_j^2 + \sum_{1 < i < j < k}^n \beta_{ijk} x_i x_j x_k + \varepsilon, \quad (2)$$

where  $Y$  is the predicted value of the response variable,  $n$  is the number of variables,  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ ,  $\beta_{ij}$ ,  $\beta_{iii}$ ,  $\beta_{ijj}$ , and  $\beta_{ijk}$  are the parameters of the model,  $x_i$ ,  $x_j$ ,  $x_k$  are variables, and  $\varepsilon$  is the remaining of the experiments.

It is necessary to use a suitable method to investigate functional conditions and their interactions to determine the factors that can be used in practice to control anaerobic digesters. This study aims to determine the optimal conditions for maximizing the efficiency of biogas production of cow manure. Therefore, three functional parameters of temperature, loading rate of organic matter, and mixing regime were investigated on biogas production and efficiency using the RSM. The innovation of this study is the use of statistical modeling to predict biogas production and the use of RSM to analyze the responses. It should be noted that the previous articles traditionally performed statistical analysis.

## 2. Materials and Methods

**2.1. Reactor Start-Up Procedure.** This CSTR has a diameter, height, and volume of 180 mm, 300 mm, and 7.6 liters, respectively. An electric hot water jacket with a thickness of 20 mm covers the reactor and maintains the temperature within the optimal range. Two steel slurry-type blades agitate the mixture by an electric motor (Figure 1).

In the start-up phase, enriched inoculum is added to the digester. As the inoculum, the decomposed cow manure from one of the reactors in the renewable energy laboratory



FIGURE 1: The CSTR pilot-measure AD system.

TABLE 1: Independent variables and their levels in CCD.

Factor	Units	−1.68	−1	0	+1	+1.68
A: OLR	kg/m <sup>3</sup> · day	1	1.61	2.5	3.39	4
B: temperature	°C	15	25.13	40	54.87	65
C: mixing	rpm	0	20	50	80	100

of the Biosystem Engineering Department was utilized [23]. The cow manure was screened to remove coarse materials such as straws and other large fibrous materials, diluted with water to obtain the desired solid concentration, and mixed thoroughly. Five liters of substrate containing 50% water and 50% (v/v) fresh cow manure were poured into the reactor. It remained in the experimental operating conditions for 15 days to start the reactor. After 15 days, continuous feeding of the reactor started. The feeding rate was semicontinuous, so the hydraulic retention time reached 15 days. The reactor was fed for 210 days to perform the tests according to the design of the experiments. Cow manure was diluted with water (50% water and 50% (v/v) fresh cow manure) and poured into the feed tank to change the organic load entering the reactor [38].

**2.2. Analytical Methods.** Based on standard methodology [39], total solids, volatile solids, total Kjeldahl nitrogen, and total organic carbon were 18.3%, 72.9%, 1.6%, and 26.8%, respectively. The Metrohm 620 pH meter (Metrohm Inc., Germany) indicated a pH value of 7.2. Also, the water displacement method was applied to measure the produced biogas.

**2.3. Design of Experiments Method.** Temperature, loading rate of organic matter, and mixing regime were considered independent variables, and biogas production rate and efficiency were dependent variables (process response). The experiments used the CCD-RSM. The three independent variables and their levels used in CCD are shown in Table 1. The 17 tests, according to the  $N = K^2 + 2K + c_p$ , are shown in Table 2. The values depend on the number of variables and can be calculated by Equation (3) [40]. Also,  $\alpha$  for 2, 3, and 4 equals 1.41, 1.68, and 2.00. All factors were studied in five levels:

$$\alpha = 2^{k/4}, \quad (3)$$

where  $K$  is the number of factors,  $c_p$  is the number of repetitions of the central point, and  $\alpha$  is the axial design point.

In Table 2, the actual and coded values of the independent variables at five levels and the response of each test are given. In general, 17 experiments were conducted, of which eight were factorial design, six were axial, and three were repeated experiments of the central point to correctly estimate the error of the experiments. The placement of various design points is shown in Figure 2. Experiments were designed using Design Expert software (version 10.0.0—produced by State-Ease Co.) with three variables and three levels.

### 3. Results and Discussion

**3.1. CCD Design Results and Regression Model Fitting.** As mentioned, 17 experiments were designed using the CCD-RSM method. The RMS design matrix for two actual and coded values of the input variables and their responses are presented in Table 2. The responses obtained were entered into the Design Expert software, and based on them, quadratic equations were obtained that predict the response of the variables well (Equations (1) and (2)). The results of ANOVA for response surface models are summarized in Table 3. The values of the correlation coefficient ( $R^2$ ) for the quadratic equations are high, which indicates that these relationships describe the process well within the desired range of changes. The insignificance of the difference between the values of  $R^2$  and the adjusted correlation coefficient (adjusted  $R^2$ ) shows that the expressions in the equations are practical. The goodness of equations is measured through  $F$ -tests of lack of fit [25].

The lack of static  $F$ -fit is not statistically significant because the  $p$ -values are smaller than 0.05. Appropriate precision is a measure of the range of the predicted response relative to the corresponding error, or in other words, the signal-to-noise ratio. The desired value of appropriate precision equals four or higher [41]. As shown in Table 3, the appropriate accuracy values for the equations obtained are acceptable. The small values of the coefficient of variation for the answers show that the tests are accurate and reliable. The actual value of the biogas production rate and its estimated value are shown in Figure 3. The actual values are obtained from the laboratory data and the estimated values are obtained from Equations (1) and (2). The closeness of the points to the 45° line indicates the good accuracy of the models for estimating the answers.

**3.2. The Effect of Temperature on the Responses.** The effect of temperature on the responses is shown in Figure 4. The impact of temperature and OLR on the biogas production rate and efficiency are demonstrated. Increasing the temperature from 25 to 40°C or decreasing it from 65 to 55°C has a positive effect on the biogas production rate and its efficiency. Different microorganisms thrive at different temperatures. Generally, warmer temperatures accelerate microbial activity, leading to faster digestion rates and higher gas production. However, extreme temperatures can also inhibit microbial activity. So, it is essential to maintain optimal conditions for efficient AD. Therefore, the best temperatures for biogas production rate are near 40 and 55°C. The average volumetric rate of biogas production was 0.44 m<sup>3</sup>/m<sup>3</sup> · day for the time when the reactor operates at 25°C, and the feed

TABLE 2: RSM design of experiments and obtained results.

Study	Space type	A (OLR) Coded (actual)	B (temperature) Coded (actual)	C (mixing) Coded (actual)	Biogas production rate (m <sup>3</sup> /m <sup>3</sup> · day)	Biogas yield (m <sup>3</sup> /kgVS added)
1	Factorial	−1 (1.61)	−1 (25.13)	−1 (~20)	0.17	0.15
2	Factorial	+1 (3.39)	−1 (25.13)	−1 (~20)	0.28	0.107
3	Factorial	−1 (1.61)	+1 (54.87)	−1 (~20)	0.32	0.231
4	Factorial	+1 (3.39)	+1 (54.87)	−1 (~20)	0.63	0.177
5	Factorial	−1 (1.61)	−1 (25.13)	+1 (~80)	0.15	0.13
6	Factorial	+1 (3.39)	−1 (25.13)	+1 (~80)	0.44	0.124
7	Factorial	−1 (1.61)	+1 (54.87)	+1 (~80)	0.2	0.196
8	Factorial	+1 (3.39)	+1 (54.87)	+1 (~80)	0.61	0.173
9	Axial	−1.68 (1.0)	0 (40.00)	0 (50)	0.1	0.144
10	Axial	+1.68 (4.0)	0 (40.00)	0 (50)	0.67	0.103
11	Axial	0 (2.5)	−1.68 (15.00)	0 (50)	0.11	0.109
12	Axial	0 (2.5)	+1.68 (65.00)	0 (50)	0.15	0.11
13	Axial	0 (2.5)	0 (40.00)	−1.68 (0)	0.41	0.16
14	Axial	0 (2.5)	0 (40.00)	+1.68 (100)	0.31	0.138
15	Central	0 (2.5)	0 (40.00)	0 (50)	0.49	0.199
16	Central	0 (2.5)	0 (40.00)	0 (50)	0.48	0.195
17	Central	0 (2.5)	0 (40.00)	0 (50)	0.50	0.198

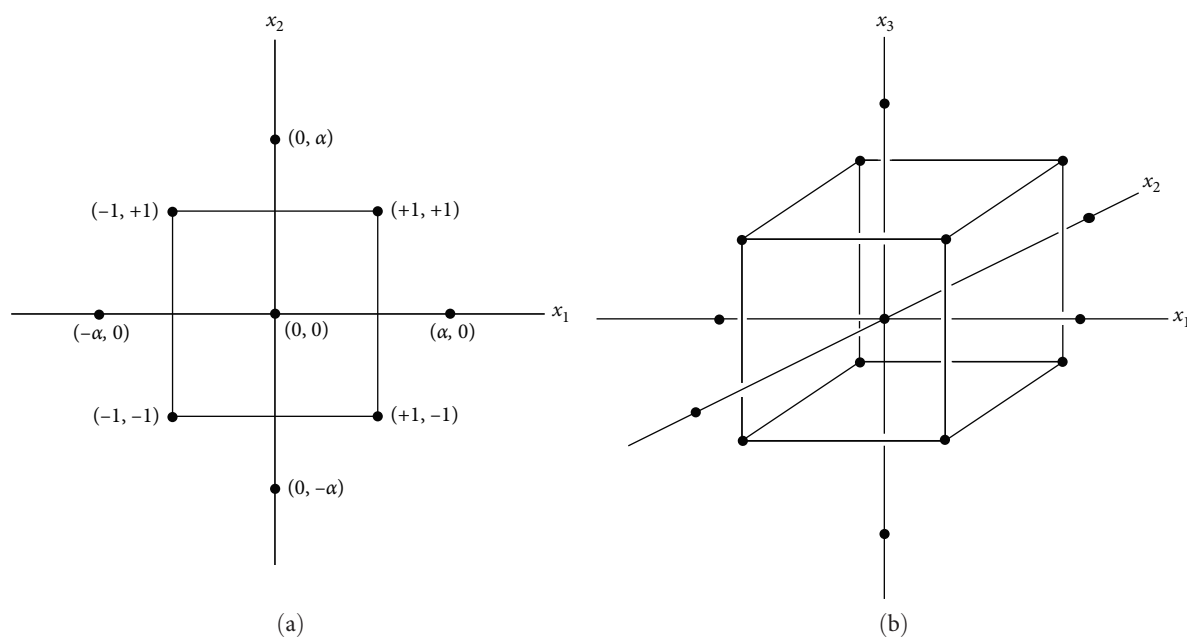


FIGURE 2: Types of design points in the CCD method: (a) for two variables ( $\alpha = 1.41$ ) and (b) for three variables ( $\alpha = 1.68$ ). Points  $(-1, +1)$ ,  $(+1, +1)$ ,  $(+1, -1)$ , and  $(-1, -1)$  factorial design points; points  $(-\alpha, 0)$ ,  $(0, \alpha)$ ,  $(\alpha, 0)$ , and  $(0, -\alpha)$  are the axial design points; and  $(0, 0)$  is the central point.

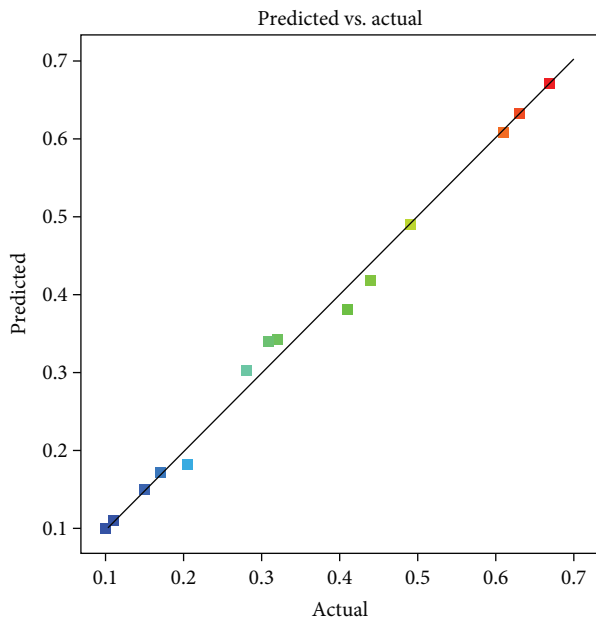
rate was 3.4 kgVS<sub>Cow manure</sub>/m<sup>3</sup> · day. While at 55°C, the production rate of biogas reached 0.63 m<sup>3</sup>/m<sup>3</sup> · day. However, as the reactor temperature increased to 65°C, the biogas production rate suddenly decreased. According to the presented results, it is pretty clear that AD is a process that strongly depends on temperature. The results of the present study are consistent with those presented by other researchers [8]. Similar results by other researchers show that AD can be performed in the temperature range of psychrophilic (less

than 30°C), mesophilic (30–40°C), thermophilic (50–60°C), and extreme thermophilic (above 60°C) [23, 38]. Although the rate of methane production from the AD of cow manure decreases at psychrophilic temperature compared to mesophilic and thermophilic temperatures [42, 43, 44]. In the temperature range between 40 and 50°C (temperature transition from mesophilic to thermophilic), the activity of methanogenic bacteria becomes problematic [45]. Due to the low activity of microorganisms and the limited activity of osteoclastic methanogenic



TABLE 3: Statistical results of the ANOVA for response surface models.

Statistical result	Estimated biogas production rate ( $Y_{1p}$ )	Biogas production efficiency ( $Y_{2p}$ )
Model $F$ -value	23.93	293.15
Model $p$ -value	<0.0408	<0.0457
Lack of fit $F$ -value	6.55	11.71
Lack of fit $p$ -value	0.1003	0.0255
$R$ -squared	0.9931	0.9997
Adj $R$ -squared	0.9516	0.9963
Std. dev.	0.043	0.0023
CV (%)	12.85	1.57
Adeq precision	14.180	56.629


FIGURE 3: Predicted vs. actual plot for biogas production rate based on  $\text{m}^3/\text{m}^3 \cdot \text{day}$ .

bacteria in psychrophilic conditions, suspended solids accumulate in the digester [46].

The estimated values of the biogas production rate ( $Y_{1p}$ ) and biogas production efficiency ( $Y_{2p}$ ) are obtained from the following equations, respectively:

$$Y_{1p} = 0.49 + 0.17A + 0.012B - 0.012C + 0.04AB - 0.037A^2 - 0.13B^2 - 0.046C^2 + 0.078A^2B - 0.029AB^2 + 0.07A^2B^2, \quad (4)$$

$$Y_{2p} = 0.2 - 0.012A + 0.003B - 0.012C - 0.004AB + 0.009AC - 0.027A^2 - 0.032B^2 - 0.018C^2 + 0.033A^2B - 0.004AB^2 + 0.038A^2B^2, \quad (5)$$

where  $A$  is the loading rate of organic substances ( $\text{kg}/\text{m}^3 \cdot \text{day}$ ),  $B$  is the temperature ( $^{\circ}\text{C}$ ), and  $C$  is the stirring regime (rpm).  $A$ ,

$B$ , and  $C$  are the coded values of the independent variables in the model.

**3.3. The Effect of Organic Substances Loading Rate on the Responses.** According to the experimental design, the loading rate of organic matter was studied at five levels (1, 1.6, 2.5, 3.4, and  $4 \text{ kgVS}_{\text{Cow manure}}/\text{m}^3 \cdot \text{day}$ ). The results showed that with the increase of OLR to above  $2.5 \text{ kgVS}/\text{m}^3 \cdot \text{day}$ , the performance of the reactor improved in the daily production of biogas (Figure 5). Although high OLR and high content of volatile solids in the input feed made the reactor more sensitive, fluctuating behavior and late stabilization. Previous studies have reported such behavior [47, 48, 49].

The results showed that organic matter's loading rate directly affects the AD process's performance. The low loading rate (less than  $1.16 \text{ kgVS}/\text{m}^3 \cdot \text{day}$ ) causes microorganisms to lack nutrients. On the other hand, excessive loading (more than  $4 \text{ kgVS}/\text{m}^3 \cdot \text{day}$ ) due to the absorption of the leading products and the transfer of toxic substances by the solid phase, it disrupts the activity of acid producers. Therefore, despite the increase in biogas production at the beginning of the work, after a while, due to the increase in the concentration of volatile fatty acids, the production of biogas decreases [23, 50, 51, 52, 53]. Due to the high loading rate, volatile fatty acids, which are essential compounds in the metabolic pathway of methane production, and their concentration shows the stability of the digester, accumulate in the digester [52, 54], and cause a decrease in pH, and applying stress effects on all microorganisms, especially methanogenic bacteria [55, 56]. If the acid concentration increases, this problem can be solved by reinoculation, temporarily stopping feeding, stirring, reducing the concentration of feed, adding lime, periodically returning the digestive fluid, periodically removing the digestive fluid, and replacing it with water [23, 53, 55].

Based on the findings derived from the AD of cow manure in the current study, it is recommended that the OLR be maintained within the range of  $1.6\text{--}2.5 \text{ kgVS}_{\text{Cow manure}}/\text{m}^3 \cdot \text{day}$  for temperatures between  $20\text{--}40^{\circ}\text{C}$  and  $2.5\text{--}3.4 \text{ kgVS}_{\text{Cow manure}}/\text{m}^3 \cdot \text{day}$  for temperatures between  $40$  and  $55^{\circ}\text{C}$ . In the references, the loading rate under mesophilic conditions ranges from  $0.4$  to  $6.4 \text{ kgVS}/\text{m}^3 \cdot \text{day}$  (with an optimal value of  $0.8\text{--}2 \text{ kgVS}/\text{m}^3 \cdot \text{day}$ ), while under thermophilic conditions, it ranges from  $1$  to  $7.5 \text{ kgVS}/\text{m}^3 \cdot \text{day}$  (with an optimal value of  $1.5\text{--}5 \text{ kgVS}/\text{m}^3 \cdot \text{day}$ ) as documented

Factor coding: actual

Biogas production rate (m<sup>3</sup>/m<sup>3</sup>·day)  
● Design points

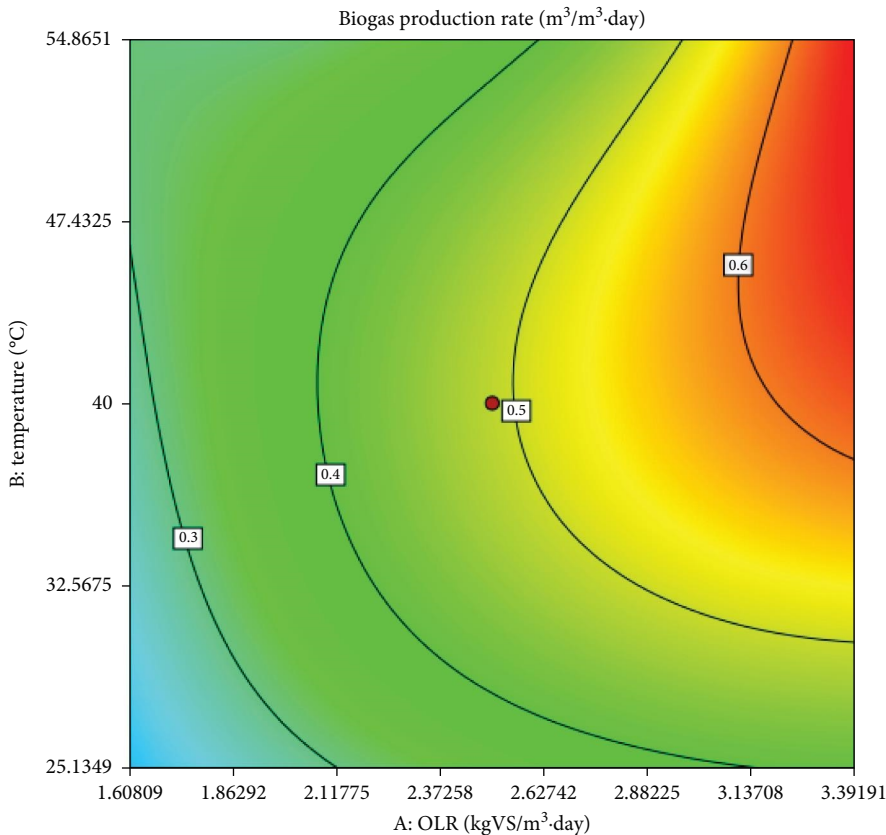
0.1 0.67

X1 = A

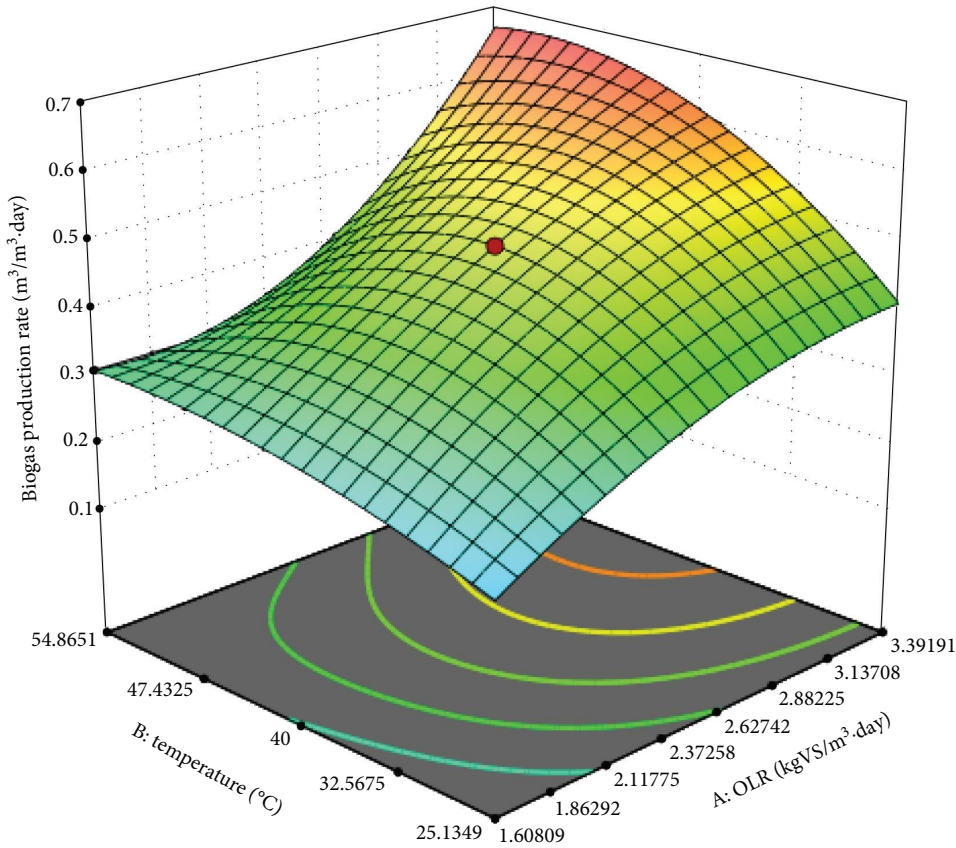
X2 = B

Actual factor

C = 50



(a)



(b)

FIGURE 4: Contour plot (a) and response surface plot (b) showing the effect of temperature and OLR on biogas production rate at mixing = 50 rpm.

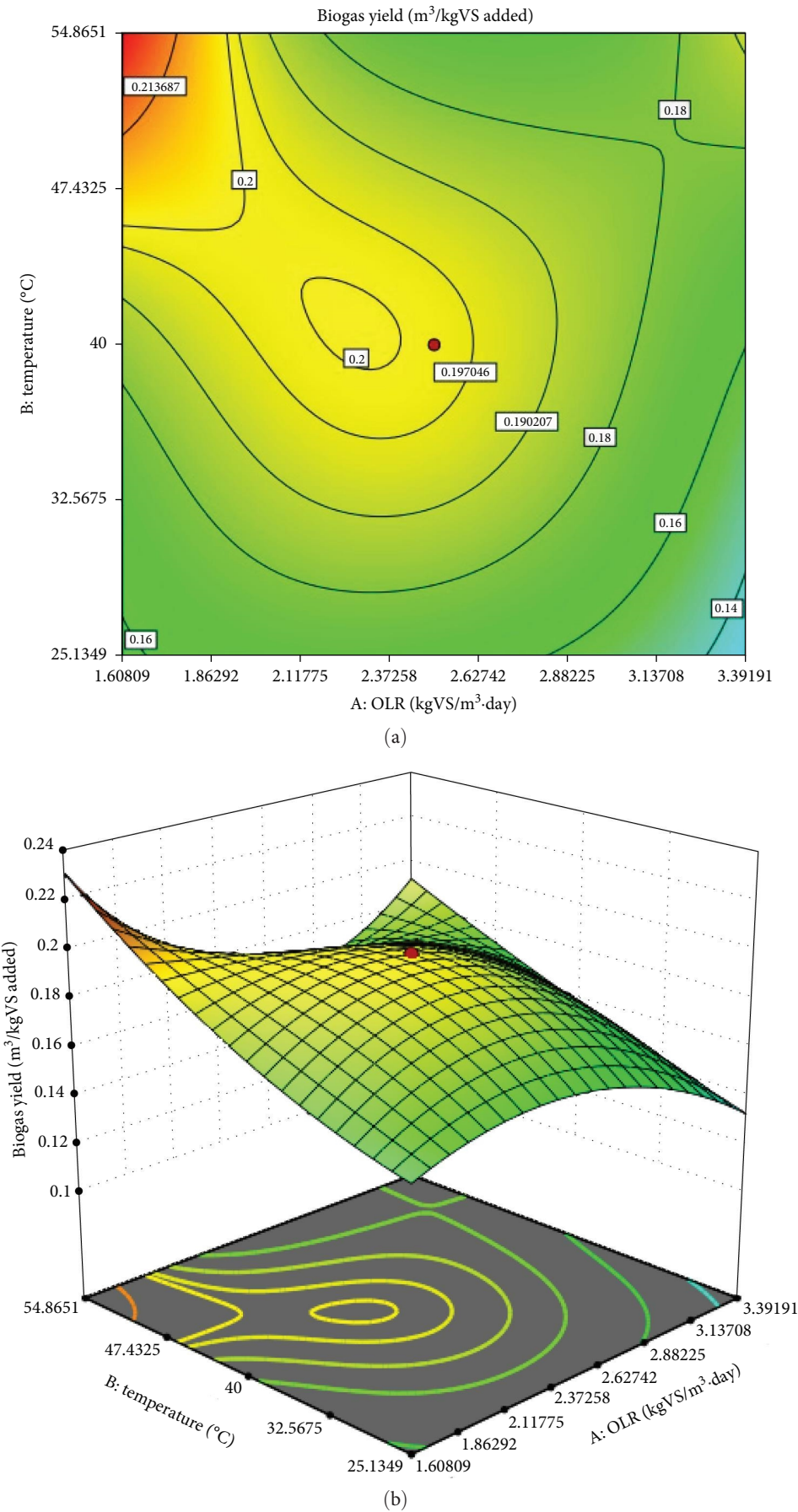


FIGURE 5: Contour plots (a) and response surface plot (b) showing the effect of temperature and OLR on biogas yield at mixing = 50 rpm.

**3.4. The Effect of the Stirring Regime on the Answers.** The current study examined the impact of five agitation protocols (20, 50, and 80 rpm for 10 min every 2 hr, continuous stirring at 100 rpm, and no agitation) on the production of biogas. Intermittent stirring with low intensity significantly enhanced biogas production and efficiency in comparison to continuous stirring and no stirring, as indicated by the results. On the other hand, the results showed that different degrees of stirring (20, 50, and 80 rpm) in intermittent stirring did not significantly affect biogas production and reactor performance, and the amount of biogas produced was the same in the three mentioned conditions. Previous research has reported similar results [15, 55].

Intermittent stirring at low intensity serves to homogenize the temperature within the reactor, facilitate contact between biomass and feed, and mitigate the inhibitory impact of by-products and metabolites, as well as inhibitory compounds in the feed on microbial activity. This practice helps to prevent the accumulation of suspended materials leading to scum formation on the surface and the deposition of nondegradable heavy solids at the reactor bottom. Additionally, it promotes the uniform distribution of microorganisms, reduces particle size, aids in the removal of biogas generated from the mixture [57, 58], and facilitates the proliferation of microbial populations within the reactor. If stirring is stopped an hour or two before feeding, heavier solids will settle to the bottom of the reactor, and the microbial population will increase there. In fact, in this case, the retention time of solids is longer than the hydraulic retention time ( $SRT > HRT$ ) [15].

## 4. Conclusions

The best model for biogas production and its efficiency was the quadratic model. The results of the analysis of variance for the response surface models showed that the correlation coefficient ( $r^2$ ) values for the quadratic relationships are high, which indicates that these relationships describe the process well within the desired range of changes. According to the presented results, it is pretty clear that AD is a process that strongly depends on temperature. Increasing the temperature from 25 to 40°C or decreasing it from 65 to 55°C has a positive effect on the biogas production rate and its efficiency. Therefore, the best temperatures for biogas production rate are near 40 and 55°C. According to the results obtained from the AD of cow manure in the present research, the OLR for the temperature range of 20–40°C is between 1.6 and 2.5  $\text{kgVS}_{\text{Cow manure}}/\text{m}^3 \cdot \text{day}$  and for the range of a temperature of 40–55°C, between 2.5 and 3.4  $\text{kgVS}_{\text{Cow manure}}/\text{m}^3 \cdot \text{day}$  is suggested. Stirring with minimal intensity and intermittently improved the interactions of nutrient interaction in the AD process compared to continuous stirring and nonstirring conditions.

## Data Availability

The data supporting this study's findings are available upon reasonable request from the corresponding author.

## Disclosure

During the preparation of this work, the authors used QuillBot software for paraphrasing, also Grammarly software, and the Isaaceditor.com website for language improvement of the entire text of the article. After using these tools, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

## Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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