Investigating the effects of biochars and zeolites in anaerobic digestion and co-digestion of cassava wastewater with livestock manure

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The tangible effects of climate change and the influence of environmental factors on climate have driven a shift towards cleaner and more sustainable energy sources. This study investigates the effects of biochar and zeolites in the anaerobic digestion (AD) and co-digestion processes of cassava wastewater (CW) with livestock manure, aiming to assess their impact on methane (CH_4) and carbon dioxide (CO_2) production, as well as process stability. The study employs design of experiments, analysis of variances, design parameter sensitivity, and differential analysis to explore how varying concentrations of biochar and zeolites influence key production parameters and optimize the overall process. Results indicate that increasing biochar and zeolite concentrations produce alternating effects on CO_2 and CH_4 generation, with CW significantly contributing to CH_4 production. Livestock manure shows similar effects on both gases, necessitating calculated trade-offs in prioritizing one over the other. Findings provide insights into enhancing biogas production and waste management, contributing to sustainable bioenergy and wastewater treatment practices. Numerical results demonstrate that increased biochar concentrations in the co-digestion mixtures increased CO_2 and CH_4 production rates respectively by an approximate factor of 2, contributing 18% and 5% overall effects respectively. Similarly, zeolite concentrations contributed 9% and 5% overall effects to the production rates of CO_2 and CH_4 respectively, boosting production rates by factors of 1 and −2. The study's significance lies in its potential to improve biogas yield and process efficiency, fostering advancements in renewable energy and environmental management.

KEYWORDS

anaerobic co-digestion, porous additives, conductive materials, parametric analysis, bioenergy, organic waste

1 Introduction

The demand for effective waste management, environmental pollution prevention, and the need for transition to sustainable green energy is steadily rising globally. This demand is driven by the changing climate, and expanding population resulting in a continuous and increasing flow of organic waste generated daily across diverse sectors of human activities—agriculture, municipalities, industries, and households. Anaerobic digestion technology—an age-long, low-cost technology—has been profitably applied to simultaneously reduce organic waste and produce renewable energy. Sevillano et al. (2021), highlighted several studies reporting the many benefits of anaerobic digestion in terms of energy and by-products from bio refineries. Anaerobic Digestion (AD) technology utilizes biological processes to break down organic matter without oxygen, facilitated by microorganisms (Batstone et al., 2002; Gujer and Zehnder, 1983; Gunnerson and Stuckey, 1986). The AD process usually occurs in an oxygen-free environment and involves the sequential activity of different microbial groups. The primary outcome of anaerobic digestion is the production of biogas, a mixture of methane and carbon dioxide. The AD process typically involves four critical stages; *Hydrolysis* (Complex organic materials are broken down into simpler compounds by enzymes); *Acidogenesis* (Organic acids and simpler compounds are further broken down into volatile fatty acids, alcohols, and other intermediate products); *Acetogenesis* (Volatile fatty acids are converted into acetic acid, hydrogen, and carbon dioxide) and *Methanogenesis* (Methanogenic microorganisms convert acetic acid, hydrogen, and carbon dioxide into methane and carbon dioxide) (Meegoda et al., 2018; Tang S. et al., 2023). Bioenergy production and recovery from organic wastes through AD processes offer a low-cost sustainable strategy for valorising various kinds of waste. While the general concept and science of AD has long been established in literature, there are still several ongoing studies focusing on improving different specific aspects of AD processes and exploring the biodegradability of different kinds of biomasses and feedstocks, such as food wastes (Paranjpe et al., 2023; Pilarska et al., 2023; Xu et al., 2018), lignocellulosic agricultural wastes (Karrabi et al., 2023; Lallement et al., 2023; Yan et al., 2017), animal manure (Silwadi et al., 2023; Song et al., 2023); and wastewater sludge (Di Capua et al., 2020; Nguyen et al., 2021). Various strategies have been applied to improve the overall AD process efficiency, yield and quality of bioenergy production from organic waste. Strategies such as; Pre-treatment of feedstock (Paudel et al., 2017; Soltanian et al., 2020; Zheng et al., 2014), mixing and homogeneity of substrate (Lindmark et al., 2014), optimal temperature (Buffière et al., 2018), inoculum to substrate ratio (Rajput and Sheikh, 2019), and by studying various microbial interactions (Tabatabaei et al., 2020) and synergy to improve the overall AD processes.

More recently, co-digestion and use of additives and nanoparticles (Achi et al., 2020; Hassanein et al., 2019; Jadhav et al., 2021; Kumar S. S. et al., 2021; Wang et al., 2023) have been applied to reduce the effects of inhibitors and enhance microbial activities, by providing an enabling environment for microbial interactions through the four stages of AD. Anaerobic co-digestion (AcoD), which refers to the simultaneous digestion of multiple organic substrates, has gained attention due to its potential to enhance biogas production and waste management efficiency (Karki et al., 2021; Khan et al., 2023; Xie et al., 2018). The (AcoD) process involves combining different organic materials, such as sewage, slurry, animal manure, lignocellulosic biomass and food wastes, to optimize methane yield and improve the overall performance of anaerobic digestion. Co-digestion has been found to address the low nutrient levels in certain wastes, which are insufficient for standalone anaerobic digestion, making it a viable option for improving methane yield (Abbas et al., 2023; Chow et al., 2020; Siddique and Wahid, 2018). Mathematical models have been developed to optimize anaerobic co-digestion processes, focusing on parameters such as co-substrate/inoculum ratio, C/N ratio, and temperature to enhance methane production and volatile solids reduction (Ghaleb et al., 2020). Studies have demonstrated the benefits of co-digestion with various substrates, including cheese whey, slaughterhouse waste, and microalgae, in improving biogas yield and biodegradation efficiency (Ferdeş et al., 2023; González et al., 2022; Kunatsa and Xia, 2022; Rincón et al., 2018). Furthermore, the addition of high-strength waste materials and wastewater, such as industrial wastewater, landfill leachate, cassava wastewater, waste activated sludge and food waste, has been explored for economic feasibility and enhanced biogas production. Co-digestion has also been shown to improve buffering capacity and methane production, particularly when combining substrates with low pH. Additionally, the co-digestion of carbon-rich substrate with nitrogen-rich substrate has been found to balance the C/N ratio of the substrate, benefiting the anaerobic digestion process (González et al., 2022; Wang et al., 2024). Beyond the potential economic benefits derived from high-strength wastes, there is an urgent need to prevent organic pollution and eutrophication resulting from poor management of high-strength industrial wastewaters in many developing countries (Achi et al., 2020).

Cassava wastewater (CW) is a typical example of a high-strength wastewater, with high concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total solids (TS), as well as a low pH (Peres et al., 2019; Zurita and Vymazal, 2023). Stabilization ponds, aerobic systems, and anaerobic digestion (AD) systems, have been utilized for the management of CW in certain regions, including Thailand, Brazil, Vietnam, India, and Nigeria (Kumar S. et al., 2021). Cassava is globally recognized for its paramount significance as the most extensively cultivated root crop in tropical regions. It consistently plays a crucial role in ensuring food security and holds the sixth position among the world's most important crops (Otekunrin and Sawicka, 2019). Additionally, cassava serves as a primary staple for more than 500 million people in Africa. Renowned for its resilience to drought and adaptability to challenging environments, cassava emerges as a vital strategy for climate change adaptation (Mupakati and Tanyanyiwa, 2017). Anaerobic digestion is currently being explored as a sustainable technology that can be deployed towards managing the huge streams of untreated wastes from cassava production. The anaerobic digestion of CW has raised concerns due to its low nitrogen concentration and rapid acidification (low pH) observed during AD process (Palma et al., 2018). Co-digestion with a nitrogen-rich substrate, such as animal manure, could decrease the carbon-to-nitrogen (C:N) ratio and provide buffering capacity for stabilizing the pH in order to increase methane (CH_4) production.

TABLE 1 Characterization of substrates: poultry litter (PL), dairy manure (DM), cassava wastewater (CW), and the inoculum source [The parameters include total solids (TS), volatile solids (VS), chemical oxygen demand (COD), pH, total Kjeldahl nitrogen (TKN), total phosphorus (TP), and the carbon-to-nitrogen ratio (C:N)].

	TS (g/kg)	VS (% TS)	COD (g/L)	pH	TKN (mg N/L)	TP (mg P/L)	C:N Ratio
CW	17.8 ± 0.7	97.2 ± 0.7	33.7 ± 0.8	5.53	375	222	27.8
PL	776 ± 1	80.0 ± 0.2	NA	8.25	3,675	1,245	13.0
DM	131 ± 2	87.3 ± 0.6	NA	7.33	3,450	603	15.2
Inoculum	29.5 ± 0.1	73.6 ± 7.0	25.1 ± 0.3	7.55	3,050	1,225	3.91

Source: (Achi et al., 2020).

TABLE 2 Biogas input/design variables matrix.

	Level		1	2	3	4	5
Design variables	A	Cassava Water (g)	0	28.9	38.6	57.8	x
	B	Zeolite (g)	0	0.3	1.5	3.4	x
	C	BioChar (g)	0	0.3	1.5	3.4	x
	D	Poultry Waste (g)	0	0.5	0.8	x	x
	E	Dairy Manure (g)	0	2.9	4.4	x	x
	F	Time (days)	1	2	3	4	5
Responses							
Carbon iv oxide (CO_2)			Bio-Methane (CH_4)				

In general, the use of additives and nanoparticles to enhance synergistic microbial interactions between different microbial species, through direct interspecies electron transfer (DIET), has received tremendous attention. Additives such as biochars, hydrochars, zeolites, trace metals, modified zeolites and biochars, conductive materials and nanoparticles (NPs), have contributed significantly in increased methane production by promoting microbial growth through electron transfer, preventing the accumulation of toxic concentrations of ammonia, encouraging the formation of biofilm/anaerobic granular sludge, providing nutrient supplements, removing H_2S, sequestering CO_2, and incorporating bio-augmentation (Arif et al., 2018; Hassanein et al., 2019). The application of additives and conductive materials in AD of cassava wastewater has not been sufficiently explored. A major research gap in the application of additives and conductive materials in AD of cassava wastewater is the lack of comprehensive studies on the stability and efficiency of these materials under varying operational conditions. Addressing this gap is crucial for optimizing the practical and sustainable application of AD technologies in real-world settings. To achieve sustainability in the management and utilization of cassava wastes and wastewater, through AD processes, there is a need to improve cassava wastewater AD system performance and predict specific outcomes in bioenergy and production, including

the development of guidelines and parameters that will aid in scaling up from pilot to large scale cassava AD systems to meet local energy needs in many developing countries.
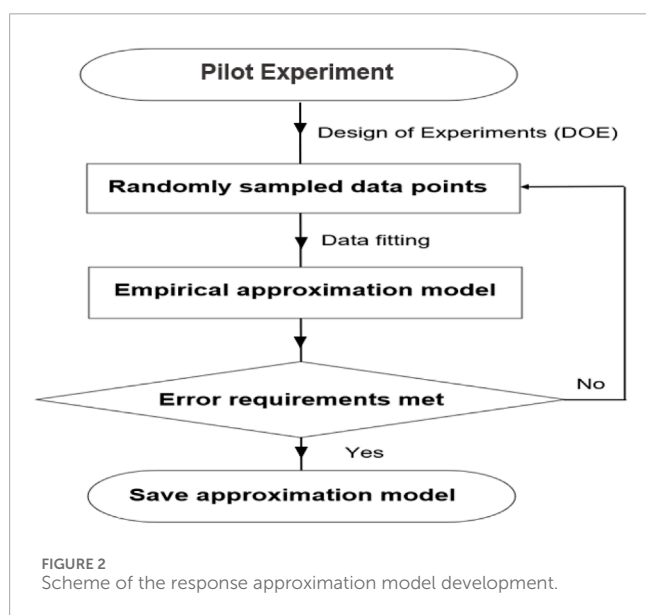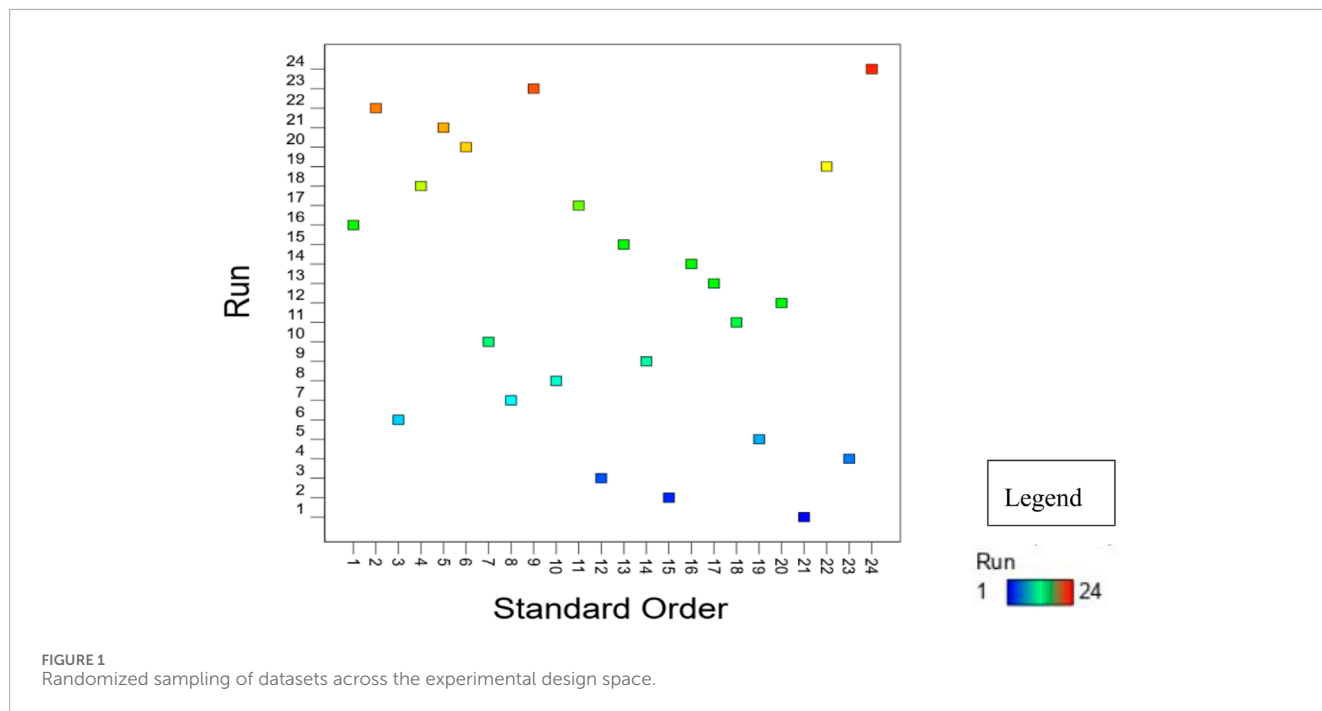
This research will build on existing literature that demonstrates the potential of biochar in optimizing anaerobic digestion (Fagbohungbe et al., 2017; Osman et al., 2023; Wei et al., 2020). Previous studies have shown that biochar can catalyze anaerobic digestion, mitigate ammonia inhibition, and enhance methane production from various organic wastes, including primary sludge and food wastes (Cai et al., 2016; Wei et al., 2020). Additionally, biochar has been found to improve microbial activity in anaerobic digestion systems and enhance nutrient availability for plant growth (Deenik and Cooney, 2016). These findings provide a strong rationale for investigating the role of biochar in the co-digestion of cassava wastewater with livestock manure. Furthermore, the study will explore the potential of zeolites in influencing the microbial and enzymatic transformations during anaerobic digestion. Zeolites have been reported to improve anaerobic digestion by influencing microbial activities, adsorbing ammonia, and supporting methanogenic activity (Montalvo et al., 2012; Paritosh et al., 2020; Tang C. C. et al., 2023). These references provide a basis for investigating the impact of zeolites in the co-digestion process and their potential to enhance biogas production from cassava wastewater and livestock manure. The experimental design involves batch experiments to assess the performance of biochar and zeolite in anaerobic digestion and co-digestion systems.

This study contributes to the understanding of the synergistic effect of biochars and zeolites on anaerobic digestion and co-digestion processes involved biogas production and methane yield, particularly within the context of additives such as cassava wastewater and livestock manure, [Dairy manure (DM) and Poultry Litters (PL)]. By synthesizing the findings from previous studies, and also applying parameter sensitivity analysis, the research will provide valuable insights into the application of biochars and zeolites for sustainable biogas production and waste management.

2 Materials and methods

2.1 Preparation of cassava wastewater and livestock manure substrates

Cassava tubers were acquired from a local market, manually peeled, and soaked for 5 days in the laboratory with 1 L/kg of

FIGURE 1
Randomized sampling of datasets across the experimental design space.

FIGURE 2
Scheme of the response approximation model development.

deionized water, replicating the traditional cassava processing steps for "fufu" production—an African dish derived from fermented cassava paste. After manual squeezing, the resulting cassava wastewater (CW) was collected for the experiments. Key characteristics of the substrate and experimental design are detailed in Tables 1, 2. The CW exhibited COD levels ranging from 29.8 to 33.4 g/L, volatile solids (VS) of 17.3 g/kg, total solids (TS) of 17.8 g/kg, and a pH of 5.5.

The characteristics of the CW substrate used in this experiment is limited by the processing conditions, considering that the fermentation was done under a controlled laboratory environment which only tried to replicate the traditional process.

The co-substrate dairy manure (DM) and Poultry Litter (PL) was collected from a livestock farm. The Poultry litter (PL) sourced from a poultry broiler farm consisted of poultry droppings and beddings from wood shavings. Both manure substrates were collected onsite and stored at 4°C until utilized. The inoculum for the experiment was derived from the digestate of a complete mixed wastewater sludge digester and was stored at 4°C before application.

2.2 Selection of biochars and zeolites

The co-treatments of biochar (B-Char) and clinoptilolite zeolite (ZEO) were introduced into the cassava wastewater (CW). The biochar (B-Char) was derived from corn stover through pyrolysis under an oxygen-free atmosphere at 500°C for 10 min (ArtiCHAR, Prairie City, Iowa, United States). The biochar particles ranged from 841 mm to <74 mm, possessing volatile solids (VS) and total solids (TS) of 690 and 980 g/kg, respectively. The zeolite used was high-purity 97% clinoptilolite zeolite from Amargosa Valley (Nye county, NV, United States), presenting as granules with an angular shape and gray color, and having a pore diameter between 4.0 and 7.0 angstroms.

2.3 Experimental design

Following the methods outlined by Moody et al. (2011), a batch digestion experiment, employing the biochemical methane potential (BMP) test, was done at the Water Quality Laboratory of the University of Maryland's Department of Environmental Science and Technology (ENST) in College Park, MD, United States. Before commencing the BMP tests, the total solids (TS)

TABLE 3 The combination of the generated sampling points.

Run	Factor A (g)	Factor B (g)	Factor C (g)	Factor D (g)	Factor E (g)	Factor F (days)	Resp. 1 (g)	Resp. 2 (g)
1	Level 3 of A	Level 4 of B	Level 4 of C	Level 2 of D	Level 1 of E	Level 1 of F	30.57	69.325
2	Level 1 of A	Level 2 of B	Level 4 of C	Level 3 of D	Level 3 of E	Level 4 of F	31.419	67.594
3	Level 2 of A	Level 1 of B	Level 2 of C	Level 2 of D	Level 1 of E	Level 2 of F	25.7	74.3
4	Level 2 of A	Level 2 of B	Level 1 of C	Level 1 of D	Level 3 of E	Level 1 of F	25.037	74.962
5	Level 3 of A	Level 3 of B	Level 3 of C	Level 3 of D	Level 3 of E	Level 2 of F	28.769	71.23
6	Level 3 of A	Level 2 of B	Level 2 of C	Level 1 of D	Level 2 of E	Level 3 of F	30.028	66.013
7	Level 4 of A	Level 1 of B	Level 3 of C	Level 2 of D	Level 3 of E	Level 5 of F	24.357	80.076
8	Level 2 of A	Level 1 of B	Level 2 of C	Level 3 of D	Level 1 of E	Level 4 of F	27.743	67.942
9	Level 4 of A	Level 4 of B	Level 1 of C	Level 1 of D	Level 3 of E	Level 2 of F	20.913	70.531
10	Level 4 of A	Level 3 of B	Level 2 of C	Level 3 of D	Level 2 of E	Level 1 of F	24.108	75.891
11	Level 3 of A	Level 1 of B	Level 1 of C	Level 3 of D	Level 2 of E	Level 5 of F	25.155	74.844
12	Level 4 of A	Level 4 of B	Level 1 of C	Level 2 of D	Level 2 of E	Level 4 of F	19.406	39.079
13	Level 1 of A	Level 1 of B	Level 3 of C	Level 1 of D	Level 2 of E	Level 1 of F	31.419	67.595
14	Level 3 of A	Level 1 of B	Level 4 of C	Level 3 of D	Level 3 of E	Level 3 of F	34.237	65.763
15	Level 2 of A	Level 3 of B	Level 4 of C	Level 1 of D	Level 2 of E	Level 5 of F	28.445	70.462
16	Level 1 of A	Level 3 of B	Level 1 of C	Level 2 of D	Level 1 of E	Level 3 of F	27.552	72.445
17	Level 4 of A	Level 2 of B	Level 3 of C	Level 3 of D	Level 1 of E	Level 5 of F	29.388	65.694
18	Level 4 of A	Level 3 of B	Level 2 of C	Level 2 of D	Level 3 of E	Level 4 of F	27.218	71.875
19	Level 1 of A	Level 2 of B	Level 4 of C	Level 2 of D	Level 2 of E	Level 2 of F	27.108	72.891
20	Level 4 of A	Level 1 of B	Level 4 of C	Level 1 of D	Level 1 of E	Level 3 of F	18.941	61.219
21	Level 2 of A	Level 4 of B	Level 3 of C	Level 3 of D	Level 2 of E	Level 3 of F	25.5	77.066
22	Level 3 of A	Level 3 of B	Level 3 of C	Level 1 of D	Level 1 of E	Level 4 of F	25.741	68.894
23	Level 2 of A	Level 2 of B	Level 3 of C	Level 2 of D	Level 3 of E	Level 3 of F	22.558	77.441
24	Level 1 of A	Level 4 of B	Level 2 of C	Level 1 of D	Level 3 of E	Level 5 of F	17.863	36.491

and volatile solids (VS) for CW, PL, DM, and the inoculum was determined and used to establish co-substrate ratios based on VS.

Before incubation, each reactor's headspace underwent a 3-min purge with N_2 to establish anaerobic conditions. The reactors were promptly sealed with a rubber septum and positioned on a shaker (120 rpm) within a controlled environmental chamber at 35°C for 48 days. Daily biogas volume was measured through volumetric displacement, employing a graduated, gas-tight 50 mL glass syringe inserted through the rubber septum. Biogas production was quantified volumetrically under normal temperature and pressure conditions using a glass gas-tight syringe, calibrated to

atmospheric pressure. All methane (CH_4) production values are reported under standard temperature and pressure conditions (1 atm and 20°C) (Achi et al., 2020).

2.4 Data generation

The parametric analysis of the effects of the inputs of the biogas production was guided by design of experiments based on a random combination of its input parameters. The use of design of experiments is hinged on its convenience and effectiveness in determining the individual or interactive effects of influential factors

TABLE 4 Fit statistics of the response surrogate models as computed from the analyses of variance (ANOVA).

Metric	CO_2	CH_4	Metric	CO_2	CH_4
Std. Dev	2.26	4.78	R2	0.7836	0.8438
Mean	26.2	68.32	Adjusted R^2	0.7072	0.7886
C.V. %	8.62	7.00	Adeq Precision	11.9980	14.1439

in a data set as well as in finding out the best operating fits of sets of data into a fitness function. Consequently, quantities of the five main ingredients of the biogas production process and a sixth quantity, Time, were computed as input factors of the process while the outputs of the process were the quantities of Carbon Dioxide (CO_2) and Bio-Methane (CH_4) generated. The resulting matrix was a Design of Experiment (DOE) matrix of 6 design variables factors of 3 – 5 levels to each combine randomly to produce multiple datasets of 2 responses as shown in Table 2.

For the avoidance of skewness in the sampling of the data space, with the hindsight of the broader objectives which require determining the design variable(s) that affect and possibly optimize the yield of corresponding response, a test of coverage was carried out on the experimental design parameters using the optimal latin hypecube sampling technique which tests and allows multiple sampling of data points, and through which more combinations can be studied for each factor. Hence, providing the comparative advantage of allowing a flexible selection in the number of designs greater than the number of factors to run. The result is a quadrant of evenly spread experiment points within the n-dimensional space defined by the n number of factors as shown in the scatter plot in Figure 1.

The scheme of highly randomized experimental parameter combinations generated from the sampling process is shown in Table 3, consequent upon which the results of the experiment conducted with each run of combinations are documented in the response columns for further computation and processing.

2.5 Data collection and analysis

The Concentrations of total solids (TS) and volatile solids (VS) for all samples were determined following the standard methods for the examination of water and wastewater (Rice et al., 2012) – TS using Method 2540B and VS using Method 2540E. The pH of substrates and inoculum was measured using an Accumet AB 15 pH meter (Fisher Scientific, Hampton, NH). Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) samples were analyzed utilizing a Lachat autoanalyzer (Quikchem 8500, Hach Company, Loveland, CO, United States) with QuikChem methods 13-107-06-2-D for TKN and 13115-01-1-B for TP. The concentration of chemical oxygen demand (COD) was measured using a Hach DR 5000 spectrophotometer (Hach Company, Loveland, CO, United States). Biogas content analysis for methane (CH_4) and carbon dioxide (CO_2) was conducted by injecting a 0.10 mL gas sample through a luer-lock, gas-tight syringe into an Agilent HP 7890 A gas chromatograph (Agilent Technologies, Santa Clara, CA, United States). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and a single HP porous layer open tubular (PLOT) Q column, with an injection temperature of 250°C, a detector temperature of 250°C, an oven temperature of 60°C, and conveyed using helium gas at a flow rate of 8.6 mL/min (Hassanein et al., 2019). The carbon content of the CW, PL, DM, and inoculum were calculated using the equation (Adams et al., 1951), where % Carbon = % VS/1.8.

$$R = \frac{Q_1(C_1 \times (100 - M_1) + Q_2(C_2 \times (100 - M_2) + Q_3(C_3 \times (100 - M_3) + \dots}{Q_1(N_1 \times (100 - M_1) + Q_2(N_2 \times (100 - M_2) + Q_3(N_3 \times (100 - M_3) + \dots}$$

Where

R = C/N ratio; Qn = mass of material n ("as is", or "wet weight"); Cn = carbon (%); Nn = nitrogen (%); Mn = moisture content (%) of material n.

3 Results and discussions

3.1 Response model

In order to adequately approximate the effect of each input parameter of the biogas production process on the outcomes, a numerical approach was deployed (Figure 2), to help in reducing the volume, cost and time incurable from the repetitive experimental evaluation that is characteristic of such a parametric study. Thus, linear regression methods were applied to the response data set of the experimental design to generate a response surface distribution fit into a surrogate empirical model representative and predictive of the experimental biogas production process. The corresponding empirical surrogate for the two biogas products under consideration, CO_2 and CH_4 are presented in Equations 1, 2 as;

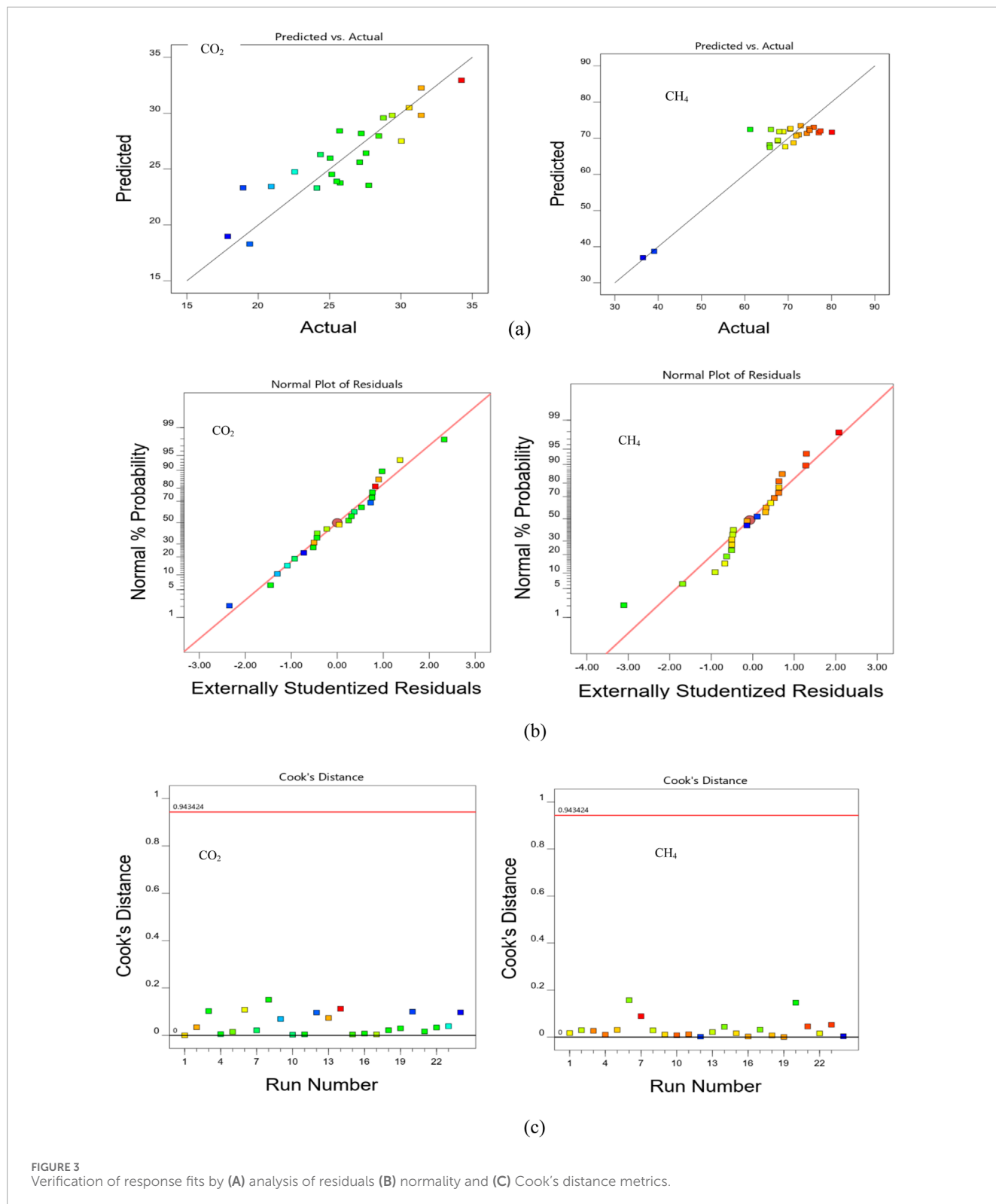$$CO_2 = 31.38 + 2.18A + 1.15B + 2.38C + 3.63D + 3.52E - 0.45F \quad (1)$$

$$CH_4 = 67.81 + 17.64A - 2.14B - 2.23C + 8.38D + 8.03E + 1.18F \quad (2)$$

Where A, B, C, D, E, F represent Cassava Water, Zeolite, Bio-Char, Poultry Litter, Dairy Manure and Time respectively. The degree of accuracy of these surrogates was verified by the analysis of the variances of the actual response values as compared to the predicted values. This is to ensure the response prediction of the fitness functions appreciably approximates the corresponding real-life experimental results and hence to a large degree reflects the underlying construct.

The R^2 values, obtained from the R-squared error analyses method is given by the expression.

$$R^2 = 1 - \frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{N} (y_i - \overline{y})^2}$$

Where N, y_i, $\hat{y}_i$, $\overline{y}$, p are the actual number of testing points within the design space, the actual value of the ith testing point, the value predicted by the approximation model, the mean of the actual

FIGURE 3
Verification of response fits by **(A)** analysis of residuals **(B)** normality and **(C)** Cook's distance metrics.

values, and the number of independent variables in the design respectively. The R^2 formulation presents the magnitude of the variance values produced by the approximation model from the experimental data, with results between 0.7 and 1 indicating a correlation with the simulation benchmark. Such high R^2 values also indicate the reliability of the data obtained by the fitness models

(Table 4). The Adequate Precision measures the signal-to-noise (SNR) ratio. A ratio greater than 4 is desirable due to its implications for measurement precision and accuracy. A higher SNR indicates that the strength of the signal is at least four times greater than the level of background noise present in the measurement (Zeng et al., 2024). The ratios of 11.998 and 14.144 indicate an adequate signal,

TABLE 5 Analysis of the Significance of Inputs from ANOVA statistics.

		CO$_2$			CH$_4$		
		F-value	*p*-value		F-value	*p*-value	
Model		10.26	<0.0001	Significant	15.30	<0.0001	Significant
Input parameter	A	5.87	0.0269	Significant	85.72	<0.0001	Significant
	B	2.26	0.1513		1.74	0.2048	
	C	5.78	0.0279	Significant	1.14	0.3000	
	D	25.41	0.0001	Significant	30.29	<0.0001	Significant
	E	23.10	0.0002	Significant	26.96	<0.0001	Significant
	F	0.4102	0.5304		0.6251	0.4401	

indicating that this response surrogate models can be used to navigate the design space.

Figure 3A presents the plots of the predicted and actual experiment data corresponding to each response. The close proximity of the data points corresponds to the results predicted by the empirical surrogates and the actual experiment to the diagonal line. The diagonal represents a perfect correlation where predicted values equal the actual values. The generality of the points falls on or close enough to the diagonal line indicating model reliability and lending credence to its validity. Figure 3B presents the distribution of residual values on normal plots in the form of studentized residuals, which is the ratio of the residuals to the estimated standard deviation of each residual. The normal distribution of the points of the plot which appreciably align with the diagonal demonstrates the adequacy of the fitness models as approximators of the experimental outcomes. Figure 3C presents the plot of the Cook's distance which describes point(s) within the design space that may be over or under-predicted, with large residuals capable of distorting the results of the model's prediction. Such points are outliers of the dataset often required to be checked and recomputed. As illustrated in the figure, all points are below the Cooks distance index Di = 1, indicating model reliability.

3.2 Results—analysis of significance and effects of biogas production inputs on outcomes

Table 5 presents the F and P values, which are indicators of design significance, derived from the analysis of variance of the response datasets. The F values are derived from the ratio of the mean square value of the respective variable to that of the corresponding residual mean square value. By statistical benchmarks, F values greater than 4, as revealed in parameters A, C, D, and E are indicators of significance on the model response for CO$_2$, while parameters A, D and E are also reported to be significant on the model response for CH$_4$.

Similarly, the P values which are also significance indicators, representing the probability values suggesting design variable effects

on the model, and by statistical benchmarks of less than the default. Value of 0.05, affirms the significance of the names inputs parameters on the respective responses. Thus in this case, this infers that the major contributors to the production of CO$_2$ in the biogas production are the inputs Cassava Water, Bio-Char, Poultry Litter and Dairy Manure as represented respectively by indicators A, C, D and E, while the major contributors to the production of CH$_4$ are Cassava Water, Poultry Litter and Dairy Manure only.

While the analysis of variance provides information on WHAT design parameters are significance on the model outcomes, it does not provide information on HOW they affect the generation of the products. Figures 4A–F which are 3D surface response graphs generated from the plot of the combination of input parameters provide such information. From Figures 4A, B, both cassava water and zeolite contribute to the yield of more CO$_2$ and CH$_4$ at constant values of other inputs. At constant quantities of zeolite, increasing cassava water input increases the yield of both products as indicated by the ascending slope of the respective graphs from left to right as the quantity of cassava water increases. This is due to its rich organic content, high biodegradability and the presence of fermentable substrates. Cassava wastewater contains high levels of organic matter, including starch, cellulose, hemicellulose, and other complex organic compounds (Khongkliang et al., 2015; Peres et al., 2019), and supported by the high volatile solids as can be seen earlier in (Table 1) which characterized cassava wastewater substrates. However, while the production of CO$_2$ increases with increasing zeolite, the rate of production of CH$_4$ decreases as the quantity of zeolite input is increased exclusively. This can be attributed to the increase in total solids concentration which potentially reduced the free available water affecting the transport of metabolites and nutrient in the vicinity of zeolite particles and associated microorganisms. Hence, elevated quantities of zeolite may elevate the apparent viscosity of the medium, impeding the efficient mass transfer between the substrate and the microorganisms crucial for the process, thereby slowing down the overall process. Moreover, alterations in the NH$_4^+$–NH$_3$ equilibrium could further impact the effectiveness of the process (Milán et al., 2001). The synergistic effect of zeolite during anaerobic digestion of organic substrate and its ability to reduce inhibition due to ammonia during AD,
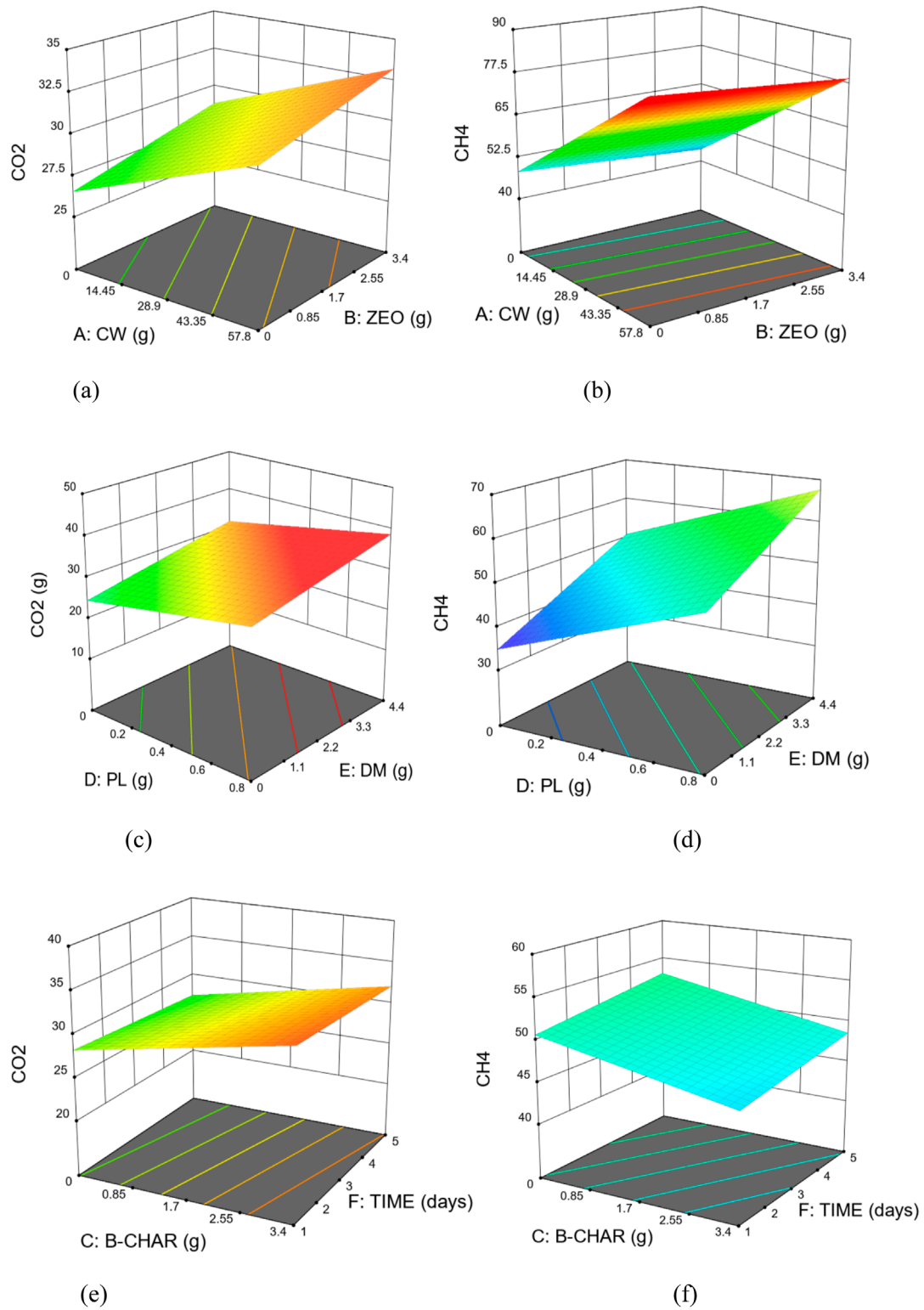
FIGURE 4
(A–F) 3D surface response graphs.

and also serve as porous medium for microbial attachment, has been extensively reported (Montalvo et al., 2012; Tang C. C. et al., 2023). The trend observed is consistent with the observation and

findings from (Milán et al., 2001) where the concentration of zeolite exceeding 6 g/L negatively affected the accumulated methane production. In our study, after keeping the quantity of zeolite

FIGURE 5
Design variable influence of product yield.
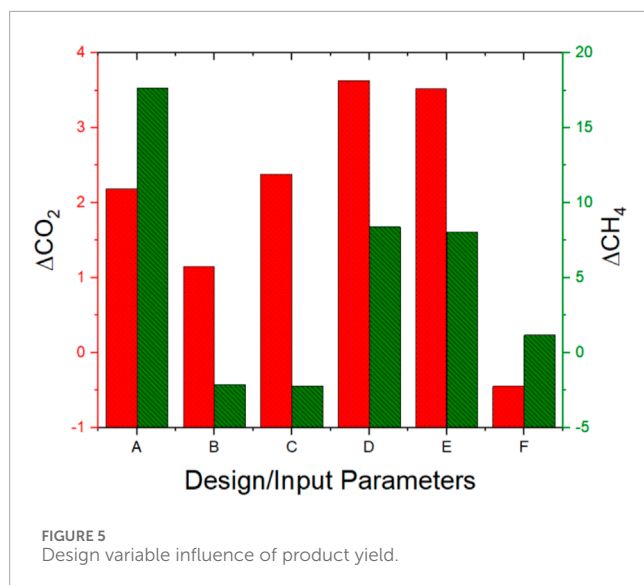
input constant while increasing cassava water input we observed an increase in the production of methane. The degree of steepness of the CH_4 graph also indicates that cassava water may be a major influencing ingredient in the generation of methane from the biogas production process.

Figures 4C, D provide information on the effect of Poultry litter and Dairy manure variations on the respective yield of CO_2 and CH_4 when quantities of other inputs are kept constant. Both inputs show the tendency to linearly increase the yield of any of the products as their quantities are increased, either independently or simultaneously. More interestingly though, the steeper slope presented by the CH_4 response plot appears to be an indication of a greater influence of both inputs on the yield of CH_4 as compared to that of CO_2. This may not be unconnected with the high nitrogen content of animal manure as seen previously in Table 1. The addition of animal dung to a carbon-rich substrate in anaerobic co-digestion has been shown to enhance the methane yield and digestion parameters, leading to improved biogas production efficiency (Renggaman et al., 2021). This improvement is attributed to the synergistic effects of combining animal dung with carbon-rich substrates, which can enhance the overall biodegradability and methane potential of the mixture (Rabii et al., 2021).

Figures 4E, F also presents the influence of "Biochar" and "Time" on the yield of CO_2 and CH_4 in the biogas production setup. While increasing the biochar content of the inputs tends to generate a relatively slight increase in the production of CO_2, the yield appears to decrease with increased time of curation of the input mixture. The highest yield of CO_2 appears to be possibly generated in this instance by the highest possible quantity of biochar input but in the least time provided. The opposite however, seems to be the case as indicated in the response of CH_4 generation. Increased input of biochar appears to be detrimental to the yield in CH_4 going by the downward slope of its response graph from left to right, whereas, allowing the mixture extended time periods contribute to further generation of CH_4. This is similar to the observation made with respect to zeolite where excessive addition of porous additives caused an increase in total solids concentration, possibly diminishing the presence

of free available water and impeding the transport of metabolites and nutrients in the proximity of biochar particles. Generally, the addition of biochar to anaerobic digestion can have varying effects on methane production. While some studies have shown that appropriate addition of biochar can significantly promote methanogenesis (Achi et al., 2020), others have indicated that high doses of biochar can negatively affect methane yield (Fan et al., 2022). According to previous reports, the alkaline functional groups on the surface of biochar can improve the buffering capacity and stability of AD process (Qi C. et al., 2021; Qi Q. et al., 2021), effectively alleviate acid accumulation and provide a stable pH environment for methanogenic microorganisms. However, it is important to highlight that anaerobic digestion (AD) requires a neutral environment. Excessive biochar presence can elevate the pH level of the digesting slurry, consequently hampering the activity of anaerobic microorganisms (Cui et al., 2021).

While the response surface plots seem to have explained how the various biogas production ingredients may affect the yield of each of the products, the extent of their influence and the hierarchy of their effects as shown in Figure 5 is of equivalent interest. The analysis of variance and surface response may have informed us about the significance or lack thereof, of the inputs, a further analysis of the differentials of the responses to the individual design variables was carried out. By simply computing the ratios of the change ΔR_i of the responses to that of the inputs ΔI_i using the formulation $\frac{\Delta R_i}{\Delta I_i}$, where i represents the ith response or input variable of the biogas production process. These where subsequently plotted as a percentage rate of change multilayer bar plot as shown in Figure 6.

Figure 6 presents the degree of influence of each input on the corresponding output. While increasing every other parameter except the amount of time utilized in the process will lead to an increase in the yield of CO_2, increasing the quantity of zeolite and time can be detrimental to the production of CH_4 The only way to simultaneously generate more CO_2 and less CH_4 appears to be by increasing the quantity of zeolite and biochar supplied into the process while keeping other inputs constant while providing an extended time for curation seems to be the only way to retard the generation of CO_2. Dairy manure and Poultry litter apparently have near same effects or influence on the generation of both CO_2 and CH_4, thus for an application of a biogas production process that supports the simultaneous yield of both products, increasing the quantities of dairy manure and poultry litter fed into the plant becomes imperative. While Cassava water may not be substantially influential in producing CO_2, it is remarkably significant to the production of CH_4. Thus, considering the inferences derived from Figures 5, 6, the hierarchy of influence of the various inputs factors, in order of decreasing influence, on the generation of the respective responses is presented in the Table 6 below.

4 Conclusion

In this parametric study, Cassava wastewater (CW) showed positive results as a viable substrate for biomethane production, and increasing the amount of CW directly correlates with an increase in CH_4 production due to its rich organic content, high biodegradability and the presence of fermentable substrates. The addition of Zeolites and biochar supported the increased yield of
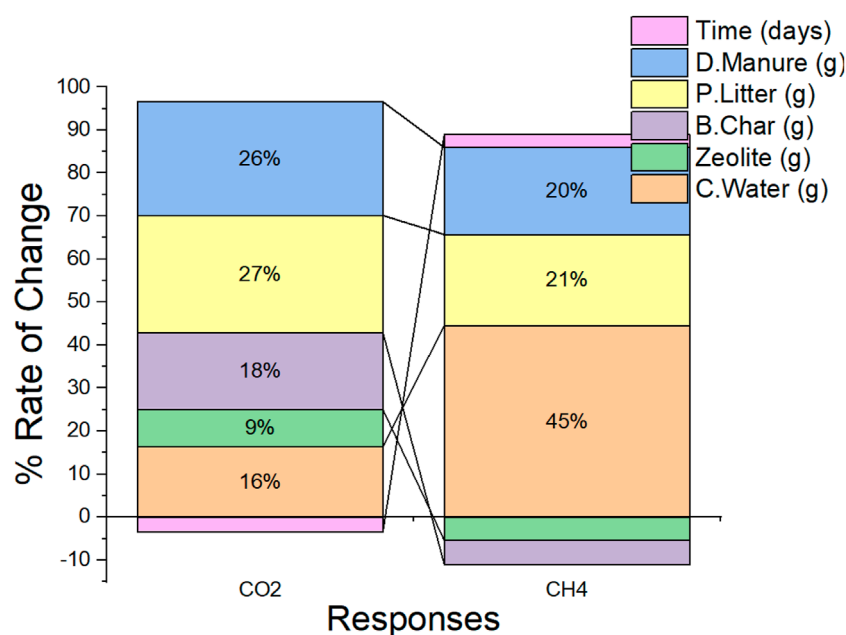
FIGURE 6
Relative degree of influence of each input on the corresponding output.

TABLE 6 Hierarchy of influences of Biogas inputs variables on the generation of CO_2 and CH_4.

	CO_2	CH_4
1st	Poultry Litter	Cassava Water
2nd	Dairy Manure	Poultry Litter
3rd	BioChar	Dairy Manure
4th	Cassava Water	BioChar
5th	Zeolite	Zeolite
6th	Time	Time

CO_2 and CH_4, although the exclusive increase in the quantity of zeolite or biochar reduced the rate of production of CH_4, due to the increase in total solids concentration which potentially reduced the free available water affecting the transport of metabolites and nutrient in the vicinity of zeolite or biochar particles and associated microorganisms. Co-digestion of carbon-rich substrate CW with Nitrogen-rich animal dung enhance the methane yield and digestion parameters, leading to improved biogas production efficiency, due to a balanced Carbon-Nitrogen ratio. In general, for all treatment mixes, an increase in time showed a decrease in CO_2 and a steady increase in CH_4. This study has the potential for a far-reaching practical application in the development of optimized AD systems for cassava wastewater treatment in agricultural and industrial settings. By incorporating specific additives and conductive materials, these systems can be tailored to maximize

biogas production, reduce waste, and lower the environmental impact. This can provide a sustainable energy source for rural communities and industries, contributing to energy self-sufficiency and reducing reliance on conventional fossil fuels.

Further studies should focus on developing efficient mechanism for the separation, capture and purification of the CO_2 component of biogas for sustainable use in food industries and for agriculture. There is a need for further studies to investigate how biochars from different lignocellulosic agricultural residues, produced at varying operating parameters can contribute in optimizing biogas production from cassava wastes. Furthermore, a comprehensive investigation is needed to understand the physicochemical attributes of biochar and interactions with microbial consortia, that significantly enhance the overall AD process.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CA: Conceptualization, Data curation, Investigation, Methodology, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. WK: Funding acquisition, Project administration, Resources, Supervision, Writing–original draft, Writing–review and editing. JS: Funding acquisition, Project administration, Resources, Supervision, Writing–original draft, Writing–review and editing. JN: Funding acquisition, Project administration, Resources, Supervision, Writing–original draft,

Writing–review and editing. FF: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing–original draft, Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer OA declared a shared affiliation with the authors to the handling editor at the time of review.

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