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Assessment of Biomethanation from Cattle Manure Through Continuous Stirred Tank Reactor (CSTR)

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ABSTRACT

This study explores biogas production through anaerobic digestion of cattle manure (CM) in a Continuous Stirred Tank Reactor (CSTR), using Response Surface Methodology (RSM) to optimize conditions for enhanced methane yield. Cattle manure, a primary substrate in biogas generation, holds untapped methane potential due to its high fiber content, which is only partially degraded in typical single-phase CSTR systems. Experiments were conducted under controlled mesophilic conditions to compare biogas outputs with and without stirring. Key variables, including Volatile Solids (VS), volatile fatty acids (VFA), total alkalinity (TA), and pH, were monitored and optimized using a Box-Behnken design. Results showed that stirring significantly increased methane yield to 0.4713 $m^3.kg^{-1}$ VS, attributed to uniform microbial activity and enhanced degradation of organic matter. The findings also show that intermittent stirring improves methane yield by 34.36%, achieving a peak value, and offer practical insights into energy-efficient alternatives to continuous mixing, with direct implications for scaling up industrial biogas systems. Statistical analysis via ANOVA confirmed the regression model's reliability, identifying significant factors influencing biogas production. This study's findings underscore the efficiency of serial CSTR configurations and optimized operating conditions for sustainable biogas production.

INTRODUCTION

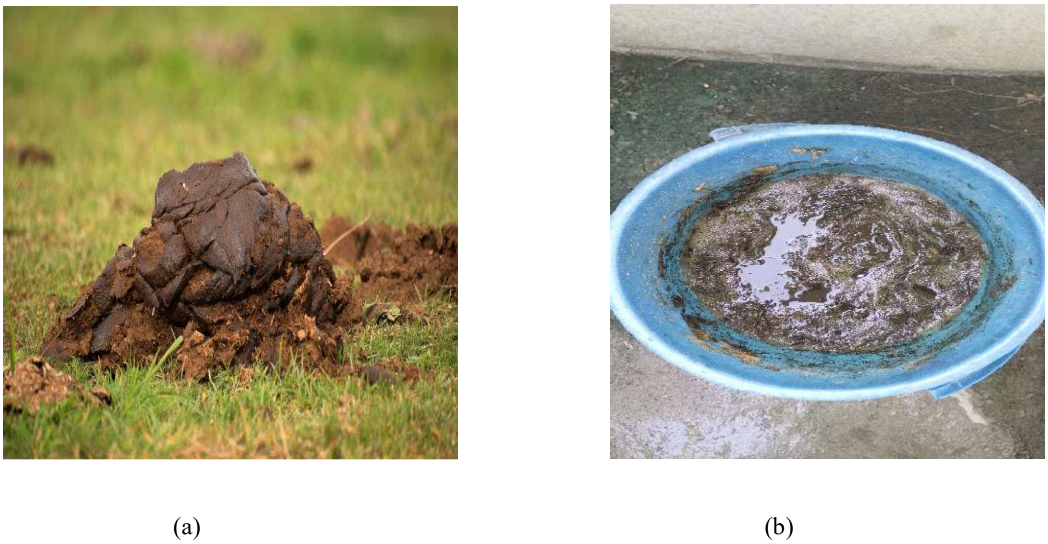
Anaerobic digestion is a natural biological process in which bacteria break down organic matter in the absence of oxygen (Cavinato et al. 2010). This process generates biogas, primarily composed of carbon dioxide and methane. These gases can be recovered and used as a renewable energy resource (Khayum et al. 2018). In anaerobic digestion, it is feasible to divert food waste from pathways that are substantial contributors to greenhouse gas emissions and landfill waste (Chanakya et al. 2009). The Continuous Stirred Tank Reactor (CSTR) is a vital component of the anaerobic digestion process. It consists of a closed tank or reactor vessel with continuously agitated and mixed contents (Li et al. 2020). Within this reactor, microorganisms responsible for anaerobic digestion are introduced and maintained under optimal conditions, allowing for efficient biogas production (Park et al. 2010). Centralized biogas facilities utilize cattle manure as one of their primary substrates (Mittal et al. 2019). A significant percentage of fibers in cattle dung is around 40–50% of the total solids (TS) (Tasnim et al. 2017). Despite the potential methane yield of 0.40–0.45 $m^3.kg^{-1}$ VS for cattle dung, only a small portion of the fibers degrades in a biogas process with a standard hydraulic retention time (HRT) of 15–30 days, generating an average methane output of 0.20–

$0.25\ m^3 \cdot kg^{-1}$ VS (Onthong & Juntarachat 2017). To recover the underutilized methane potential of manure, which makes up around 25% of the theoretical output, many approaches to improving biogas production have been investigated (Mittal et al. 2018). This study focused on improving the reactor configuration. Continuous-flow stirred tank reactors (CSTRs) are widely used in the anaerobic digestion of livestock waste products for biogas production (Warade et al. 2025). Loss of degradable organic matter can be attributed to "short-circuiting," in which a portion of the feed is retained in the reactor for less than the specified retention time (Warade et al. 2023). A typical single-batch CSTR is easier to operate but less effective in terms of effluent quality than a two-phase system, which consists of an acidogenesis step with a lower HRT and a methanogenesis stage with a higher HRT (Havukainen et al. 2014). Nevertheless, the two-phase system has been widely recommended as a means of improving digestion performance (Anon 2014). Conversely, it is susceptible to substrates with a high organic load that are easily degradable (Matheri et al. 2017). In such cases, a single CSTR can achieve a yield similar to that of the two-phase system (Neri et al. 2024). Numerous methods have been investigated to enhance biogas production from manure in the CSTR process, including increasing hydraulic retention time, adding activated carbon to reduce ammonia toxicity, pretreating feedstock to improve the degradability of recalcitrant materials, and co-digesting with other organic wastes or feedstocks (Agrawal et al. 2025, Abdulhameedi et al. 2024a, 2024b, Agha et al. 2024). In the current study, the potential to enhance biogas production by implementing a novel serial CSTR configuration was examined on a laboratory scale. This study also compared stirred and non-stirred conditions in the same laboratory-scale CSTR system, providing a direct performance evaluation under controlled mesophilic conditions, which is rarely reported in prior literature. While traditional CSTRs rely on continuous stirring, our study introduced an intermittent stirring strategy (15 min per hour at 20–30 rpm) and compared it with a non-stirred batch setup, assessing not just gas yield but also VS reduction, pH stability, VFA/alkalinity ratios, and temperature behavior. Therefore, the focus of this investigation was to evaluate biogas production in a continuous stirred tank reactor through wet fermentation processes. Additionally, the Response Surface Methodology (RSM) was implemented to determine biogas production from cattle manure with and without the stirring procedure. The number of trials under optimization conditions was analyzed, and control parameters such as VS, VFA, TA, and pH were monitored.

MATERIALS AND METHODS

Feedstock Supply and Its Combination

The methane-rich biogas produced from animal manure is being used as a reliable and sustainable energy source in rural parts of India and worldwide (Corré & Conijn 2016). The primary uses of cattle manure for organic farming, on the one hand, and the burning of cow-cakes for fuel, on the other hand (Ramos-Suárez et al. 2019). Cattle manure is a source of nutrients that is effective for the growth of plant material, as well as an option for improving soil structure and restoring the quality of the soil (Achinas et al. 2017). The cattle manure, as shown in Fig. 1 (a & b) for this study, was obtained from a nearby agricultural field.



(a) (b)

Fig. 1: (a) Raw Cattle manure, (b) Prepared slurry of cattle manure.

Table 1: Characterization of the substrate.

Parameters	Unit	Cattle Manure
MC	%	83
TS	%	17
VS	%	84
Fixed Solid	%	16
pH	--	6.9

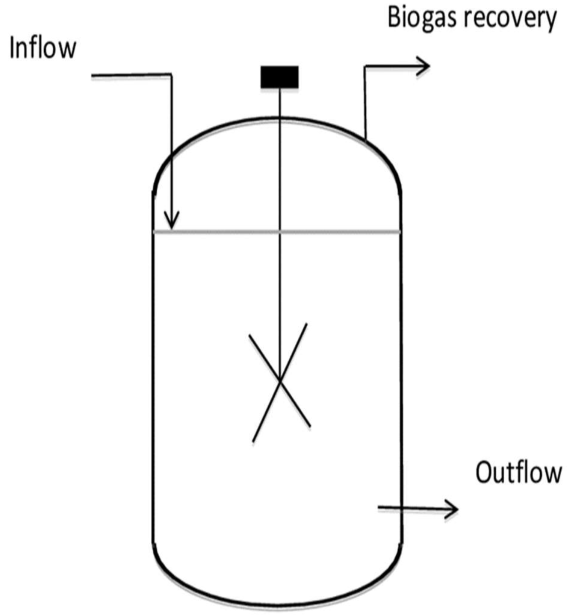
Feedstock Characteristic

Feedstock utilized in the investigation was cattle manure from a pilot-scale biogas facility. The received feedstock has been mixed with water at a proportion of 1:1 and fed into the CSTR. A standard method was implemented to analyse the primary materials collected for pH, moisture content (MC), TS, VS, VFA, and TA, outlined in Table 1. The quality of the biogas produced was analysed using a portable methane analyser, and the quantity of biogas generated has been measured using the water displacement method. Before conducting measurements, the methane analyser was calibrated with a reference gas that contained a specified quantity of methane. TS was measured by retaining the sample in the oven at 105^{o}C. However, remains from the TS measurement are burned at 550^{o}C to calculate the VS. Titration with NaOH has been used to determine the total VFA and TA.

Cattle manure was selected as the sole substrate for its wide availability, stable composition, and relevance in rural and agricultural waste management systems. It typically has a C:N ratio of approximately 25:1, which is considered optimal for anaerobic digestion, minimizing ammonia inhibition while supporting microbial growth. In this study, the collected cattle manure had a Total Solids (TS) content of 17% and a Volatile Solids (VS) content of 84%, indicating a high proportion of biodegradable organic matter suitable for methane production.

Laboratory Scale CSTR

The CSTR model, as shown in Fig. 2 (a & b), was constructed from double glass in a box arrangement, with glass panels fixed at the top and bottom. The stirring mechanism, motor, stirrer, outlet point, temperature sensor, and sampling port were mounted on the upper plate. A stable CSTR temperature of 35°C (mesophilic range) was maintained. Cattle manure and water were fed into the CSTR in batch mode at a 1:1 ratio. Because the reactor was constructed from acrylic material, it lacked an internal heating jacket. Instead, heat was transferred externally from the water bath to the reactor walls, ensuring uniform thermal distribution. Temperature readings were recorded daily, and no significant variation was observed, as the system remained within ±1°C of the target temperature throughout the entire digestion period. The laboratory-scale reactor used in the study was a cylindrical acrylic tank with a working volume of 10 L, a total height of 42 cm, and an inner diameter of 20 cm. A 3 L headspace



(a) (b)

Fig. 2: (a) Continuous Stirred Tank Reactor (CSTR). (b) Arrangement of the stirring mechanism in CSTR.

was reserved for biogas collection, and all fittings were placed on the top lid, including the stirrer, gas outlet, thermocouple, and sampling port. The reactor was stirred at a constant speed of 20 to 30 rpm for 15 min every hour to ensure proper mixing without over-agitation, which could inhibit microbial activity. Previous studies on CSTR-based anaerobic digestion have shown that stirring within the range of 30 to 100 rpm can significantly influence substrate homogenization and biogas yield. 30–90 rpm was identified as a safe and effective range that minimizes shear stress on microbial communities while ensuring adequate mixing. Biogas from the feedstock was recorded daily by the water displacement method. A hydraulic retention time (HRT) of 60 days was selected based on standard retention periods used in mesophilic anaerobic digestion of cattle manure, as well as the high fiber content and slow biodegradation rate of the substrate. This duration ensured sufficient time for complete VS reduction and stable methane production under both stirred and non-stirred conditions.

Specifically, the experiments were conducted in two distinct phases (with and without stirring), and each condition was tested in triplicate under identical reactor configuration and operational parameters. Variations among replicates were minimal, with less than 5% deviation in cumulative methane yield, confirming the consistency of the experimental outcomes.

Analytical Methods

The pH, VS, TA, and VFA were measured by extracting samples from the CSTR, two to three times weekly. A pH meter was utilized to determine the pH of the reactor daily. The ideal pH range for anaerobic digestion was 6.8-7.2, maintained by adding sodium bicarbonate ($NaHCO_3$) if necessary. The VFA analysis was done by the titration method, adding 0.1 M HCl and NaOH of 0.01 M. The VFAs concentration was monitored to ensure that the accumulation did not inhibit methanogenic activity. The TS and VS of the feedstock and digestate were determined using standard gravimetric methods (APHA 2005). TS was measured by drying the samples at 105°C, and VS was determined by igniting the dried samples at 550°C.

Experimentation Design and Optimization

The current study optimized the variables by assembling a CSTR in a 1:1 feedstock combination. The most effective method to produce biogas from a CSTR with and without stirring was determined using the Design-Expert (version 13.0.5.0, Stat-Ease) program. The Box-Behnken design (BBD) was used for optimizing the production of biogas via cattle manure mixing with water. Compared to full factorial or central composite designs, BBD requires fewer experimental runs, especially when dealing with four independent variables, as in our case (VS, VFA, TA, and pH). This makes it cost-effective and time-efficient without compromising statistical power. BBD is specifically suited for developing second-order (quadratic) models, which are ideal for capturing nonlinear interactions among variables influencing methane yield. Unlike central composite designs, BBD does not include extreme (corner) points, which helps prevent unsafe or unstable operational conditions, particularly important in anaerobic digestion, where factors like excessive VFA or extreme pH can inhibit microbial activity. The ranges of TA (A), VS (B), VFA (C), and pH (D) were taken into consideration for optimization, as indicated in Table 2.

Table 2: Levels of factors and variables used for optimization.

Variables	Parameters	Levels		
		-1	0	1
A	TA [mg.L^{-1}]	2354	2791	3087
B	VS [%]	90.16	91.9	93.82
C	VFA [%]	413	552	699
D	pH	6.3	6.7	7.3

For each variable under consideration, the three levels were assigned as -1, 0 and +1 in coded factors. Biogas production ($m^3.kg^{-1}$ VS) has been evaluated under two different situations as a response variable. Seven replications of the center locations were used in a total of twenty separate experiments (Table 3). Additionally, a regression model with a p-value < 0.05 and an F-value with a 95% confidence level was developed by fitting a second ordinal polynomial model function for optimal point prediction to the experimental results using analysis of variance (ANOVA). The appropriateness of the optimization model was represented by the coefficient of determination (R2). If the parameters are modified at the same time, a relationship between them will impact the outcomes. In order to determine the ideal proportion of variables, the software was used to develop 3D graphs that explored the impacts of each variable separately as well as their relationship to one another. Due to maximum responses, numerical and point prediction methods have been used to optimize the outcomes of each variable (Fig. 4). To verify the model's accuracy in a range of RSM scenarios, the outcomes of the laboratory tests have been compared with those that the model suggested.

RESULTS AND DISCUSSION

Gas Production

This study was conducted using a laboratory-scale Continuous Stirred Tank Reactor (CSTR) to evaluate and

Table 3: Real value of variables and response variables.

Std	Run	Factor 1 Day [A]	Factor 2 VS% [B]	Factor 3 VFA mg.L^{-1} [C]	Factor 4 TA mg.L^{-1} [D]	Factor 5 pH [E]	Factor 6 Temperature in degrees Celsius [F]	Gas Production without CSTR [m³.kg^{-1} VS] Response 2	Gas Production with CSTR [m³.kg^{-1} VS] Response 2
10	1	3	92.47	413	2364	7.3	30.5	0.01117	0.00667
11	2	6	91.34	428	2645	7.3	30.5	0.01500	0.02056
4	3	9	91.64	421	3020	7.2	31.0	0.01972	0.04389
16	4	12	91.45	426	3131	7.2	31.0	0.02039	0.04833
19	5	15	92.42	532	3542	7.0	31.0	0.02100	0.04360
1	6	18	91.34	564	3087	7.1	31.5	0.02150	0.04278
2	7	21	91.34	566	3016	6.9	30.0	0.02320	0.04389
13	8	24	90.16	579	2983	6.8	31.2	0.03060	0.04111
6	9	27	92.41	594	2648	6.6	26.5	0.02389	0.02640
5	10	30	90.47	679	2534	6.6	33.0	0.02250	0.02100
14	11	33	93.67	648	2645	6.5	30.5	0.01300	0.02889
9	12	36	92.14	699	2548	6.5	34.5	0.02111	0.01340
3	13	39	89.66	753	2498	6.5	34.5	0.02333	0.01722
20	14	42	93.14	642	2634	6.5	33.5	0.01500	0.01720
17	15	45	92.64	621	2354	6.3	30.5	0.01278	0.01240
18	16	48	91.49	601	2594	6.4	31.0	0.01210	0.00100
8	17	51	92.43	504	2683	6.5	31.0	0.01120	0.01000
15	18	54	93.82	514	3019	6.5	31.0	0.01220	0.01100
7	19	57	92.57	434	3055	6.3	31.5	0.01100	0.01000
12	20	60	91.44	432	2831	6.3	30.0	0.01010	0.01200

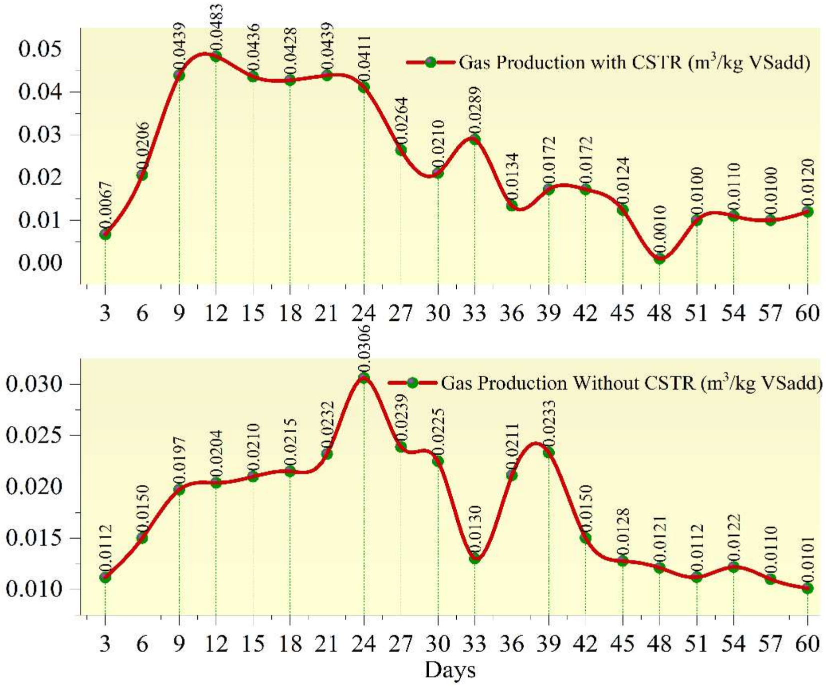


Fig. 3: Gas production with and without the CSTR mechanism.

compare biogas production under stirred and non-stirred conditions. Fig. 3 presents two graphs that compare gas production over time (in days) under two different conditions: one using a Continuous Stirred Tank Reactor (CSTR) and the other without it. Gas production measures in m³.kg^{-1} of VS added over 60 days.

The gas production with CSTR starts low, around 0.0067 m³.kg^{-1} at day 3 and rises to the highest being around 0.0439 m³.kg^{-1} on days 9, 12, and 15. After this peak, there is a general decline with fluctuations, dropping to as low as 0.0100 m³.kg^{-1} around day 48. The total biogas volume is 0.4713 m³.kg^{-1} VS by day 60. Gas production without CSTR also starts low, around 0.0112 m³.kg^{-1} on day 3, and increases to a peak of 0.0306 m³.kg^{-1} on day 24. Following this, it decreases with more visible fluctuations compared to CSTR. The production rate reaches lower values of about 0.0101 m³.kg^{-1} VS and total volume is 0.3508 m³.kg^{-1} VS by day 60. It was observed that gas production was increased with the stirring mechanism, as degradation occurs uniformly due to continuous mixing. A significant portion of the VS would have already been degraded after day 15, reducing the availability of energy sources for methanogens. As a result, methane yield naturally declines even if stirring continues. The experiment was conducted in triplicate under both stirred and non-stirred conditions using the same reactor design and operational parameters. Daily biogas production and methane content were monitored, and high consistency was observed across the three parallel trials, with less than 5% variation in cumulative methane yield. The mean values of methane yield and cumulative gas production have been presented along with their respective standard deviations (SD). Some key performance parameters, such as maximum methane yield (0.4713 m³.kg^{-1} VS), and the standard error of the mean (±0.011) have been included in the results. The optimized methane yield achieved in this study was 0.413 m³.kg^{-1} VS, under mesophilic conditions with intermittent stirring. This yield is comparable to or slightly higher than those reported in similar studies. For instance, El-Mashad & Zhang (2010) reported a methane yield of 0.22–0.27 m³.kg^{-1} VS for dairy manure in a continuously stirred CSTR at 35°C. Khoshnevisan et al. (2018) obtained yields in the range of 0.25–0.30 m³.kg^{-1} VS using mesophilic single-phase digestion. Kasinath et al. (2021) observed a yield of 0.30–0.35 m³.kg^{-1} VS in lab-scale digesters with optimized co-digestion strategies. While higher methane yields (up to 0.50–0.55 m³.kg^{-1} VS) have been reported in two-phase or thermophilic systems, these often require more complex configurations and energy input. Therefore, the methane yield achieved in this study demonstrates that simple, cost-effective process modifications such as intermittent stirring can significantly enhance methane recovery from cattle manure, approaching the efficiency of more complex systems without the associated operational burden.

Volatile Solids

VS are of the highest importance among all other solids present in the feedstocks, as they directly indicate the amount of energy generation (Singh et al. 2019). Additionally, the conversion of VS indicates the quantity of methane available in biogas (Hagos et al. 2017). Fig. 4 presents the percentage

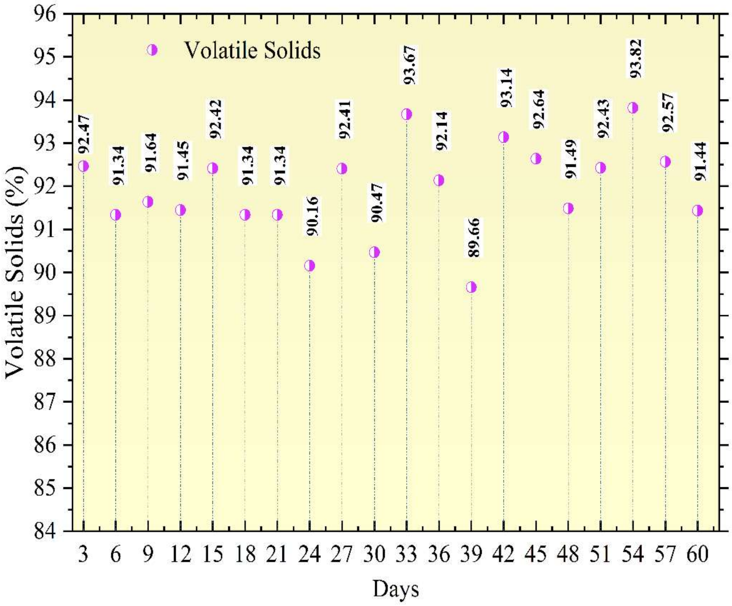


Fig. 4: VS in slurry.

of VS over time, measured at regular intervals (every 3 days) across 60 days. There is a gradual decrease over the first 21 days, reaching a minimum of 90.16% on day 21. The reduction suggests some degradation of VS during this period. After day 21, there is a slight recovery in the volatile solids, with values increasing to 92.41% on day 24, followed by small fluctuations. A notable drop occurs at day 33, where the VS percentage reaches its lowest value, i.e., 89.66%. This likely reflects a more intense phase of VS reduction or microbial activity. By the end of the period (day 60), the VS percentage is recorded at 91.44%, showing that the solids have stabilized at this value after some fluctuations. The reduction and fluctuation of VS over time suggest that degradation or conversion of organic matter occurred throughout the 60 days. The lowest VS percentage, i.e., 89.66% on day 33, indicates a critical point of degradation, possibly corresponding to a peak in microbial activity or anaerobic digestion.

pH and Temperature

The optimal range of pH for anaerobic degradation, such as acid-forming or methane-forming bacteria, is 6.8 to 7.6, whereas the normal pH range is 6.5 to 7.5 (Warade et al. 2019a). The rate at which methane is produced might be decreased when the pH is in the lower range, i.e., up to 6.1, and higher up to 8.0 (Lohan et al. 2015). High concentration of hydrolysis and acidogenesis was obtained by AD of cattle manure with 86% TOC and 82% COD, with a pH of around 7.0 (Li et al. 2018). The ideal pH range has been maintained by adding lime, bicarbonate, or carbonate salt (Emmanuel et al. 2013). Fig. 5 shows the variation of pH and temperature over time during 60 days. This visualization helps to observe how the pH and temperature changed and possibly interacted throughout the experiment. The initial drop in pH (from 7.2 to 6.2) reflects the production of acidic byproducts, such as VFA, during microbial digestion. The gradual stabilization of pH in the latter half of the experiment suggests that the system reached equilibrium, likely due to the depletion of easily degradable substrates or buffering mechanisms that stabilized the acidity. The increase in temperature from day 21 to day 33, followed by fluctuations, suggests periods of high microbial metabolic activity, especially during peak degradation phases. The eventual stabilization of temperature indicates that microbial processes slowed down or reached a steady state after day 45.

VFA and Total Alkalinity

Acetate, propionate, butyrate, and lactate are among the intermediate compounds known as VFA that are generated during the acidogenesis stage (Warade et al. 2019b). The AD process, which lowers pH, is severely affected by the excess VFA generated in the CSTR (Filer et al. 2019). The alkalinity of Cattle manure is high, but if VFA generation occurs in large amounts during AD, it has a detrimental effect on the AD process, which ultimately affects the production of biogas (Muralidharan 2017). A VFA to alkalinity ratio in the range of 0.05 to 0.25 is optimal (Mukwane et al. 2024). When VFA increases in proportion to alkalinity at a ratio

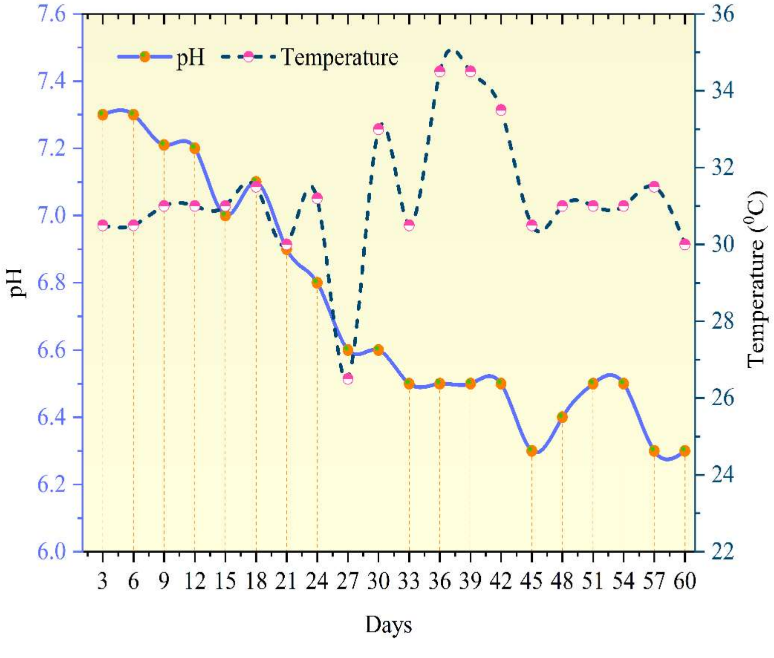


Fig. 5: Variations in pH and temperature.

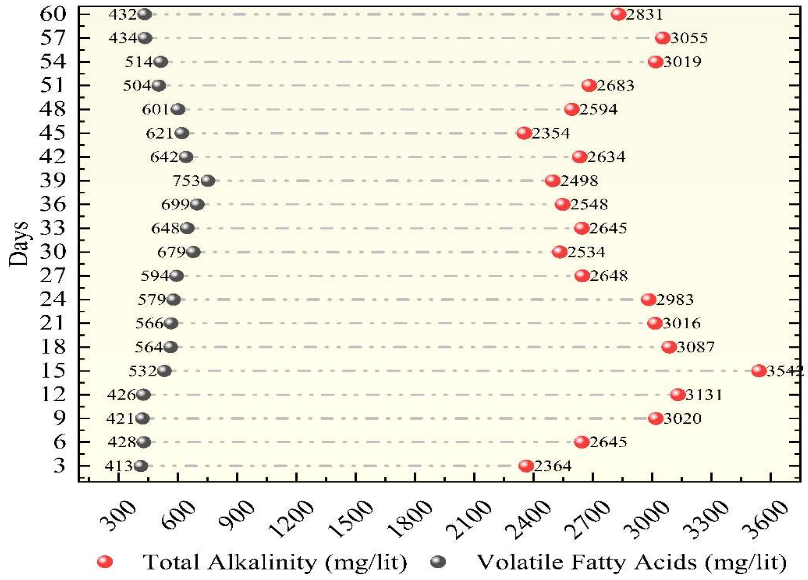


Fig. 6: VFA Alkalinity ratio.

greater than 0.25 is cause for considerable concern in AD (Warade et al. 2019a, 2019b). It must be prevented by a proper neutralization, such as water or lime (Warade et al. 2021). Furthermore, the amount of methane that controls the production of biogas in specific feedstock has a negative correlation with VFA generation (Achinas & Euverink 2016). The alkalinity ratio and VFA with and without CSTR have been shown in Fig. 6. In every proportion trial, the appropriate values were obtained when the reactor was in a stable condition. The rapid rise in VFAs and the relatively stable alkalinity during the early phase (up to day 15) suggest an intense phase of acidogenesis, where organic matter is rapidly converted into VFAs. The stable alkalinity during this time helps to buffer the system, preventing an immediate pH drop. The peak of VFA at day 15 indicates the highest level of acid production, after which the concentration starts to decrease as VFAs are likely being converted into biogas. The rise in alkalinity during this phase (day 21–36) suggests increased buffering to neutralize the acids, preventing the system from becoming too acidic and ensuring continued microbial activity. Both parameters stabilize after day 36. VFAs remain at lower levels compared to the peak, indicating a reduction in acid production and possibly a more stable anaerobic digestion process. Meanwhile, the high alkalinity ensures that the system remains stable, allowing for the final breakdown of organic matter without any major disruptions to pH.

Regression Analysis and Modelling

The twenty cycles of projected and actual experimental responses for coded CCD models used in biogas production with and without CSTR are displayed in Table 3. To evaluate the quadratic regression model, researchers use four variables: TA (A), VS (B), VFA (C), and pH (D). CCD's quadratic design structure substantially enhanced modelling efficiency while requiring fewer RSM trials. Equations (1), (2), (3), and (4) were used by CCD to demonstrate the ideal production of biogas in terms of actual and projected responses for each variable using the mathematical model for AD as per the RSM. The mathematical model's accuracy, significance, and error reduction were assessed using ANOVA. Responses for a certain set of factor values, which are described in their natural units, are predicted by this equation expressed in terms of fundamental factors. Although the point of intersection doesn't reside in the design center, the equation's coefficients were scaled to be undefined; as a result, they cannot be used to evaluate the relative importance of each component. Using an ANOVA, the model's effectiveness was evaluated using the F test, wherein the F-value indicates whether or not the second-order polynomial equation is statistically significant. It displays a value of 12.11, which indicates the model's significance. In addition, the quadratic regression model's ANOVA table had an average value below 0.05, indicating that the results were highly significant. The conclusion would be that these variables are significant since they were all controlled variables with lower p-values indicating a higher relevance level; all of them display values less than 0.05. Subsequent regression analysis revealed that while the linear term and the interaction at (A, D) were not significant at $p > 0.05$, terms in the linear, quadratic, and interaction models were significant, $p < 0.05$. According to Table 5, the polynomial

Table 4: Regression model for gas production with and without CSTR.

Responses	Factor	Regression Model	Eq.
Sqrt (Gas Production without CSTR)	Coded	+0.1765-0.0188A-0.0834B+0.0243C+0.0324D-0.0443E-0.0117F	1
	Actual	+0.984572-0.000890A-0.008703B+0.000127C+ 0.000034 D -0.019804E-0.001837F	2
Sqrt (Gas Production with CSTR)	Coded	+0.1519-0.0536A-0.0463B+0.0262C+0.1110D-0.1238E-0.0215F	3
	Actual	+0.746073-0.002543A-0.004826B+0.000137C +0.000116D-0.055349E-0.003366F	4

Table 5: Analysis of variance (ANOVA) of the model without CSTR.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0078	6	0.0013	12.11	0.0001	Significant
A-Day	0.0002	1	0.0002	1.97	0.1834	
B-VS	0.0014	1	0.0014	12.9	0.0033	
C-VFA	0.0008	1	0.0008	7.25	0.0185	
D-TA	0.0015	1	0.0015	14.38	0.0022	
E-pH	0	1	0	0.3043	0.5905	
F-Temperature	0.0001	1	0.0001	0.9925	0.3373	
Residual	0.0014	13	0.0001			
Cor Total	0.0091	19				

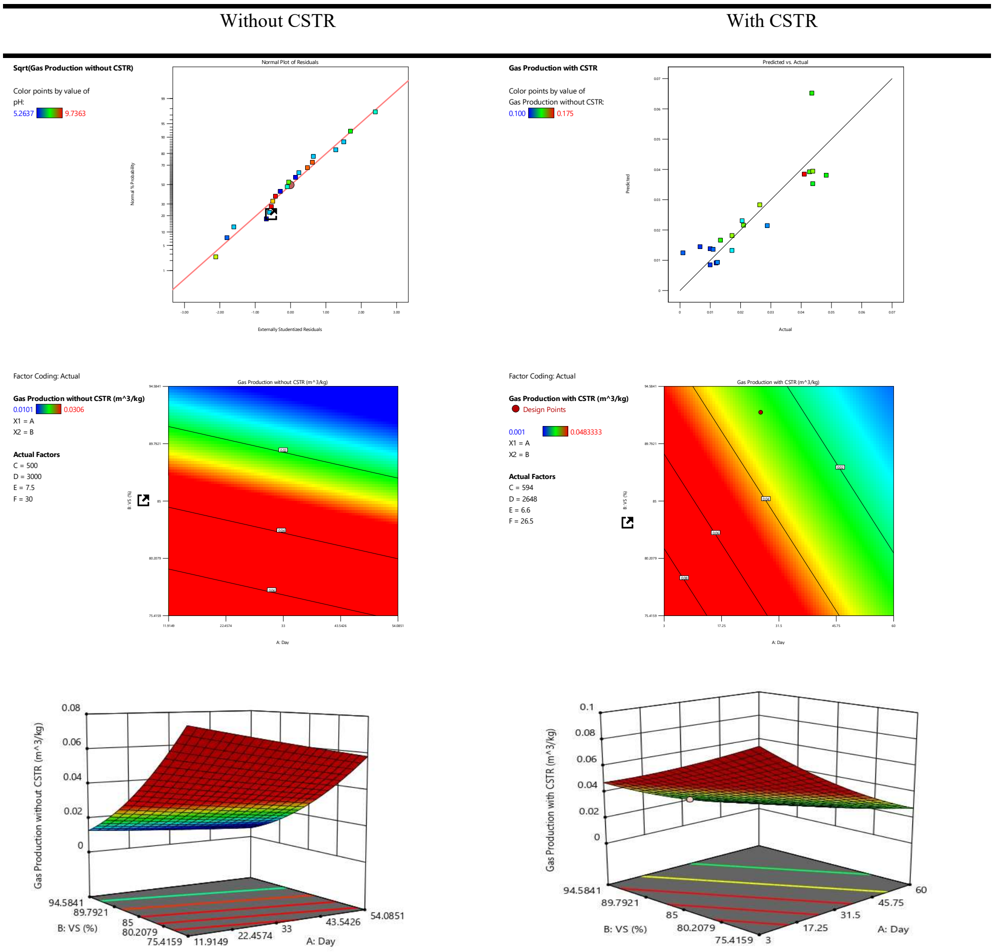


Fig. 7: The effects of variables independently and their relationships in determining the optimal level of variables with or without CSTR.

Table 6: Analysis of variance (ANOVA) of the model with CSTR.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0392	6	0.0065	6.50	0.0024	Significant
A-Day	0.0017	1	0.0017	1.71	0.2131	
B-VS	0.0004	1	0.0004	0.4215	0.5275	
C-VFA	0.0009	1	0.0009	0.8978	0.3606	
D-TA	0.0181	1	0.0181	18.00	0.0010	
E-pH	0.0003	1	0.0003	0.2527	0.6236	
F-Temperature	0.0004	1	0.0004	0.3540	0.5620	
Residual	0.0131	13	0.0010			
Cor Total	0.0522	19				

equation's R2 coefficient was 0.9499. However, Table 6 shows an F value of 47.85, revealing the model's validity. At $p < 0.05$, the quadratic regression model's ANOVA was significant. The experimental design's regression analysis revealed significant terms for the linear and quadratic models, as well as interaction models, at $p < 0.05$. With a p-value > 0.05, there was no significant difference between the linear term and the interaction term at (A, D). The value of R2 was 0.8482 (Table 7).

Equation (1) was used to express the system response as the biogas production without CSTR in terms of coded factors & Equation (2) was used to express the system response as the biogas production without CSTR in terms of actual factors.

Equation (3) was used to express the system response as the biogas production with CSTR in terms of coded factors & Equation (4) was used to express the system response as the biogas production with CSTR in terms of actual factors (Table 4).

Fig. 7 compares gas production during anaerobic digestion of cattle manure with and without a Continuous Stirred Tank Reactor (CSTR). The top row of scatter plots shows predicted versus actual gas production values, with points aligning closely to the diagonal, indicating good predictive accuracy for both setups. Gas production is more efficient with CSTR, as indicated by the color gradient. The middle row contour plots display gas production levels based on days and VS % for each setup. The CSTR setup shows a stronger response, with higher gas production (red areas) compared to the non-stirred system. The bottom row of 3D surface plots further illustrates that while gas production without CSTR is limited and highly sensitive to changes in VS % and days, the CSTR setup maintains more consistent and higher gas yields across these parameters. Overall, the results highlight that CSTR significantly enhances biogas production efficiency and stability under varying conditions.

Table 7: Statistics of the Gas Production with & Without CSTR.

Statistics	With CSTR	Without CSTR
R^2	0.9499	0.8482
Adjusted R^2	0.6345	0.7782
Predicted R^2	0.4030	0.5366
Adequate precision	8.9203	9.6930

Optimization

Fig. 8 reveals that most parameters in the anaerobic digestion process for biogas production in a CSTR are highly optimized. The duration (Day), VFA, TA, pH, and temperature all show near-perfect desirability scores, indicating these conditions are well-suited for maximizing biogas output. Gas production with CSTR achieves a score of 1, demonstrating full optimization, while production without CSTR slightly trails at 0.951805. The VS parameter has a lower desirability score of 0.412274, suggesting room for improvement in this area. Overall, the combined desirability score of 0.871656 reflects a highly optimized system, with enhancements to VS processing offering potential for further gains in biogas efficiency (Fig. 9). TS % shown significant positive influence on biogas yield up to an optimal threshold, beyond which substrate inhibition may occur. Its significance was supported by a low p-value (< 0.05) and a strong linear term in the regression model. Intermittent stirring at controlled speeds (20–30 rpm) had a notable effect on improving gas-liquid contact, enhancing substrate breakdown. We observed diminishing returns at higher RPM levels, suggesting that overly vigorous mixing may disturb microbial communities. The interaction between VFA and TA, and between pH and RPM, was also found to be significant, highlighting the importance of balancing biological and mechanical factors in maximizing methane production.

CONCLUSIONS

The current study demonstrated that anaerobic digestion of cattle manure in a Continuous Stirred Tank Reactor (CSTR), especially with optimized stirring, can significantly enhance biogas production by 34.36%. By maintaining optimal

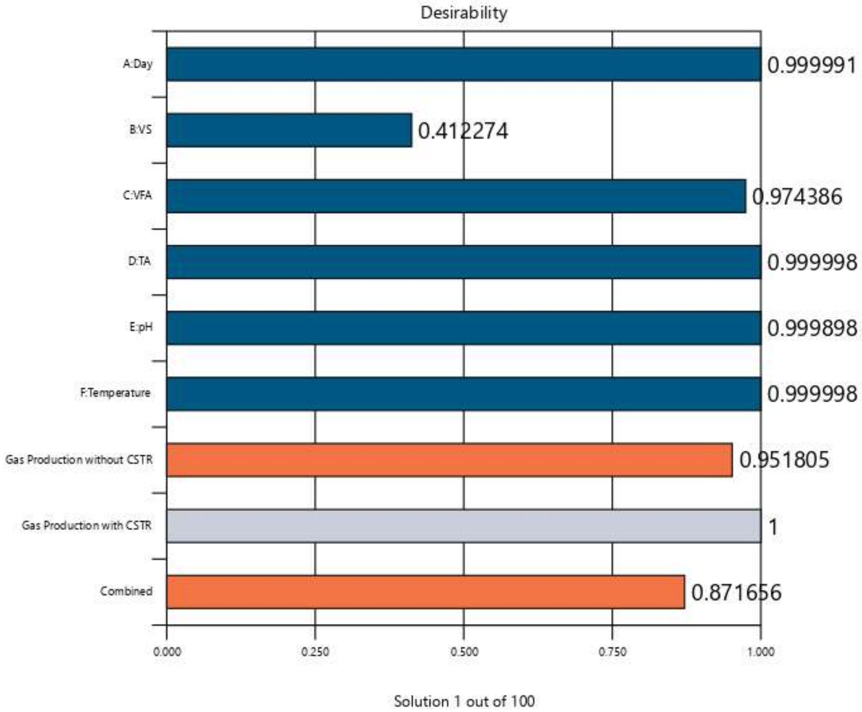


Fig. 8: Optimization and desirability in key parameters.

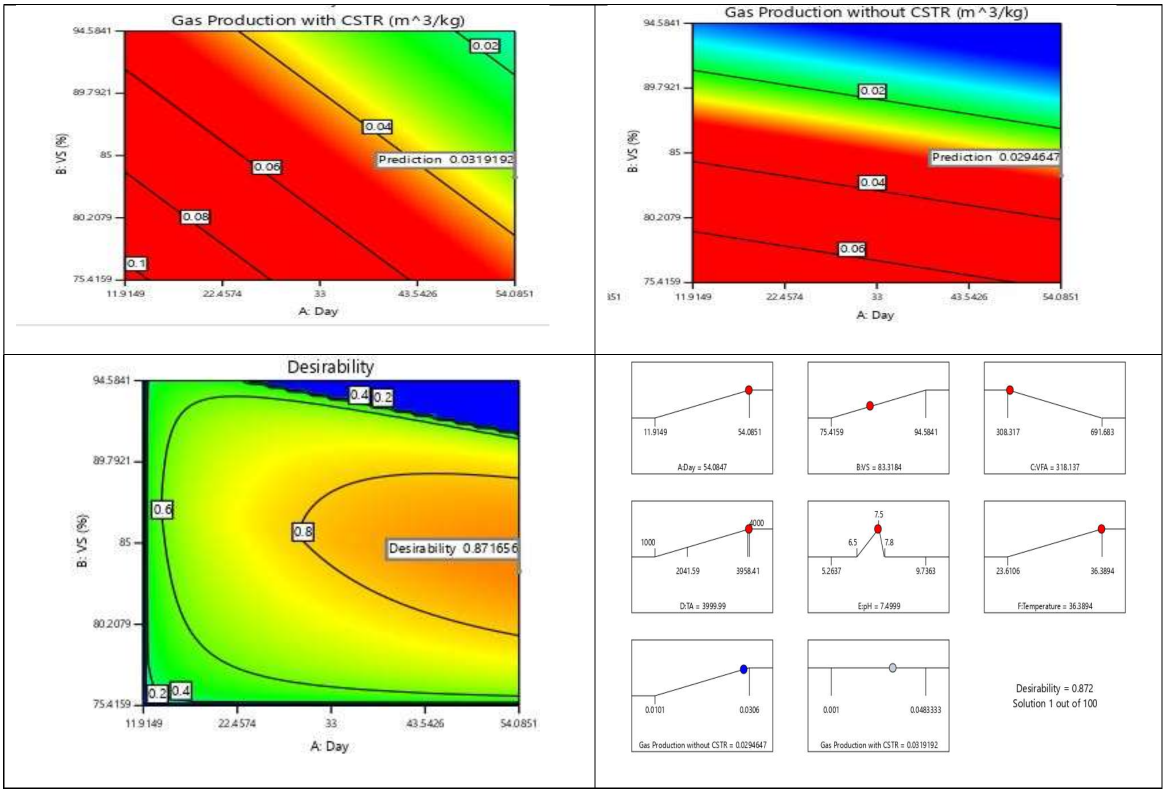


Fig. 9: Optimization and desirability contours.

conditions for variables such as VS, VFA, TA, and pH, methane yield improved due to increased microbial activity and better degradation of organic matter. The RSM and Box-Behnken design successfully optimized these parameters, as confirmed by ANOVA, validating the reliability of the regression model. The results indicate that a stirred CSTR configuration, compared to non-stirred systems, achieves higher biogas efficiency, i.e., up to 0.4713 $m^3.kg^{-1}$ VS, suggesting its potential for sustainable energy production from organic waste in centralized biogas facilities. Future research could explore scaling these findings for larger biogas operations and the use of diverse organic substrates to further maximize yield.

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