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Optimization of biomethane production from pet coke through anaerobic digestion using microbial inoculum and Fe_2O_3 nanoparticles: A response surface methodology approach

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ABSTRACT

The search for alternative renewable energy sources has mounted interest in biomethane as a viable substitute for fossil fuels. This study explores the anaerobic digestion of petroleum coke, a recalcitrant byproduct of oil refining, enhanced by coal mine-derived microbial inoculum and magnetic iron oxide (Fe_2O_3) nanoparticles (NPs). The Fe_2O_3 NPs were obtained through the coprecipitation technique. A central composite design within Response Surface Methodology was employed to optimize three variables; pet coke concentration, inoculum size, and NPs dosage. Scanning electron microscopy results of the synthesized NPs showed quasi-spherical morphology, particle aggregation, and distinct crystalline. X-ray diffraction peaks indicative of spinel-type ferrites, confirming a magnetite-based structure. Analysis of variance results of the linear model present a moderate coefficient of determination ($R^2 = 0.5799$) for the model, indicating its adequacy for prediction. The optimized conditions for biomethane production were determined as follows: Feedstock (Pet coke) concentration of 8 g/L, Inoculum of 8 % (v/v), and 40 mg/L of magnetic iron oxide NPs. Under the optimized conditions, the model predicted a biomethane yield of 33.2%, which closely aligned with the experimentally observed yield of 32 %; the difference was not statistically significant ($P = 0.158$) reliability. Validation experiments substantiated the model reliability. The gas chromatography analysis of the generated gas revealed a methane concentration of 55.86 wt%, thereby illustrating significant bioenergy potential. The integration of microbial consortia and NPs strategies offers a promising alternative for converting industrial residues, such as pet coke into sustainable biofuels.

1. INTRODUCTION

Bioenergy constitutes a crucial category of renewable energy obtained from biological substances, including but not limited to wood, animal waste, straw, and an array of agricultural by-products [1]. It is considered one of the most feasible short to medium strategies for mitigating greenhouse gas emissions and substituting traditional fossil fuels. The increasing global energy demand and mounting environmental concerns associated with fossil fuel consumption have intensified the search for sustainable and clean energy alternatives [2]. Among the diverse spectrum of renewable energy sources, biomethane has surfaced as a noteworthy biofuel owing to its elevated energy density and its congruity with the pre-existing natural gas infrastructure [3]. Anaerobic digestion (AD), a microbial process that breaks down organic matter to produce biogas, mainly methane and carbon dioxide, has been increasingly adopted in recent years [4,5]. Conventionally used for agricultural and municipal waste treatment, the scope of AD has expanded to include complex and energy-dense feedstocks. One such unconventional yet underexplored feedstock is petroleum coke (pet coke), a carbon-rich byproduct of oil refining that presents both a disposal challenge and an opportunity for bioenergy valorization [6].

Pet coke is characterized by its high calorific value and carbon content but poses substantial barriers to microbial degradation due to its low biodegradability, aromatic structure, and high sulfur content [7]. To circumvent these limitations, biological pre-treatment and enhancement strategies, such as the incorporation of specialized microbial consortia and functional nanomaterials, have been recently explored. In particular, microbial consortia derived from coal mine environments have shown unique metabolic adaptability and resilience under harsh conditions, making them ideal inocula for tackling recalcitrant carbonaceous substrates, such as pet coke [8,9]. These microbial communities often possess anaerobic hydrocarbon degrading capabilities that can facilitate the breakdown of pet coke components into intermediate compounds suitable for methanogenesis.

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Furthermore, the incorporation of magnetic iron oxide nanoparticles (NPs) into the AD system introduces a novel approach to improving process efficiency [10]. These NPs have the capacity to augment microbial activity through the facilitation of electron transfer, the enhancement of enzyme accessibility, and the potential reduction of toxic intermediates [11]. Their magnetic attributes also allow for easier recovery and recycling, adding a layer of economic and environmental sustainability. So also, the utilization of magnetic Fe_2O_3 NPs to improve AD of pet coke represents a novel approach, as the interaction between iron-based nanocatalysts and a resistant carbon-rich substrate has not been thoroughly explored. However, despite their potential of these enhancements, the synergistic effects of biomass concentration, inoculum size, and NP dosage remain largely unoptimized and poorly understood.

To systematically investigate and optimize these critical parameters, this research employs Response Surface Methodology (RSM), a robust statistical and mathematical tool used for modeling and analyzing problems in which multiple variables influence the desired response. RSM facilitates the development of empirical models that can predict biomethane yield under various operational conditions and identify optimal combinations of the input variables [12]. This methodology not only augments the efficacy of the experimental framework by diminishing the quantity of necessary trials but also elucidates the interactive influences among variables, which are frequently neglected in conventional one-factor-at-a-time approaches [13,14].

The objective of this study is to optimize biomethane production from pet coke using AD, with a specific focus on three key variables: Biomass (pet coke) concentration, inoculum size (coal mine microbial consortium), and magnetic Fe_2O_3 NPs concentration. Across the application of RSM, the study aims to maximize methane yield. This research not only contributes to advancing sustainable biofuel production from industrial residues but also offers a strategic framework for integrating novel materials and microbial consortia into AD systems. In addition, the successful optimization of such a system could pave the way for valorizing pet coke waste streams, reducing environmental burdens, and creating a feasible pathway for decentralized renewable energy generation.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals

All chemicals used in this work were of analytical grade, purchased from Sisco Research Laboratories Pvt. India and used as received without further purification**.** All chemicals were of analytical grade (≥99% purity) and used without further purification.

2.1.2. Feeds stock

Petroleum coke (pet coke) used in this study was sourced from Chennai Petroleum Corporation Limited (CPCL), Chennai, Tamil Nadu, India. The material was ground and sieved to a uniform particle size (<250 µm) to attain homogeneity and improve surface area for microbial action. A preliminary hydrogen sulfide (H_2S) screening test t was conducted to assess potential inhibition by the feedstock. The pet coke was also characterized before use for key parameters, including elemental composition (C, H, S, N, and O), moisture content, and ash content using standard methods (ASTM D3176 and D5865). All parameters were analyzed and triplicate and the mean standard deviation was calculated.

2.1.3. Microbial inoculum

The microbial inoculum was procured from anaerobic sludge sampled from coal mine effluent sedimentation tanks in Telangana district (Coordinates: 17.20613, 80.79979). The microbial community was enriched under strictly anaerobic conditions for three weeks in a basal mineral medium supplemented with coal-derived organics to promote the proliferation of hydrocarbon-degrading anaerobes.

2.1.4. Magnetic iron oxide (Fe_2O_3) NPs

Magnetic Iron oxide (Fe_2O_3) NPs were synthesized through coprecipitation of ferrous and ferric salts in an alkaline environment, followed by functionalization with ionic liquids comprising biocompatible cations and anions, as elucidated by [15,16]. The NPs were characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD) recorded using a PANlytical X'Pert PRO Diffractometer with Kα radiation (λ = 1.5406 Å), to confirm morphology, crystalline structure, and surface functionality. The NPs were added in varying dosages (mg/L) based on experimental design.

2.2. Fermentation Experiments and Analytical Methods

Batch AD experiments were conducted using a 500 mL Erlenmeyer flask with a working volume of 400 mL. Each bottle was loaded with the required amount of pet coke, inoculum, and NPs as per the experimental design (Figure 1). The medium was adjusted to a pH of 7.0 ± 0.2 and subjected to nitrogen gas purging for 10 min to ensure anaerobic conditions; all reactors were maintained at 37 ± 1°C for 30 days in a controlled shaker (100 rpm), with controls lacking pet coke, inoculum, or NPs to assess baseline methane production. Biogas production was monitored daily by the water displacement method. The methane concentration within the biogas was analyzed through gas chromatography (GC) (HP-PLOT Q (30 m × 0.32 mm, 20 µm film, Helium at 1.2 mL/min) integrated with a thermal conductivity detector alongside a stainless-steel packed column. The calibration was performed using standard gas mixtures with R^2 > 0.995.

2.3. Experimental Design and Statistical Analysis

A two-level central composite design factorial design, under RSM was employed to optimize the process parameters affecting biomethane yield. The independent variables selected were pet coke concentration (A: 4–10g/L), inoculum size (B: 5–2-8% (v/v)), and magnetic Fe_2O_3 NPs dosage (C: 10–40 mg/L), which are shown in Table 1. The ranges were determined based on preliminary experiments, ensure optimal biomethane yield. The dependent variable (response) was cumulative methane production (CH_4 %). Design-Expert software (version 13)

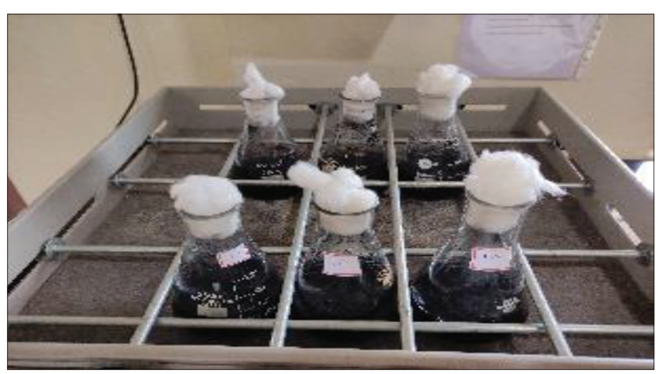

Figure 1: Digital photos showing parts of sample during biomethane production experiment.

Table 1: Independent-dependent variables and limit values.

Name	Unit	Type	Standard deviation	Minimum	Maximum
Pet coke	g/L	Independent	0	4	10
Inoculum size	%(v/v)	Independent	0	2	8
Magnetic iron oxide nanoparticles	mg/L	Independent	0	10	40

Table 2: Experimental design for RSM.

Standard	Run	Factor 1: Pet coke (A) (g/L)	Factor 2: Inoculum size %(v/v)	Factor 3: Magnetic Fe_2O_3 NPs (mg/L)	Actual response: Biomethane (%)	Predicted response values (%)
7	1	4	8	40	27.8	25.2
9	2	6	6	25	26.4	20.9
6	3	8	4	40	32.6	28.4
12	4	6	6	25	25.3	20.9
4	5	8	8	10	18.7	21.5
8	6	8	8	40	32.1	33.3
1	7	4	4	10	16.3	8.4
10	8	6	6	25	16.5	20.9
3	9	4	8	10	14.3	13.3
2	10	8	4	10	13.6	16.6
5	11	4	4	40	12.8	20.3
11	12	6	6	25	13.9	20.9
18	13	6	6	50.2	28.6	30.1
17	14	6	6	0.23	5.7	11.1
19	15	6	6	25	28.7	21
16	16	6	9.4	25	24.8	25.1
14	17	9.4	6	25	27.9	27.9
13	18	2.6	6	25	10.2	14.2
20	19	6	6	25	26.8	21.01
15	20	6	2.6	25	15.4	16.9

was used for the design matrix generation, regression analysis, and response surface modeling. The respective variables are shown in Table 1, while the generated experimental designed with predicted and actual response are shown in Table 2.

3. RESULTS AND DISCUSSION

3.1. Proximate Analysis of Pet Coke

Proximate analysis provides insights into the thermal decomposition and organic content of pet coke, which are critical for assessing its suitability in AD [17]. The result of proximate analysis for pet coke are shown in Table 3. The extremely low moisture content indicates that pet coke is a dry, hydrophobic material [18]. While this property is advantageous for storage and combustion, it presents a challenge in AD, where water is essential for microbial activity. The negligible volatile matter content suggests that pet coke contains very few compounds that can be easily gasified or metabolized by microorganisms [19]. This aligns with its highly condensed aromatic structure, which is resistant to biological breakdown.

3.2. SEM and XRD of Magnetic Fe_2O_3 NP

The SEM image of the synthesized magnetic ionocide NPs is presented in Figure 2a, revealing a relatively uniform and dense particle distribution. The NPs exhibit a quasi-spherical morphology and tend to aggregate a typical behavior observed in magnetic NPs due to dipole-dipole interactions [20]. Based on the scale bar (~500 nm), the individual particles appear to range between 40 nm and 80 nm in size, though aggregation may lead to an overestimation of their actual dimensions. In addition, the particles exhibit a rough texture, which could influence their surface area and reactivity.

The XRD pattern [Figure 2b] displays distinct peaks with corresponding 2θ values and Miller indices suggesting the crystalline nature of the NPs. The notable peaks at 218, 220, and 308 corresponds to plane, which are characteristic of spinel-type cubic ferrite structures, especially magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), confirming magnetic iron oxide NPs [16,21]. The sharpness and intensity of the peaks indicate good crystallinity. So also, the most intense peak at 311 at an angle theta ~35.8° typically indicates the presence of magnetite [22].

3.3. RSM Model Equation

RSM is a widely applied statistical and mathematical approach used to model and optimize processes influenced by multiple variables [23]. Its primary objective is to determine the optimal conditions that maximize or minimize a desired response. In this context, the variables that influence the process are referred to as independent variables, whereas the outcomes or results are termed dependent variables [24]. For instance, biomethane yield (response) may be influenced by variables, such as X_1 (biomass concentration) and X_2 (inoculum size), with the yield varying across different combinations of these variables. The selection of independent variables and their respective limits is typically based on previous experimental studies or literature. In RSM, the experimental data are fitted to appropriate statistical models, which may include linear, quadratic, cubic, or two-factor interaction (2FI) models [14]. The model coefficients comprise a constant term, linear coefficients (A, B, C), interaction coefficients (AB, AC, BC), and quadratic coefficients (A^2, B^2, C^2). The highest biomethane yield was obtained at a feedstock concentration of 8 g/L pet coke, with 8% (v/v) inoculum and 40 mg/L of magnetic iron oxide NPs. Under these conditions, the predicted biomethane yield was 33.2%, whereas the observed yield was 32.1%. Paired T-test confirmed no statistically significant difference between the two ($P = 0.158$).

The adequacy of the fitted model is evaluated through various statistical parameters, including the coefficient of determination (R^2), adjusted R^2, and adequate precision [25]. A model is generally considered statistically significant and reliable if the P-value < 0.05, the lack of fit P-value > 0.05, $R^2 > 0.9$, and adequate precision >4. Furthermore, analysis of variance (ANOVA) is employed to assess the statistical significance of the model and the differences between treatment means [26].

Table 3: Proximate and ultimate analysis of pet coke.

Parameter	Pet coke (wt.%)
Moisture	0.46±0.02
Ash	13.22±0.01
Volatile matter	1.91±0.03
Carbon	72.54±0.1
Hydrogen	3.46±0.02
Sulfur	6.70±0.04
SiO_2	1.20±0.01
AL_2O_3	0.22±0.01
CaO	0.14±0.04
MgO	0.02±0. 01
TiO_2	0.0 2±0.01
Na_2O	0.17±0.03
Fe_2O_3	0.16±0.02
K_2O	0.003±0.01

3.4. Model Test Result

The selection of an appropriate model is a critical step in RSM as it significantly influences the accuracy and reliability of predictions related to biomethane yield [27]. In the present research work, various models, including linear, two-factor interaction (2FI), quadratic, and cubic, were evaluated to identify the best fit for the experimental data obtained from the AD of pet coke.

As presented in Table 4, the linear model demonstrates statistical significance with a $P = 0.001$, signifying that the model is significant and capable in explaining a substantial variation of biomethane production. The non-significant lack of fit $P = 0.5857$ indicates that the model sufficiently fits the empirical data, exhibiting minimal unexplained variability. Moreover, the model's Adjusted R^2 (0.5799) and predicted R^2 (0.4135) values demonstrate moderate levels of explanatory and predictive efficacy, respectively. The relatively close alignment between these two metrics further substantiates the appropriateness of the linear model [28]. However, the 2FI model yielded a higher $P = 0.4083$, signifying that the interaction terms between factors did not significantly enhance model performance. As shown in Table 4 the adjusted R^2 value (0.5837) of the 2FI model was marginally higher than that of the linear model and the predicted R^2 (0.1032) was notably low, reflecting poor predictive ability. The non-significant lack of fit ($P = 0.5804$) does suggest that the model was not overfitting the data, but the weak prediction performance rendered it less favorable [29]. In addition, the quadratic model was found to performed poorly as evidenced by a high P-value (0.7433), a negative predicted R^2 (−0.2606), and a relatively low adjusted R^2 (0.513). These results imply that the model neither captures the variability in the response nor has predictive reliability. The insignificant lack of fit ($P = 0.4492$) was not sufficient to offset its poor performance metrics. Similarly, the cubic model showed the least reliability, with a high P-value (0.7287), a negative adjusted R^2 (0.38), and an extremely poor predicted R^2 (−57.6972), indicating severe overfitting or model misspecification. Furthermore, despite a non-significant lack of fit ($P = 0.1583$), this model was deemed aliased, meaning some terms are confounded and cannot be uniquely estimated, further disqualifying it for practical use.

Table 4: Lack of fit test.

Model	*P*-value	lack of fit *P* value	Adjusted R^2	Predicted R^2	
Linear	0.001	0.5857	0.5799	0.4135	Suggested
2FI	0.4083	0.5804	0.5837	0.1032	
Quadratic	0.7433	0.4492	0.513	−0.2606	
Cubic	0.7287	0.1583	0.38	−57.6972	Aliased

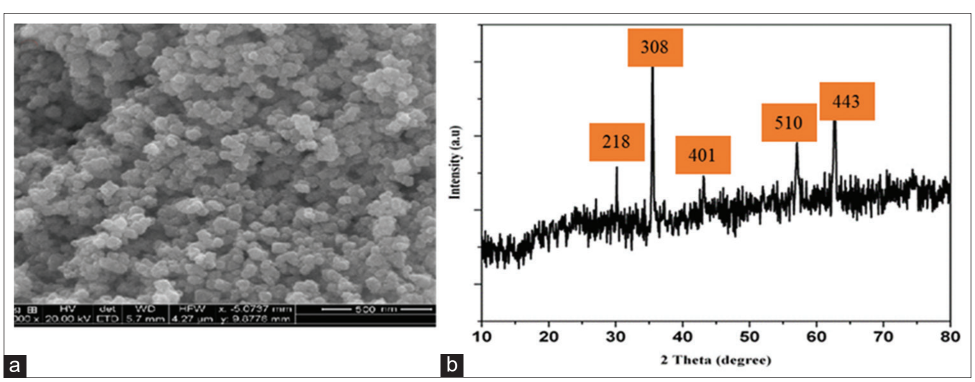

Figure 2: SEM (a) and XRD (b) analysis of magnetic Fe_2O_3 nano particles.

3.5. ANOVA analysis for linear model

The ANOVA results presented in Table 5 provide essential insights regarding the factors affecting biomethane production in AD of pet coke. The model shows statistical significance, exhibiting an F-value of 9.28 and a $P = 0.001$. This indicates that the selected process variables, pet coke concentration (A), inoculum size (B), and magnetic ionocide NPs (C), collectively have a significant effect on biomethane yield, validating the adequacy of the fitted model in describing the experimental data. Among the individual factors, pet coke concentration exhibited a significant effect on biomethane production (F = 8.00, $P = 0.0127$). Variations in substrate load notably affect microbial activity and methane production. However, magnetic Fe_2O_3 NPs exhibited the highest significance (F = 16.96, $P = 0.0009$) among the tested variables. This is likely linked to their ability to enhance microbial metabolism and improve AD efficiency. Inoculum size was not significant (F = 2.89, $P = 0.1097$) at the 95% confidence level. Its impact may be more evident under varied conditions than those examined here. This can be supported with evidence that microbial processes are widely affected by multiple factors [30,31]. The insignificance indicates that optimization should focus on pet coke concentration and magnetic Fe_2O_3 NPs dosage for enhanced biomethane yield. Moreover, the lack of fit test was found to be non-significant with p value equating to 0.5857. This denotes that the developed model fits the experimental data and that the variation in the response can be explained by the model terms [12,32]. The relatively low pure error and residual mean square values support the model's reliability and predictive capability. Overall, the statistics confirm that the model is moderately predictive and suitable for further optimization of biomethane production.

The regression coefficient analysis is presented in Table 6 revealing C (Fe_2O_3) factor had the most significant influence on biomethane yield with a coefficient of 5.92 and a $P = 0.0009$, respectively. Both factors showed a positive and statistically significant effect, indicating that increasing these parameters enhances biomethane production during the AD of pet coke. However, factor B exhibited a positive but statistically insignificant effect $P = 0.1097$, implying that its individual contribution to the response may be limited or dependent on interaction with other variables.

3.6. Model Validation and Diagnostic Analysis

The performance of the RSM based model was evaluated through validation experiments and diagnostic plots. The objective was to assess how well the model could predict biomethane yield based on selected process variables: Pet coke (biomass) concentration, inoculum size, and magnetic Fe_2O_3 NPs dosage. Table 7 presents the experimental validation of the model using three sets of operating conditions predicted by the model development phase. The comparison between the predicted and actual biomethane yields demonstrates a high degree of agreement, indicating the model robustness and predictive accuracy. For example, in Run 6, where 8 g of biomass, 8 mL of inoculum size, and 40 mg of magnetic NPs were used, the actual Biomethane yield was 32.4%, closely aligning with the model's prediction. Similarly, Run 16 (6 g biomass, 9.4 mL inoculum, and 25 mg NPs) produced 24.3% biomethane yield and Run 17 (4 g biomass, 8 mL inoculum, and 40 mg NPs) resulted in 27.6% biomethane yield, both in close proximity to the corresponding model predictions. The low absolute error between predicted and experimental values across all runs confirms the model's capability to accurately forecast biomethane output across the design space. These results validate the effectiveness of the second-order polynomial equation in representing the experimental system.

This scatter plot [Figure 3a] evaluates the model predictive performance by comparing predicted and experimental values. The close clustering of data points along the 45° line (Y = X) signifies a high correlation and indicates that the model neither systematically overpredicts nor underpredicts the response. The near-linear fit validates the accuracy of the RSM model and reinforces the strength of the regression relationship. So also, the differences between observed and predicted values [Figure 3b], are plotted against the predicted values to assess model assumptions. A random scatter of residuals around the zero line is evident with no discernible pattern, indicating that the model satisfies the assumption of homoscedasticity (constant variance) [33]. This further proves that the variance of the prediction errors is stable across the range of predicted values, supporting the reliability of the model. In addition, Figure 3c depicts how residuals behave with respect to the order of experimental runs. The distribution of residuals appears to be randomly scattered without any visible trends, cycles, or drift. This suggests that there are no lurking variables or time-related biases affecting the experimental runs. The consistency across run order confirms proper randomization of experimental trials and adds confidence in the validity of the experimental design.

3.7. 3D Surface and Contour Plot Analysis

The three-dimensional (3D) response surface plots and their corresponding contour plots provide significant insights into the interactive effects of key process parameters, pet coke concentration, inoculum size, and magnetic ionocide NP dosage on biomethane yield during AD. These visual tools are instrumental in identifying optimal

Table 5: ANOVA analysis for linear model.

Source	Sum of squares	df	Mean square	F-value	*P*-value	
Block	0.1141	1	0.1141			
Model	787.21	3	262.4	9.28	0.001	Significant
A-pet coke	226.1	1	226.1	8	0.0127	
B-inoculum size	81.73	1	81.73	2.89	0.1097	
C-magnetic Fe_2O_3 NPs	479.39	1	479.39	16.96	0.0009	
Residual	423.97	15	28.26			
Lack of fit	304.75	11	27.7	0.9296	0.5857	Not significant
Pure error	119.21	4	29.8			
Cor total	1211.29	19				

Table 6: Coefficients of factors.

	Intercept	block 1	A	B	C
Biomethane	20.9354	−0.077083	4.06885	2.44631	5.92472
P-value			0.0127	0.1097	0.0009

Table 7: Model validation results.

Run No.	Biomass (g/L)	Inoculum size% (v/v)	Magnetic Fe_2O_3 NPs (mg/L)	Biomethane yield (%)
16	6	9.4	25	24.3
6	8	8	40	31.1
17	4	8	40	27.5

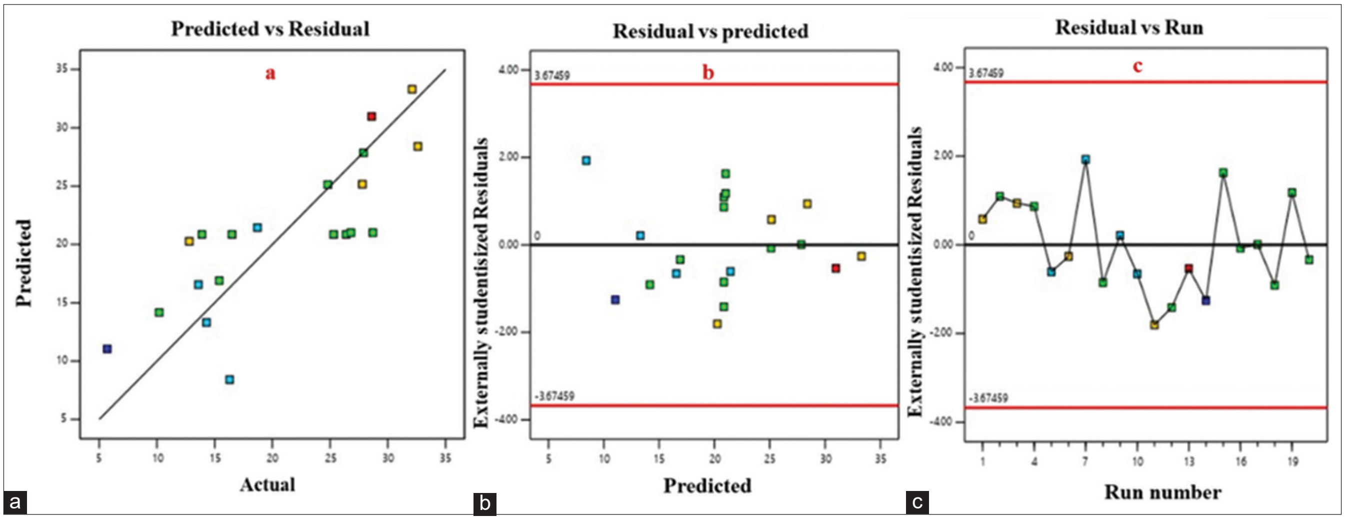

Figure 3: (a) Graph of predicted and actual values, (b) Graph of predicted and residual values, (c) Graph of predicted and run values - experiment deviations.

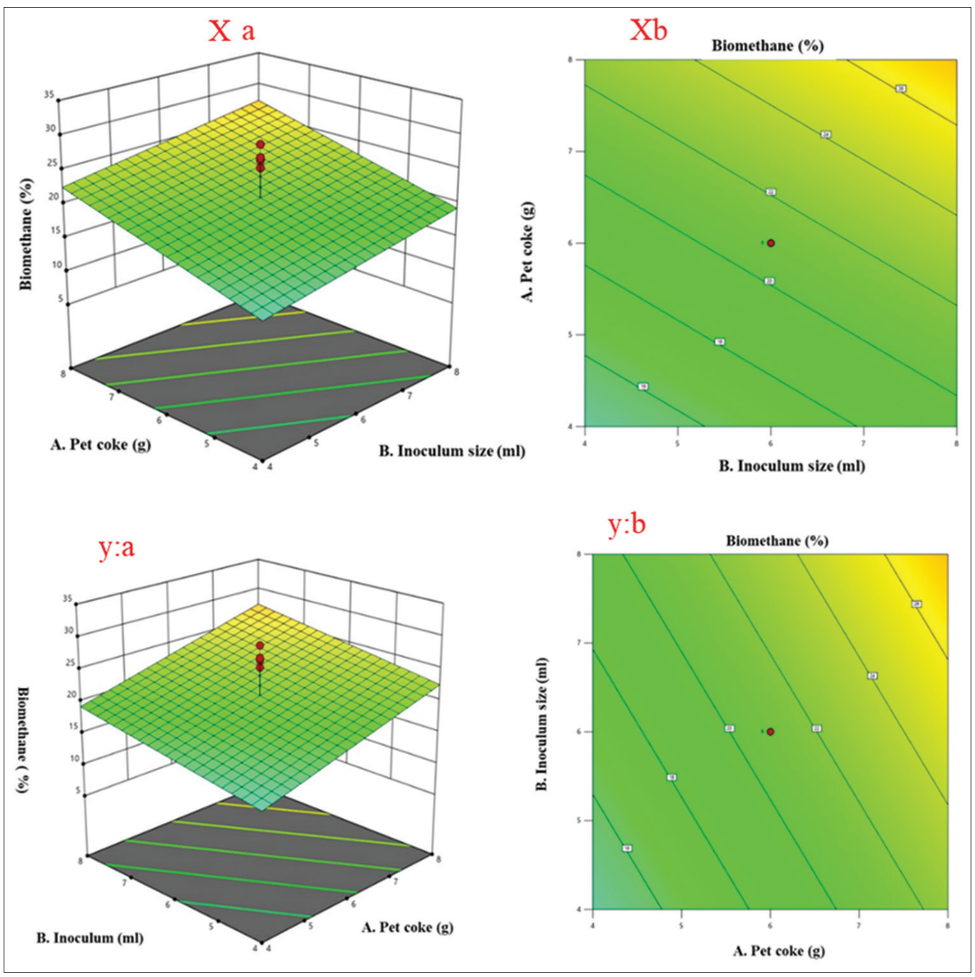

Figure 4: 3D and contour surface interaction of pet coke and inoculum size (X: a and Y: a) and (Xb: a and Y: b).

operating conditions and understanding the non-linear behavior of the system under investigation [34]. The interaction between pet coke concentration and inoculum size [Figure 4X: a and b)] revealed a distinct curved response surface, indicating a significant interaction effect. Biomethane yield was observed to increase with moderate levels of pet coke and higher inoculum size. This trend can be attributed to the fact that low concentrations of pet coke may not provide sufficient carbon substrate to support microbial activity, whereas excessively high concentrations might inhibit digestion due to substrate overloading or an imbalance in the carbon-to-nitrogen (C/N) ratio. In contrast, increasing inoculum size enhanced microbial population density, thereby improving substrate degradation and gas

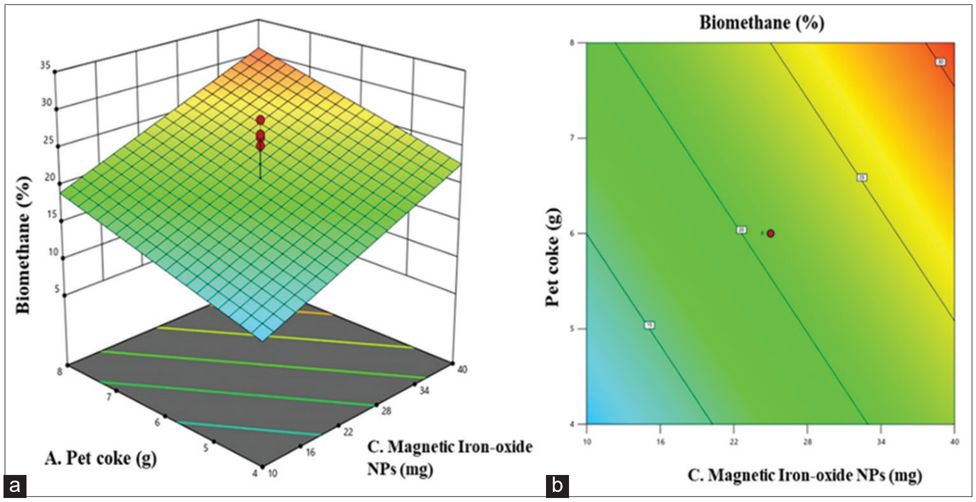

Figure 5: 3D and contour surface interaction of pet coke and Magnetic Iron oxide NPs(X: a and Y: a) and (Z: b and Y: b).

production. Similar behavior regarding this was previously reported by [31] using organic waste feedstock and nano magnetite particles as additives. The elliptical nature of the contour plot further confirmed a strong interaction between these two variables [35], implying that their effects on biomethane production are interdependent rather than additive.

A similar pattern was observed in the interaction between pet coke concentration and magnetic Fe_2O_3 NPs dosage [Figure 4Y: a and b]. The biomethane yield improved with moderate values of both parameters, suggesting a synergistic effect. Magnetic NPs, at optimal levels, likely stimulate microbial metabolism, enhance enzyme activity, or promote sludge flocculation, all of which contribute to better digestion efficiency. This agrees with previous findings of [36], who reported Magnetic NPs exert effects on microbial metabolism. However, high concentrations of NPs may exhibit inhibitory or toxic effects on microbial consortia as per previously reported studies [37], thereby reducing methane production. Again, the elliptical contours indicated a statistically significant interaction, reinforcing the importance of jointly optimizing these two variables.

Moreover, the third interaction, between inoculum size and magnetic Fe_2O_3 NPs [Figure 5], demonstrated a similar synergistic response. Maximum biomethane yield was achieved when both variables were maintained within a specific optimal range of (Inoculum size; 9.4% (v/v): Magnetic NPs 25mg/L). At low inoculum levels, the stimulatory effects of NPs could not be fully realized due to insufficient microbial biomass [38]. Conversely, high levels of both factors did not lead to further improvements and may have introduced inhibitory effects, possibly due to saturation or oxidative stress from excess NPs. The tightly curved contours reflect a narrow range within which both factors must be controlled to achieve peak performance [35]. Overall, these response surface analyses underscore the complexity of optimizing biomethane production, where multiple interacting variables must be carefully balanced. The non-linear and interactive nature of these effects validates the application of RSM and the use of a second-order polynomial model to accurately describe and predict system behavior.

3.8. GC Analysis of Produced Biomethane

The GC analysis of the biogas generated through AD of pet coke is presented in Table 8. Methane yield (wt%) was calculated from GC output normalized to total biogas volume using standard biomethane chromatogram as shown in Figure 6. The primary constituent of the produced biomethane was methane (CH_4), which accounted for 55.86 wt%, indicating a high energy potential of the biogas. This finding aligns closely results obtained by [39], who reported a biomethane yield ranging from 56.8% to 60.97% using Lime water and under acetic acid treatment. Methane is the main combustible component of biogas [40], and its proportion above 50% is generally considered adequate for most energy applications, including combustion for heat and electricity generation [41]. The second major component was carbon dioxide (CO_2), constituting 18.62 wt%. CO_2 is a non-combustible gas

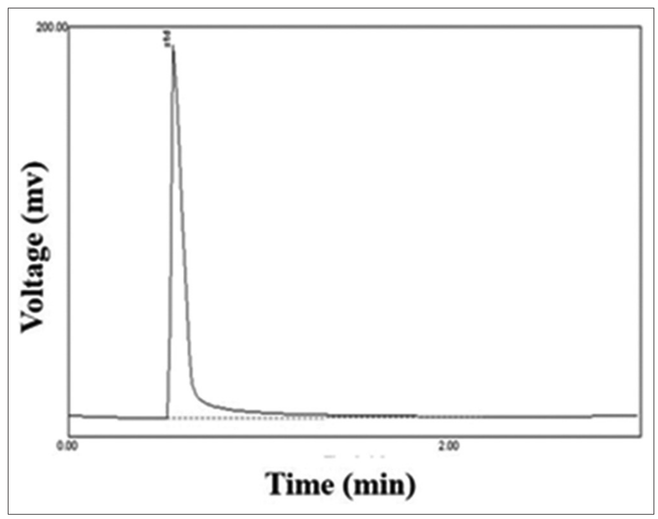

Figure 6: Standard chromatograph for the estimation of produced biomethane.

Table 8: Gas chromatography of produced Biomethane.

Element	Composition (wt. %)
CH_4	55.86
CO_2	18.62
H_2S	2.64
NH_3	1.84
N_2	3.35
O_2	0.58
H_2O	4.22

that dilutes the energy content of biogas but is a typical by product of AD [42]. The observed concentration is within the expected range, reflecting effective organic matter degradation and carbon conversion pathways. A notable presence of hydrogen sulfide (H_2S) was detected at 2.64 wt%, which raises concerns due to its corrosive nature and toxicity. The concentration of H_2S suggests that sulfur-containing compounds in the pet coke feedstock or microbial sulfate reduction pathways contributed to its formation. Pre-treatment of the substrate or post-treatment of the biogas (e.g., scrubbing, adsorption) may be necessary to reduce H_2S to levels compliant with safety and emission standards [43]. Ammonia (NH_3) was found at 1.84 wt%, which is relatively high and may have originated from nitrogenous compounds in the feedstock or microbial protein degradation. Elevated NH_3 can inhibit microbial activity in the digester and also contributes to odor problems, necessitating careful management [44]. The gas also contained minor quantities of nitrogen (N_2) at 3.35 wt% and oxygen (O_2) at 0.58 wt%. These likely originated from residual air during sample collection or incomplete anaerobic conditions. Water vapor (H_2O) constituted 4.22 wt%, typical of biogas under normal temperature and pressure conditions. The presence of moisture requires gas drying before usage, especially if the biogas is to be compressed or used in internal combustion engines [45].

4. CONCLUSION

This study successfully demonstrates the optimization of biomethane production from pet coke using AD enhanced by coal mine microbial inoculum and magnetic Fe_2O_3 NPs. The application of RSM enabled efficient exploration and identification of optimal operational conditions, with pet coke concentration and Fe_2O_3 NPs dosage emerging as statistically significant variables. The highest biomethane yield of 32.2% was observed with 8 g of pet coke, 8 mL of inoculum concentration, and 40 mg of Fe_2O_3 NPs. The linear model showed strong predictive performance and was validated through experimental trials, while GC confirmed the biogas energy-rich composition, particularly its high methane content. These findings establish pet coke as a feasible substrate for bioenergy generation, offering a sustainable pathway for its exploration. Future research should explore long-term operational stability, scale-up feasibility, and assess sulfur toxicity more comprehensively to understand its impact on microbial activity and biomethane yield.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

10. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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