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Optimising Biogas Production from the Anaerobic Co-digestion of Primary Sewage Sludge with Intermediate Landfill Leachate using Response Surface Methodology

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Anaerobic co-digestion has emerged as an effective approach for treating municipal and industrial wastewater, offering the added benefit of renewable energy generation through biogas production. This study investigated the optimisation of biogas yield via the co-digestion of sewage sludge and intermediate landfill leachate using Response Surface Methodology (RSM) based on a Box-Behnken Design (BBD). Three key parameters; co-substrate loading ratio (1:20–1:5 v/v), inoculum-to-substrate ratio (ISR; 1:2–1.5:1), and temperature (25-55 °C)—were examined for their influence on biogas yield (mL/gVS), chemical oxygen demand (COD) removal, and volatile solids (VS) reduction. Numerical optimisation identified the optimal conditions as a leachate (co-substrate loading) of 1:20, an ISR of 1.5:1, and a temperature of 42.21 °C, achieving a desirability of 90.10 %. Under these conditions, biogas yield reached 90.76 mL/gVS, with COD and VS removals of 33.62 % and 43.25 %. The developed RSM models showed strong predictive capability ($0.9 < R^2 < 1$) and were validated experimentally. Observed values closely matched predicted values, with discrepancies below 5 %, confirming the model's reliability. The model validation experimental runs resulted in an average biogas production of 87.90 mL/gVS, with corresponding chemical oxygen demand (COD) and volatile solids (VS) removal efficiencies of 35.16 % and 39.90 %. These findings underscore the potential of optimised co-digestion for enhanced biogas production and effective waste treatment.

1. Introduction

Rapid industrial growth in developing regions has resulted in a marked increase in the production of municipal and industrial wastewater, much of which is rich in organic pollutants (Moretta et al. 2022). When properly treated, such wastewater presents a viable opportunity for renewable energy generation, particularly through biogas production via anaerobic digestion (AD) (Kato and Kansha, 2024). Wastewater, defined as water contaminated by human-related activities, originates from sources such as household use, industrial operations, sewage discharge, and surface runoff (Singh et al., 2023). Domestic wastewater typically contains about 1 % solids, with the remainder being water. High-strength wastewater, often defined by COD concentrations exceeding 10,000 mg/L and BOD values above 5,000 mg/L, presents significant treatment challenges for many industries (Chan et al., 2016). To comply with environmental discharge regulations, these streams often require multi-stage treatment processes. Anaerobic digestion has long been established as a practical solution for treating such high-load wastewaters while simultaneously generating biogas, a form of sustainable energy. The Division of Water Technology has undertaken extensive studies demonstrating the feasibility and benefits of AD for municipal and industrial wastewater management (Montwedi et al., 2021).
Sewage sludge, a byproduct of wastewater treatment processes, is a particularly valuable substrate for AD due to its high methane generation potential. It has been estimated that the energy contained in sewage sludge may be up to tenfold greater than the energy required for its treatment (Bachman, 2015). This sludge typically includes both primary and secondary components. Primary sludge is collected via sedimentation and is richer

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in organics, yielding up to 300–400 Nm³ of methane per tonne of organic dry matter under optimal digestion conditions (Davidson, 2007). In contrast, secondary sludge, produced during the aeration stage, contains less organic material and produces less biogas. On a dry weight basis, sewage sludge has an energy density of approximately 19 MJ/kg (equivalent to 5.2 kWh/kg), placing it in the same energy range as low-grade coal (Musvoto et al., 2018).

Another waste stream of interest for co-digestion is landfill leachate, a complex effluent formed as water filters through decomposing municipal solid waste (MSW). This leachate contains a variety of contaminants (Lindamulla et al., 2022) including dissolved organic matter, heavy metals, inorganic salts, and synthetic organic compounds, depending on landfill age, composition of waste, climate, and hydrology (Vaverková, 2019). These compounds can include nitrates, ammonia, chlorides, phenols, phthalates, and trace metals such as zinc and mercury (Sossou et al., 2024).

Landfill leachate is commonly classified based on landfill maturity: leachate from newer sites (1–5 years) is typically acidic and rich in volatile fatty acids and readily degradable organics, while older leachate (from sites older than 10 years) contains more stable compounds such as humic and fulvic acids and tends to be more alkaline (Mojiri et al., 2020). Treating landfill leachate can be technically demanding and costly. A wide range of treatment technologies has been employed, including physical-chemical techniques (e.g., coagulation, filtration, adsorption, oxidation), biological methods (both aerobic and anaerobic), and integrated systems that combine several processes (Nabi et al., 2022). AD is especially suited for treating younger or intermediate leachates due to their higher biodegradability. However, its application is often limited by operational issues such as acid build-up, microbial inhibition, and the presence of slowly degradable compounds. These factors contribute to the relatively small number of studies exploring landfill leachate as a substrate for anaerobic digestion, particularly in co-digestion settings, despite its potential for resource recovery and waste minimisation.

Hence, this study aimed to evaluate the optimum conditions for biogas production while also considering factors such as COD and VS removal as key performance indicators.

2. Materials and Methods

2.1 Feedstock characterization

In this study, sewage sludge was collected from the primary settling tanks (PSTs) of a local wastewater treatment plant (WWTP), while the inoculum was obtained from the facility's secondary anaerobic digesters. Landfill leachate was sourced from the influent pipeline of the leachate treatment facility at the Lovu landfill site, situated within the eThekwini Municipality, South Africa. Prior to conducting anaerobic digestion experiments, the sludge, inoculum, and leachate were characterized for key physicochemical properties, including pH, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD), using standard methods prescribed by the American Public Health Association (APHA, 2005). Following characterisation, all samples were stored at 5 °C to preserve their integrity until use.

2.2 Experimental Design

A Box-Behnken Design (BBD) was employed using Design-Expert software (version 12.1.2.0) to investigate the effects of three key operational variables on the anaerobic digestion process. The process performance was evaluated based on three response variables: biogas yield and the percentage removal of demand COD and VS. RSM was applied to statistically analyse the data, allowing for the assessment of interaction effects among the selected parameters on the Biological Methane Potential (BMP). Predictive regression models were developed based on the experimental data obtained from the Box–Behnken Design (BBD) trials by fitting a generalized second-order polynomial equation, in accordance with the methodology outlined by Ghaleb et al. (2020). The input variables and their corresponding coded levels are presented in Table 1.

Table 1: BBD coded factors

Input Variables	Coded Level	Factor Code		
		-1	0	1
Temperature (°C)	A	25	37	55
ISR (gVS/gVS)	B	1:2	1:1	1.5:1
Leachate loading rate (v/v)	C	1:20	1:10	1:5

The experimental data were fitted to a second-order polynomial equation to develop a predictive model for the response variables. The general form of the multivariate regression model used in the Response Surface Methodology (RSM) is expressed in Eq(1) below:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \varepsilon \quad (1)$$

Where:

- Y is the predicted response.
- A, B, and C are the input variables.
- β_0 is the intercept term.
- β_1, β_2, β_3 are the linear coefficients.
- β_{11}, β_{22}, β_{33} are the quadratic coefficients.
- β_{ij} are the interaction coefficients.
- ε is the residual error.

2.3 Experimental Set-up

The experiments were conducted using 1 L Schott bottles, which served as batch bioreactors. Each reactor was a graduated bottle with a total volume of 1,000 mL and measurement markings up to 950 mL to ensure accurate loading. The bottles were sealed with screw caps fitted with silicone tubing to allow gas flow while preventing liquid leakage. Unused ports were securely closed with additional screw caps. To establish anaerobic conditions, each bioreactor was purged with nitrogen gas (N_2) for approximately 4–5 minutes before sealing, as described by Wu et al. (2016) and Yukesh Kannah et al. (2020). This step was critical to remove residual oxygen and enable the anaerobic microbial community to function effectively.

Biogas was collected using the downward water displacement method, where the gas was routed via silicone tubing into a graduated collection cylinder. Prior to gas measurement, the digesters were stirred using magnetic stirrers for two hours to ensure homogenous mixing of the substrate and inoculum. Daily biogas production was recorded throughout the experiment.

3. Results and Discussion

3.1 Feedstock characterisation

Table 2 below presents the results for the characterisation of each substrate.

Table 2: Physicochemical Characterisation of Feedstocks and Inoculum

Parameter	Sludge	Leachate	Inoculum
pH	5.5	7.85	7.83
COD (mg/L)	59 040 ± 31,5	5 346 ± 46	42 080 ± 24,5
TS (gTS/L)	39.02 ± 7.1	2.56 ± 0.5	53.4 ± 6.5
VS (gVS/L)	29.72 ± 6.6	4.385 ± 9.8	27.96 ± 5.8
VS/TS	0.76	-	0.52

Li et al. (2017) reported typical TS and VS values for primary sludge (PS) between 30–60 gTS/L and 30–50 gVS/L. In this study, PS had 39.02 gTS/L and 29.72 gVS/L, aligning with expected ranges. A VS/TS ratio above 50 % indicates good biodegradability. The inoculum had the lowest ratio (0.52), reflecting a higher microbial content and confirming its suitability as an inoculum rather than a substrate.

3.2 Response Surface Methodology

Design-Expert software recommended a quadratic model for all response variables—Biogas Yield, COD removal, and VS removal—based on the input factors: Temperature (A), Inoculum-to-Substrate Ratio (ISR) (B), and Co-substrate Loading (C). Each model incorporated the intercept, linear terms (A, B, C), quadratic terms (A^2, B^2, C^2), and interaction terms (AB, AC, BC). The sequence of influential factor interactions for maximizing biogas yield was AC > AB > BC. For COD and VS removal, the order of significance was AB > AC > BC. Linear terms represented the primary effects of individual variables, while interaction and quadratic terms captured the relationships and curvature among predictors. The regression equations for each response, based on coded factor levels, are provided below in Eq(2), Eq(3) and Eq(4).

$$\text{Biogas Yield} = 59.9696 + 3.33743\,A + 6.57608\,B - 0.192024\,C - 9.97907\,AB - 4.1192\,AC - 25.0815\,BC - 24.005\,A^2 - 1.9206\,B^2 + 1.75371\,C^2 \quad (2)$$

$$\text{COD Removal (\%)} = 31.8143 + 2.70051A - 2.14382B + 1.71722C + 8.65452AB + 6.825AC - 2.03937BC - 7.12343A^2 + 1.98463B^2 + 1.13413C^2 \quad (3)$$

$$\text{VS Removal (\%)} = 26.3367 + 5.1165A + 1.82245B - 0.111514C + 2.29363AB - 3.77627AC - 1.25255BC - 1.90203A^2 + 6.20249B^2 + 5.9890\,C^2 - 3.17536A^2B \quad (4)$$

Model performance was assessed using the coefficient of determination (R^2), adjusted R^2, and predicted R^2. All R^2 values were close to 1, indicating excellent model fit. The R^2 values for biogas yield, COD removal, and VS removal were 0.9971, 0.9853, and 0.9932. The differences between adjusted and predicted R^2 values were less than 0.2, further supporting model reliability. The adequacy of the developed models was confirmed by the Adequate Precision values, which assess the signal-to-noise ratio. All values exceeded the commonly accepted threshold of 4, indicating that the models can be used to navigate the design space effectively. Specifically, the Adequate Precision values for biogas yield, COD removal, and VS removal were 52.85, 21.39, and 23.23, reflecting high signal strength and reliable model performance. A summary of these results is provided in Table 3.

Table 3: Statistical Summary of Regression Model Performance for Response Variables

Parameter	Biogas Yield	COD Removal	VS Removal
R^2	0.9971	0.9853	0.9932
Adequate Precision	52.85	21.39	23.23
LOF	> 0.1 (Good Fit)	> 0.1 (Good Fit)	> 0.1 (Good Fit)
p-value	< 0.05 (Significant)	< 0.05 (Significant)	< 0.05 (Significant)

3.3 Response Surface Plots.

Figure 1 presents 3D surface plots that depict how the selected process variables influenced each response variable. The plots provide a graphical interpretation of the regression models, highlighting the significance of temperature, inoculum-to-substrate ratio (ISR), and co-substrate loading. Among these, temperature and ISR emerged as the most critical factors affecting process performance, although co-substrate loading also showed a meaningful contribution. The combined effects of ISR, temperature, and co-substrate loading on biogas production are shown. Biogas yield increased with higher ISR, peaking at the maximum tested value of 1.5:1. Maintaining an appropriate ISR is vital to prevent the buildup of volatile fatty acids (VFAs), which can disrupt anaerobic digestion. The inoculum also enriches the system with nitrogen and micronutrients, improving the C/N balance and supporting enzymatic activity that boosts methane production. Temperature had a strong influence on biogas yield, with an upward trend observed between 25 °C and 37 °C (within the mesophilic range). Beyond 37 °C, yield declined, likely due to increased VFA concentrations and reduced stability under thermophilic conditions.

An increase in co-substrate loading also enhanced biogas output, likely due to nutrient supplementation from the landfill leachate, which helped offset nutrient limitations in the primary sludge.

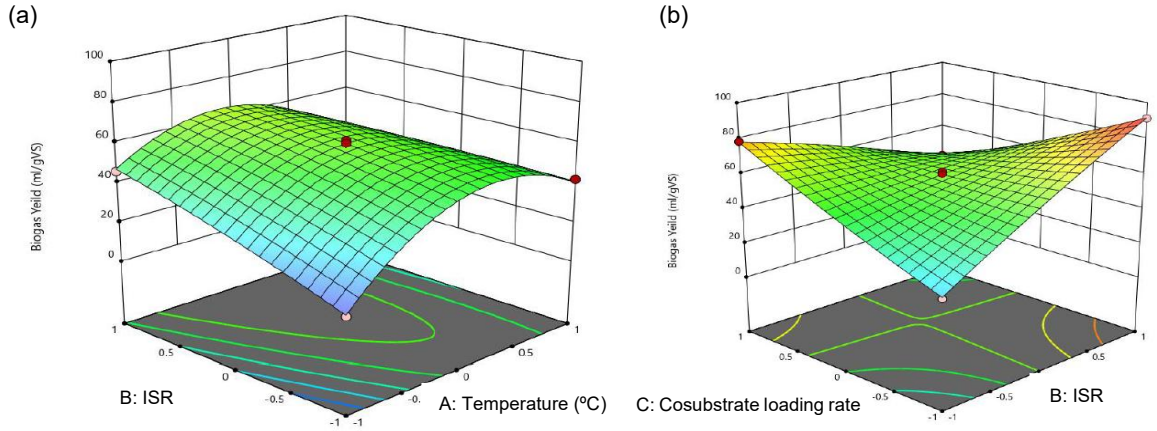

Figure 1: 3D surface plots of Interaction Effects of ISR and (a) Temperature; (b) Co-Substrate Loading on Biogas Yield

3.4 Process optimisation

Numerical optimisation using RSM was applied to identify optimal values for temperature, ISR, and co-substrate loading. As shown in Figure 2, the optimum conditions — 42.21 °C, ISR of 1.5:1, and a co-substrate loading ratio of 1:20 achieved a high desirability of 90.10 %. Under these conditions, biogas yield reached 90.76 mL/gVS, with COD and VS removal efficiencies of 33.6 2 % and 43.25 %. The model was validated through three experimental runs at these optimal settings.

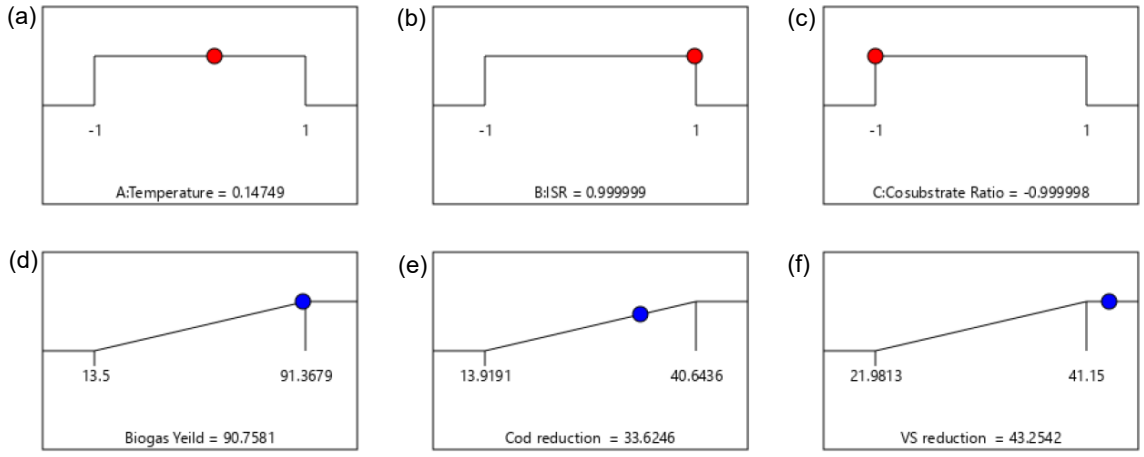

Figure 2: Ramp Plot of Optimum Operating Conditions for the BMP System of (a) Temperature; (b) ISR, and (c) Co-substrate Ratio on (d) Biogas yield, (e) COD reduction, and (f) Volatile Solids (VS) reduction

3.5 Model validation.

Table 4 presents the three optimal experimental runs selected for each process to evaluate the validity and predictive capability of the developed models.

Table 4: Model validation statistical analysis

Analysis	Predicted mean	Standard deviation	n	SE Pred	95 % PI Data low	mean	95 % PI high
Biogas Yield (ml/gVS$_{added}$)	91.6524	1.79585	3	1.86918	86.8475	87.9	96.4572
COD Removal	33.1114	1.58299	3	1.64763	28.876	35.1633	37.3468
VS Removal	41.7148	1.00895	3	1.10908	38.6355	39.3033	44.7941

The mean biogas yield, COD removal, and VS reduction were 87.9 mL/gVS, 35.1633 %, and 39.3033 %. The experimental results deviated by less than 5 % from the predicted values, indicating a strong correlation between observed and simulated outcomes. These findings confirm that the models reliably represent the system behaviour and possess robust predictive accuracy.

4. Conclusions

This study successfully identified the optimal operating conditions for the co-digestion of sewage sludge with intermediate landfill leachate (ILL) using Response Surface Methodology (RSM). Numerical optimisation determined that a temperature of 42.21 °C, an inoculum-to-substrate ratio (ISR) of 1.5:1, and a co-substrate loading ratio of 1:20 produced the most favourable outcomes. Under these conditions, a high biogas yield of 90.76 mL/gVS was achieved, along with 33.62 % COD removal and 43.25 % VS reduction, corresponding to an overall desirability of 90.10 %. These findings demonstrate the potential of anaerobic co-digestion as an effective strategy for energy recovery and organic matter reduction in high-strength wastewater treatment systems.

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