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Statistical Modeling and Optimization of Biogas Yield under Different Seeding Conditions Using ANOVA and Response Surface Methodology

Andrew Ngbeneme[1], Samuel Batet[2], Efe Justic Igbagbon[3,*]

[1] Department of Mechanical Engineering, Faculty of Engineering, University of Delta, Agbor, Delta State, Nigeria; andrew.ngbeneme@unidel.edu.ng.

[2] Department of Mechanical Engineering, Faculty of Engineering, Nigeria Maritime University, Okerenkoko, Delta State, Nigeria; batet.samuel@nmu.edu.ng.

[3] Department of Mechanical Engineering, College of Engineering, Igbinedion University, Okada, Edo State, Nigeria; igbabon.efe@iuokada.edu.ng.

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Abstract

The optimization of Anaerobic Digestion (AD) is essential for improving renewable energy production and enhancing the sustainable management of biodegradable wastes. This study investigated the influence of different seeding conditions on biogas yield using Analysis of Variance (ANOVA) and Response Surface Methodology (RSM). Laboratory-scale batch AD operated under mesophilic conditions (36–37 °C) was employed. The batch AD was used for the treatment of organic waste seeded with cow dung, organic waste seeded with Talinum triangulare, and an unseeded control. Biogas production was monitored through gas pressure measurements and flame combustion tests over the digestion period. Statistical analyses were performed to determine the significance of seeding conditions and to develop predictive models for process optimization. The results revealed that all seeded digesters enhanced biogas production compared with the unseeded system. Also, cow dung exhibited the highest mean biogas pressure (10.01 psi), followed by Talinum triangulare (9.90 psi), while the control recorded the lowest value (9.38 psi). The RSM response surface and contour plots identified an optimum digestion period of approximately 20–35 days for maximum biogas generation. The developed models demonstrated satisfactory predictive capability and confirmed the significant interaction between retention time and seeding condition. The findings establish cow dung as the most effective inoculum for improving AD performance.

Keywords: Anaerobic digestion, Biogas yield, Response surface methodology, Analysis of variance, Seeding conditions, Cow dung inoculum.

1 | Introduction

The growing global demand for clean, affordable, and sustainable energy has intensified research into renewable energy technologies. Research has shown that such technologies are capable of simultaneously

Corresponding Author: igbabon.efe@iuokada.edu,ng

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addressing energy security, environmental sustainability, and waste management challenges [1], [2]. Besides, rapid industrialization, urbanization, and population growth have significantly increased global energy consumption while accelerating the generation of municipal, agricultural, industrial, and livestock wastes [3]. More so, the continued dependence on fossil fuels has contributed substantially to greenhouse gas emissions and climate change [4]. This dependence has, on the other hand, contributed to environmental degradation and energy insecurity [5]. Thus, there is a need to develop an alternative energy source that is environmentally friendly and economically viable [6]. Among the various renewable energy technologies, Anaerobic Digestion (AD) has emerged as one of the most sustainable biological processes for converting biodegradable organic materials into valuable energy products. The AD technology utilizes naturally occurring microorganisms to decompose organic matter under oxygen-free conditions [7]. The process produces biogas primarily composed of Methane (CH_4) and Carbon Dioxide (CO_2), together with nutrient-rich digestate that can be utilized as an organic fertilizer [8–11]. Unlike many renewable energy systems that solely generate electricity or heat, the AD process simultaneously provides renewable energy [12]. The AD technology also reduces organic waste disposal problems, lowers greenhouse gas emissions, minimizes environmental pollution, and supports the principles of the circular bioeconomy [13–17]. Biogas has become increasingly important in achieving global carbon neutrality and sustainable development objectives because of its versatility and renewable nature [18]. The CH_4 contained in biogas can be utilized for electricity generation, domestic cooking, industrial heating, combined heat and power production, and upgraded to biomethane for injection into natural gas grids or as transportation fuel [19], [20]. Furthermore, AD contributes significantly to CH_4 emission reduction from uncontrolled decomposition of organic wastes in landfills while improving sanitation and resource recovery [21], [22].

Furthermore, recent advances in AD research have focused on maximizing CH_4 production through optimization of operational conditions and feedstock characteristics [22]. Several factors influence biogas productivity, including substrate composition, particle size, moisture content, carbon-to-nitrogen ratio, organic loading rate, hydraulic retention time, temperature, pH, mixing intensity, and inoculum characteristics [23]. Among these variables, seeding plays a particularly significant role because they directly influence microbial adaptation, enzymatic activity, degradation kinetics, and process stability throughout the digestion process [24]. The inoculum serves as the microbial catalyst responsible for initiating and sustaining the sequential biochemical reactions involved in AD, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. A suitable inoculum contains highly active microbial communities capable of rapidly colonizing the substrate. It also shortens the lag phase, accelerates organic matter degradation, stabilizes pH fluctuations, and enhances CH_4-producing archaea populations. Conversely, inadequate seeding conditions may result in prolonged startup periods, volatile fatty acid accumulation, process inhibition, low CH_4 concentration, and eventual digester failure [25]. The inoculum-to-substrate ratio has consequently become one of the most extensively investigated parameters in AD research [24]. Appropriate inoculum loading ensures sufficient microbial biomass for the rapid degradation of biodegradable compounds [24]. Inoculum loading also helps in preventing substrate overloading that may inhibit methanogenic microorganisms [24]. Numerous investigations have demonstrated that optimizing inoculum concentration significantly improves cumulative biogas production, CH_4 yield, volatile solids reduction, and digestion efficiency across various feedstocks [24–26]. Agricultural residues remain one of the largest underutilized renewable biomass resources worldwide. Large quantities of crop residues, animal manure, and agro-processing wastes are generated annually. The conversion of these wastes into renewable biogas offers an effective strategy for waste valorization while simultaneously contributing to rural electrification, sustainable agriculture, and climate change mitigation [27], [28].

Despite the numerous advantages associated with AD, achieving consistently high biogas yields remains challenging because the biological process involves complex interactions among physicochemical, biochemical, and microbiological factors [29], [30]. Traditional optimization methods that vary one experimental factor while keeping others constant are often inefficient because they fail to capture interaction effects among process variables [31]. Such approaches require large numbers of experiments, consume significant resources, and frequently fail to identify true optimum operating conditions [32]. Consequently, statistical optimization techniques have gained widespread acceptance in AD research. Statistical experimental design enables simultaneous evaluation of multiple process variables, quantification of factor interactions, reduction in experimental costs, and development of reliable predictive models [32]. Such models are capable of estimating system performance under different operating conditions [33]. These advantages have made statistical modelling indispensable for modern bioenergy research and industrial process optimization [34]. Analysis of Variance (ANOVA) constitutes one of the most powerful statistical tools used for determining whether experimental variables significantly influence process performance. Within AD studies, ANOVA enables researchers to identify statistically significant operational parameters, evaluate interaction effects, quantify model adequacy, and assess the reliability of predictive equations. The technique provides objective evidence for determining whether observed improvements in biogas yield are attributable to experimental treatments or merely random experimental variation [35]. Complementing ANOVA, Response Surface Methodology (RSM) has become one of the most widely applied optimization techniques because of its ability to model nonlinear relationships between independent variables and process responses. The RSM integrates carefully designed experiments with mathematical regression analysis to generate predictive equations capable of estimating optimum process conditions. Common experimental designs employed within RSM include the Central Composite Design (CCD) and the Box–Behnken Design (BBD), both of which substantially reduce the number of experimental runs while maintaining high predictive accuracy [36]. Recent studies have demonstrated that combining ANOVA with RSM provides an effective framework for optimizing inoculum conditions, substrate composition, pH, temperature, retention time, and organic loading rate in AD systems. Such statistical approaches facilitate the identification of optimal operational windows that maximize CH_4 production while minimizing experimental costs and process instability. Moreover, the integration of statistical modelling with modern computational techniques has improved prediction accuracy and enhanced the scalability of laboratory findings for industrial applications [37]. Although considerable progress has been achieved in optimizing AD processes, significant knowledge gaps remain regarding the combined effects of different seeding conditions on microbial activity, substrate degradation kinetics, and cumulative biogas production. Variations in inoculum source, microbial diversity, inoculum age, inoculum to substrate ratio, and acclimatization often produce inconsistent experimental outcomes across different feedstocks. Consequently, robust statistical modelling approaches capable of simultaneously evaluating these interacting factors remain essential for improving process efficiency and supporting large-scale commercial implementation of AD technologies. Against this background, the present study focuses on the statistical modelling and optimization of biogas yield under different seeding conditions using ANOVA and RSM. The study seeks to develop statistically reliable predictive models that describe the relationship between seeding conditions and biogas production while identifying optimum operating conditions for maximizing CH_4 generation. The findings are expected to contribute to the development of efficient AD systems, improve renewable energy production, enhance organic waste valorization, and support global efforts toward sustainable energy development and environmental protection.

2 | Materials and Methods

The materials used for this study consisted of biodegradable organic waste substrates, AD containers, seeding agents, measuring instruments, and gas monitoring accessories. The experimental setup was designed to evaluate the influence of different seeding agents on biogas yield under mesophilic operating conditions.

2.1 | Substrate Materials

The biodegradable feedstock used for the AD process comprised selected organic wastes collected from domestic and agricultural sources. The wastes were sorted manually to remove non-biodegradable materials such as plastics, metals, stones, and glass particles before use. The substrate was then reduced to smaller particle sizes to enhance microbial degradation and improve digestion efficiency.

2.2 | Seeding Agents

Three different digestion conditions were investigated:

I. Sample A: Organic waste seeded with cow dung.

II. Sample B: Organic waste seeded with Talinum triangulare.

III. Sample C: Organic waste without seeding agent (control sample).

Fresh cow dung was selected because of its high microbial population and proven effectiveness in AD systems. Talinum triangulare was selected as an alternative organic inoculum due to its biodegradability and nutrient composition.

2.3 | Digester System

Laboratory-scale batch anaerobic digesters were used for the experiment. The digesters were airtight containers designed to maintain anaerobic conditions throughout the digestion process. Each digester was fitted with:

I. Gas collection outlet.

II. Pressure gauge.

III. Gas release valve.

IV. Feeding inlet.

V. Thermometer port.

The digesters were operated under mesophilic temperature conditions ranging between 36°C and 37°C.

2.4 | Equipment and Instruments

The following instruments and equipment were used during the study:

Table 1. List of equipment and instruments.

Equipment	Function
Anaerobic digester	Biogas production
Pressure gauge	Measurement of gas pressure
Thermometer	Monitoring digestion temperature
Digital weighing balance	Measurement of substrate mass
Gas hose and burner	Flame test evaluation
Measuring cylinder	Slurry preparation
Stirrer	Homogenization of substrate
Computer software	Statistical and optimization analysis

2.5 | Experimental Procedure

The collected organic waste materials were cleaned and shredded into smaller particles to increase surface area and improve microbial accessibility. Water was added to the processed substrate to form a slurry suitable for AD.

The slurry was divided into three equal portions corresponding to the three experimental samples:

I. Sample A (cow dung seeded).

II. Sample B (talinum triangulare seeded).

III. Sample C (without seeding).

The seeding agents were thoroughly mixed with the substrate to ensure uniform microbial distribution. Each prepared slurry was introduced into separate airtight digesters. Adequate headspace was allowed inside the digesters for gas accumulation. The digesters were tightly sealed to prevent oxygen infiltration and gas leakage. The digesters were maintained under mesophilic conditions throughout the experimental period. Manual stirring was performed periodically to ensure proper mixing and uniform microbial activity. Biogas production was monitored daily through pressure measurements and flame combustion tests. Gas pressure readings were obtained using calibrated pressure gauges attached to each digester. The quality of biogas generated was evaluated using flame tests and categorized as no gas, yellow flame, and blue flame. Blue flame production indicated higher CH_4 concentration and better combustion quality. Biogas yield values were also recorded periodically during the digestion process. The experiment continued for the complete retention period until gas production stabilized.

2.6 | Statistical Analysis

ANOVA was used to determine the statistical significance of the effect of different seeding agents on biogas production. The pressure readings obtained from the digesters were analyzed at a 95% confidence interval.

The ANOVA model used is expressed as:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}, \quad (1)$$

where:

Y_{ij}= observed biogas pressure response.

μ= overall mean response.

τ_i= treatment effect.

ϵ_{ij}= experimental error.

The statistical significance was evaluated using p-values and F-statistics.

RSM was employed to model and optimize the relationship between retention time, seeding conditions, and biogas pressure generation.

A second-order polynomial model was developed as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \epsilon, \quad (2)$$

where:

Y = predicted biogas pressure.

X_1= retention time.

X_2= seeding condition.

β_0= intercept term.

β_1, β_2= linear coefficients.

β_{11}, β_{22}= quadratic coefficients.

β_{12}= interaction coefficient.

ϵ= residual error.

The developed model was used to generate response surface plots and determine the optimum operating conditions for maximum biogas production.

Optimization was carried out based on maximum biogas pressure, stable blue flame production, enhanced CH_4 quality, and improved digestion efficiency. The optimum condition was identified from the response surface model predictions and experimental observations. Based on the optimization results, cow dung seeding was identified as the most effective inoculum for enhanced biogas generation.

3 | Results and Discussion

The variation of biogas pressure with retention time for the three experimental digesters is presented in Fig. 1. The results showed that all digesters initially recorded zero gas production during the early retention period due to microbial adaptation and stabilization of AD conditions. Gas production commenced gradually after the fourth day of digestion. The cow dung-seeded digester exhibited the fastest increase in pressure generation and maintained relatively higher pressure values throughout the digestion period. This improved performance was attributed to the high microbial population and enhanced methanogenic activity introduced by cow dung inoculation. The initial period of digestion was characterized by negligible gas production across all digesters. This observation may be attributed to the microbial acclimatization phase, during which hydrolytic and methanogenic microorganisms adapt to the AD environment before substantial CH_4 generation occurs. Similar observations were reported by [38], who explained that the lag phase in AD systems is associated with microbial stabilization and substrate hydrolysis before active CH_4 production begins. As digestion progressed, the cow dung-seeded digester exhibited faster pressure build-up and earlier flame ignition compared to the other treatments. The superior performance of cow dung may be attributed to its rich microbial consortium and high methanogenic bacterial population. Cow dung naturally contains anaerobic microorganisms capable of accelerating substrate degradation and CH_4 conversion. Similarly, it was reported that cow dung inoculation significantly enhanced CH_4 concentration and digestion efficiency in food waste anaerobic AD biodegradation systems, as reported by [34].

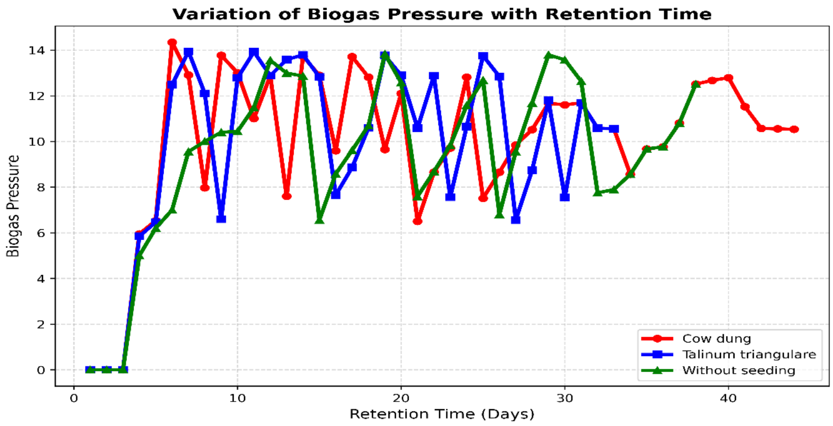

Fig. 1. Variation of biogas pressure with retention time for the three experimental digesters.

The progressive increase in biogas pressure with increasing retention time indicates active microbial metabolism and continuous biodegradation of the organic substrate. Retention time is a critical operational parameter affecting digestion efficiency and CH_4 productivity. Zhan and Zhu [36] reported that prolonged digestion periods enhance microbial decomposition and increase CH_4 generation until optimum substrate conversion is achieved. Similarly, Maakoul and Boulal [39] observed that optimum retention periods significantly improve gas yield and digestion stability during anaerobic treatment of biodegradable wastes. The transition from yellow flame to stable blue flame observed in the seeded digesters further confirms the improvement in CH_4 quality during digestion. Blue flame combustion is generally associated with higher CH_4 concentration and cleaner combustion characteristics. The cow dung-seeded sample consistently produced stable blue flames earlier than the other digesters, indicating improved methanogenic activity and enhanced substrate biodegradation.

Basak et al. [40] reported similar findings and concluded that inoculated digesters produce higher CH_4 purity and combustion efficiency compared to untreated systems. *Fig. 2* presents the mean biogas pressure generated under different seeding conditions. The cow dung-seeded sample recorded the highest average pressure, followed closely by Talinum triangulare, while the unseeded digester produced the lowest average pressure. The calculated mean pressure values were 10.01 psi for cow dung, 9.90 psi for Talinum triangulare, and 9.38 psi for the unseeded system.

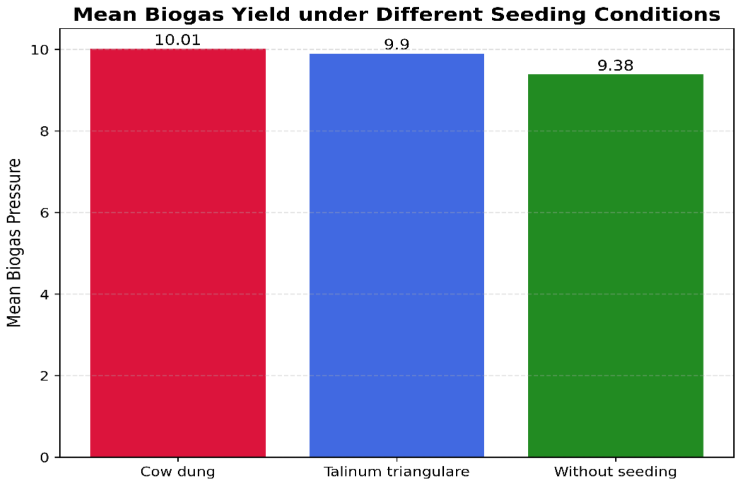

Fig. 2. Mean biogas pressure generated under different seeding conditions.

The boxplot analysis presented in *Fig. 3* illustrates the distribution and spread of pressure values under different seeding conditions. The cow dung-seeded digester showed relatively stable pressure distribution with higher median pressure values. The Talinum triangulare sample also demonstrated stable performance, while the unseeded system showed comparatively wider fluctuations and lower pressure development.

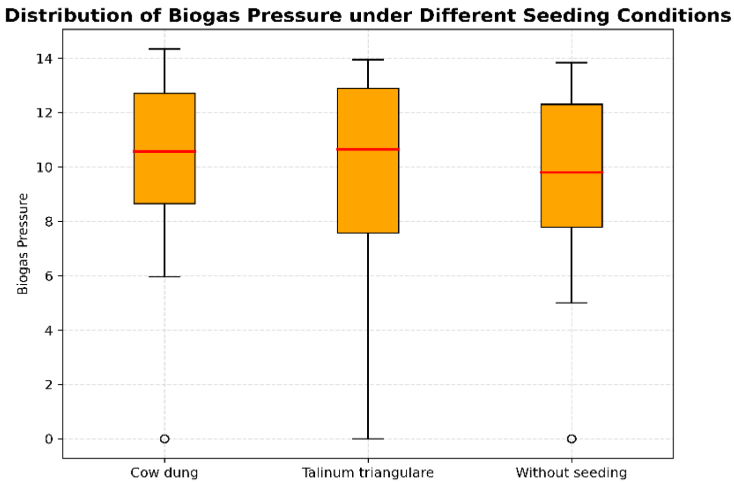

Fig. 3. Boxplot analysis showing the distribution and spread of pressure values under different seeding conditions.

The ANOVA results indicated that the seeding condition influenced biogas pressure generation. Although all seeded digesters demonstrated improved gas production compared to the unseeded sample, cow dung inoculation showed the best overall performance due to enhanced microbial digestion efficiency. Statistical modeling techniques such as ANOVA have been widely applied in recent AD optimization studies to evaluate the significance of operational variables on CH_4 yield. Bensegueni et al. [41] employed ANOVA to evaluate digestion variables and reported that inoculum concentration and retention time significantly affect CH_4 productivity and system stability.

Table 2. ANOVA analysis.

Source	Sum Sq	df	F-value	P-value
Seeding	8.813	2	0.324	0.724
Residual	1525.363	112		

The 3D response surface plot shown in *Fig. 4* illustrates the interaction between retention time and seeding condition on biogas pressure generation. The response surface revealed increasing pressure development with increasing retention time up to an optimum region. Beyond the optimum point, pressure generation became relatively stable. The response surface further confirmed that cow dung seeding produced superior optimization performance compared to Talinum triangulare and the unseeded system. The optimum operating region was observed between approximately 20 and 35 days of digestion under mesophilic temperature conditions. Besides, the experimental findings demonstrated that seeding significantly enhanced biogas pressure generation, flame stability, and CH_4 quality compared to the unseeded control system. Among the investigated treatments, cow dung inoculation produced the best overall performance, followed by Talinum triangulare, while the unseeded digester exhibited the least performance (*Fig. 2*). These findings are consistent with the observations of [34], who reported that cow dung inoculation improved CH_4 yield and digestion stability due to enhanced microbial activity.

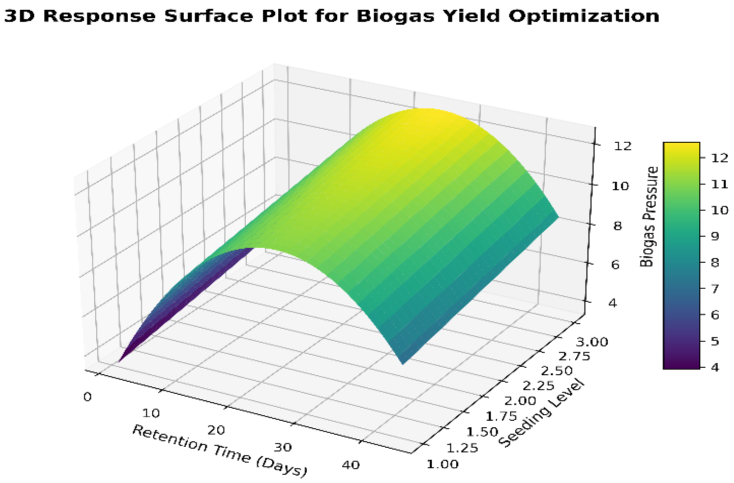

Fig. 4. 3D response surface plot for biogas optimization.

The contour plot illustrates the interaction between retention time and seeding condition on biogas pressure generation during AD. The contour regions indicate the optimization zones associated with increasing biogas yield. Higher pressure regions were observed at extended retention times and seeded conditions, particularly under cow dung inoculation. The contour distribution revealed that optimum biogas production occurred within the digestion period of approximately 20–35 days under mesophilic operating conditions. The closely spaced contour regions indicate rapid pressure development within the optimum operating zone. The generated response surface and contour plots revealed strong interaction effects between retention time and seeding conditions on biogas pressure development.

The response surface demonstrated increasing pressure generation with increasing digestion time until an optimum region was attained. Beyond the optimum zone, pressure development became relatively stable, suggesting gradual depletion of biodegradable organic matter. Similar optimization trends were reported by [42], who observed that quadratic RSM models effectively predicted optimum CH_4 production conditions in AD systems. The contour plot further illustrated closely spaced contour regions within the optimum operating zone, indicating strong interactions between retention time and seeding conditions. Such contour behavior is characteristic of highly responsive digestion systems where process variables significantly affect CH_4 generation.

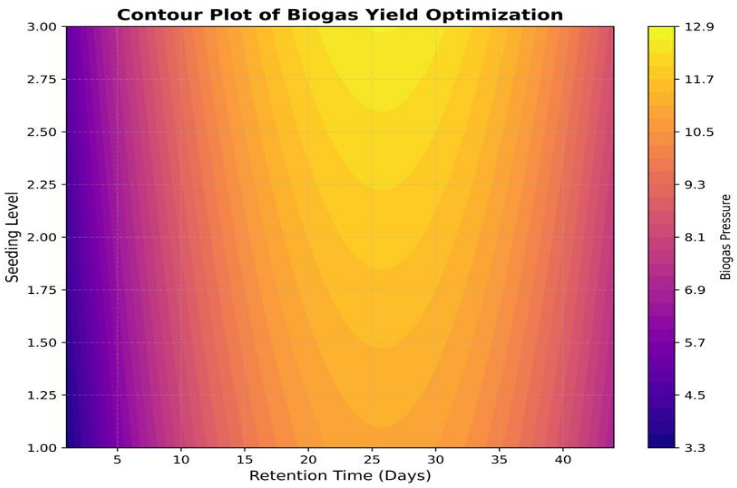

Fig. 5. Contour plot of biogas optimization.

The contour analysis further confirmed the superiority of seeded digesters over the unseeded system. Cow dung seeding demonstrated the highest optimization performance due to improved microbial activity and enhanced CH_4 generation. The experimental investigation demonstrated that seeding significantly improved biogas generation and combustion quality. The seeded digesters achieved earlier gas ignition and stable blue flame production compared to the unseeded system. Among all the investigated treatments, cow dung seeding was identified as the best-performing inoculum due to its higher CH_4 generation potential and enhanced microbial activity.

4 | Conclusion

This study evaluated the influence of different seeding conditions on biogas production from biodegradable organic waste through laboratory-scale AD experiments. Statistical analyses using ANOVA and RSM were employed to assess the significance of the experimental factors and optimize the digestion process. The results demonstrated that the addition of suitable inocula substantially enhanced biogas production compared with the unseeded control. Among the treatments investigated, cow dung proved to be the most effective seeding agent, producing the highest average biogas pressure of 10.01 psi, followed by Talinum triangulare (9.90 psi), while the unseeded digester recorded the lowest average pressure of 9.38 psi. The improved performance of the cow dung-seeded digester was attributed to its abundant methanogenic microbial population, which accelerated substrate degradation and enhanced CH_4 generation. The developed ANOVA model provided valuable insight into the influence of seeding conditions on biogas production. Also, the RSM model successfully described the interaction between retention time and inoculum type. The response surface and contour plots identified an optimum digestion period of approximately 20–35 days under mesophilic conditions. During this period, the maximum biogas production and improved combustion characteristics were achieved. The predictive capability of the statistical models demonstrates the effectiveness of RSM as a robust optimization tool for AD processes. Overall, this research confirms that inoculum selection plays a crucial role in improving the efficiency, stability, and productivity of AD systems. The integration of ANOVA with RSM provides a reliable and cost-effective approach for modelling and optimizing biogas production. It also reduces the need for extensive experimental trials while improving process predictability. The findings contribute to the growing body of knowledge on renewable energy production from organic waste and offer practical guidance for the design, optimization, and scale-up of AD systems. Therefore, the adoption of optimized seeding strategies, particularly the use of cow dung as an inoculum, can enhance sustainable waste management practices, increase renewable energy generation, and support the transition toward a circular bioeconomy and reduced greenhouse gas emissions.

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Author Contributaion

A. N.: Conceptualization, methodology, experimental design, RSM modelling, validation, data analysis, results generation, and manuscript review. S. B.: Experimental investigation, laboratory analyses, data collection, manuscript writing, and participation in data interpretation and results analysis. E. J. I.: Literature review, data preparation and presentation, results compilation, manuscript writing, and editing.

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Data Availability

The data will be made available by the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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