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Anaerobic digestion of *Gliricidia sepium* inoculated with pig dung using a portable bio-digester for process optimization

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ABSTRACT

Energy is essential for development, and sustainable energy systems are required for long-term development. Anaerobic digestion (AD) provides a sustainable approach to managing organic waste and supplying energy. This study focus on the optimization of anaerobic digestion of *Gliricidia sepium* with pig dung using response surface methodology (RSM). Central Composite Design was used for experimental design to generate the best process level and predict the optimal process. The three variable used were: mixing ratios of substrate/inoculum (S/I) ratios (1:1, 1:1.55, and 1:3.5), hydraulic retention time (HRT) (20 to 30 days), and temperature) using 25 Litre-biogas plastic digesters. Substrate characterization was performed using standard methods to assess physico-chemical and microbial properties. The model's viability was statistically tested using ANOVA to identify significant differences. The hemicellulose concentration reduced from 9 to 3 after the thermo-alkaline pretreatment. T.alkalinity, T.nitrogen, T.phosphate, T.carbon, Iron, zinc, aluminum, copper, BOD, COD, as well as potassium, sulphate, calcium, magnesium, total solids, volatile solids and manganese all showed an increase after pre-treatment. The difference between Predicted R^2 (0.8966) and Adjusted R^2 (0.9848), the model shows strong correlation and agreement with experimental data. The model's significance is confirmed by a p-value of 0.0001. This indicates a high degree of correlation between experimental data and the model. Co-digesting *Gliricidia sepium* and pig manure yields 0.0764 m^3/kg with 58.26 % methane content, suitable for small-scale applications.

1. Introduction

Inadequate energy supply and environmental degradation are huge challenges confronting Nigeria and many other emerging nations of the world [1,2]. The projection of the world energy consumption is 6.6×10^{20} and 8.6×10^{20} J in 2020 and 2040, respectively [3]. The ongoing energy crisis has precipitated significant economic constraints, thereby hindering national growth and development [4,5]. Increasing energy supply is of significant importance in Sub-Saharan Africa (SSA) because currently fewer than 45 % of the population has access to power [6,7]. The situation is simply growing worse: SSA population growth is only 3 % each year [8]. Nigeria is the most populated country in Africa, with population of over 200 million people and the vast majority of people live on less than $1.0 per day [9,10]. Only 40 percent of Nigeria's population is linked to the national energy grid; the connected population has power outage 60 percent of the time [11]. Energy is essential for development, and sustainable energy systems are required for long-term development [12,13]. Over 80 % of Nigerians rely on fuel wood for cooking and warmth, and Nigeria's current energy strategy has deemphasized the usage of fuel wood for energy [14,15]. The convergence of multiple factors, notably the depletion of conventional energy resources, volatility in international oil markets, increasing pressure to mitigate carbon emissions, and the quest for diversified energy sources among developed and emerging economies, shows a substantial decline in Nigeria's petroleum-derived revenue in the foreseeable future [16,17]. Nigeria's sustainability as a nation would be compromised unless new sources of energy are utilized to close the financial gaps caused by the declining money supplied by fossil-based sources, and to supply energy

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sources for power generation in the country [18,19]. The world's ever-increasing energy consumption has resulted in certain environmental issues, such as air pollution and global warming [3,20]. The prospect of Nigeria's bioenergy resources for bioelectric power generation and the role of bioenergy in alleviating the country's electricity issue are promising [21–23]. Anaerobic digestion of biodegradable waste efficiently produces high-energy, eco-friendly biogas, outperforming fossil fuels. This innovative approach tackles waste pollution, a major global environmental challenge, particularly in developing countries like Nigeria, Togo, and Cameroon. Africa must urgently transition to local, renewable energy sources to safeguard human well-being and drive national growth [24,25]. Animal waste represents a substantial feedstock opportunity for bioenergy generation in Nigeria, constituting a readily accessible and renewable resource [22,26]. One of which is pig dung deposit from livestock farmlands in southwest and eastern region [27]. Extensive studies have investigated the feasibility of biogas production from animal manure [28–30]. Oladejo et al., [31] conducted an investigation on the potential of anaerobic co-digestion for bioenergy generation, utilizing a mixture of food wastes, cow dung and piggery dung as feedstocks. The biogas digester output from corn-chaff and cow dung was optimized according to Iweka et al., [32] among so many others.

West Africa, specifically Nigeria, Sierra Leone, and Ghana, serves as a habitat for *Gliricidia* tree species (Lamiaceae and Fabaceae) [33,34]. These deciduous trees are widely cultivated in plantations across various landscapes. In local terminology, *Gliricidia sepium* is recognized as 'Agumaniye' [35]. *Gliricidia sepium* has grown throughout the arid regions from its original area to support cultivation products such as cocoa. The abundance of lignocellulosic biomass offers a significant opportunity for sustainable bioenergy and biofuel production. This organic material can be sourced from agricultural, municipal, industrial, and others [36]. Nigeria possesses a diverse range of lignocellulosic biomass resources, specifically; wood, forage grasses and shrubs and livestock manure [26,37].

Previous studies, notably alkali-treated corn stover and poultry manure using artificial neural network and response surface methodology [36], have investigated the parameters impacting biogas yield. According to Khan et al., [38], the findings indicate that factors such as digester temperature, retention time, fermentation pH value, digester pressure, significantly influence anaerobic digestion. In addition, biogas production has been modelled and optimized using response surface methodology (RSM). The optimization of biogas yield from alkali-treated corn stover and poultry manure [39], mixed canola residues with cattle manure [40], were carried out using RSM. Previous studies haven't explored using *Gliricidia sepium* digestate and pig dung as inoculum, nor optimized it using Central Composite Design (CCD). Key factors like temperature, pH, and retention period can improve biogas output.

This study investigates the optimization of sustainable energy production through alternative means, focusing on pig manure and *Gliricidia sepium* to generate pure energy, considering factors such as mixing ratio, temperature and retention time using RSM.

Nomenclature

AD	anaerobic digestion
RSM	response surface methodology
S/I	Substrate/Inoculum
G. sepium	*Gliricidia sepium*

2. Materials and methods

2.1. Materials collection

Gliricidia sepium was sourced locally from the Omu-Aran community in Kwara State, Nigeria, while the pig manure was collected from Landmark University Farms, also located in Omu-Aran, Kwara State.

2.2. Pre-treatment processes

Physical pre-treatment methods, including milling, pyrolysis, and mechanical extrusion, can effectively enhance the surface porosity of lignocellulosic biomass while concurrently reducing cellulose crystallinity and polymerization degree.

A combined pre-treatment approach, integrating mechanical and alkaline methods, was adopted from Oladejo et al., [31]. The biomass was grinded using a mill, sun dried to remove moisture form them and thereafter grinded and sieved into fine particles and then a 70 min thermal treatment at 80 °C using the EDIBON water bath as shown in Fig. 1, in line with the method of Dahunsi et al., [36].

Biomass like agricultural residues and organic waste is typically high in lignin, cellulose, and hemicellulose, which are resistant to microbial degradation [41,42]. Hydrothermal pre-treatment reduces the crystallinity and polymerization of cellulose and partially dissolves hemicellulose, enhancing the substrate's digestibility for anaerobic microbes in biogas production [43]. Hydrothermal pre-treatment reduces the amount of available cellulose and hemicellulose, leading to greater biogas yield.

2.3. Experimental set up of digester

Anaerobic fermentation was carried out for the pretreated samples and this was done using 25 Litre-anaerobic digester tanks each of height 0.5 m and diameter 0.25 m were made from a 25-litre gallon that was sufficiently strong to withstand pressures exerted by the mixed substrates as shown in Fig. 2. The substrates were thoroughly mixed to achieve homogeneity, before being poured into the digester. Each tank is positioned above ground level and airtight, with each tank connected to a gas collection system via a plastic hose, allowing biogas to be transferred to a storage tube.

2.4. Experimental design

Central Composite Design (CCD) was employed to optimize the experimental conditions, investigating the interactive effects of three critical parameters: retention time, mix ratio, and substrate temperature. The retention time ranges from 20 to 30 days as shown in Table 1. The mix ratio of inoculum to substrate is 1:1, 1:1.5 and 1:3 while temperature ranges from 25 to 28 °C (mesophilic temperature). Mesophilic temperatures (typically around 30–40 °C) are widely used in anaerobic digestion for biogas production due to the stability, cost-effectiveness, and efficiency they provide [44]. Mesophilic temperatures support a diverse group of bacteria responsible for breaking down organic materials in anaerobic conditions [45]. 10 litres of water was used to mix the combined inoculum to substrate mixture from the CCD design before loading into the digester. The CCD design matrix efficiently generated a set of 20 optimal experimental runs, summarized in Table 2, to investigate the study's parameters.

2.5. Characterization of substrate

To analyze the morphological and elemental surface, it was carried out through an SEM. SEM micrograph was made using an SEM machine at 30 magnifcation of the substrate samples before and after hydrothermal treatment.

2.6. Determination of structural components and physio-chemical properties of substrate

2.6.1. Determination of pH

A 10 g sample was mixed with 10 ml distilled water, stirred, and left to settle for 30 min. After re-stirring for 2 min, the pH was measured using a calibrated Dwyer Model WPH1 pH Meter. The pH of the waste suspension was then measured using the Electrometric pH

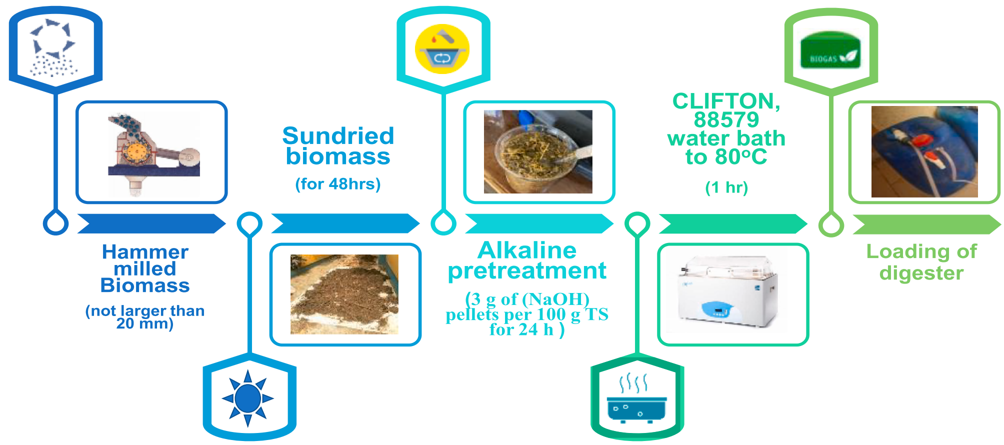

Fig. 1. Pretreatment process for *G. sepium* Co-digested with pig dung.

Fig. 2. 25 liters anaerobic digester.

Table 2
Process variables from RSM.

Run	Retention Time	Mix ratio	Temp.
1	30.00	1.00	25.00
2	20.00	1.00	25.00
3	20.00	1.00	28.00
4	25.00	0.65	26.50
5	30.00	0.30	28.00
6	25.00	0.65	26.50
7	25.00	0.65	29.02
8	25.00	0.65	26.50
9	25.00	0.06	26.50
10	30.00	0.30	25.00
11	25.00	0.65	23.97
12	20.00	0.30	28.00
13	25.00	0.65	26.50
14	25.00	0.65	26.50
15	25.00	0.65	26.50
16	20.00	0.30	25.00
17	30.00	1.00	28.00
18	25.00	1.23	26.50
19	16.59	0.65	26.50
20	33.40	0.65	26.50

Table 1
Factors and their levels for central composite design.

Variable	Symbol	Coded factor level		
		1	0	1
Retention Time	X1	20	25	30
Mix Ratio	X2	1:1	1:1.5	1:3
Temperature	X3	20	26.5	30

determinations method [46]. All determinations were performed in triplicate. Previous studies shows that bacteria thrive best within a pH range of about 6.5 to 8 [47,48]. Lower or higher pH above the range, creates an environment becomes too acidic for the methanogenic (methane-producing) bacteria or the environment becomes too alkaline, which can negatively impact the biogas production process respectively [47,49].

2.6.2. Total solids and volatile solids

For total solids (TS) analysis, samples of the substrates was dried at 105 °C to constant weight. In contrast, for volatile solids (VS), a known weight of dried sample is ignited to constant weight at temperature of 575 ± 25 °C. Following prescribed standards [50]. Total solids and Volatile solids were computed using Eqs. (1) and 2, respectively. The Physico-chemical parameters determined are as shown in the Table 1.

$$\text{Total Solid (TS) g} \Big/ \text{kg} = \frac{z_3 - z_1}{z_2 - z_1} \times 1000 \tag{1}$$

$$\text{Volatile Solid (VS) g} \Big/ \text{kg} = \frac{(z_3 - z_1) - (z_4 - z_1)}{z_2 - z_1} \times 1000 \tag{2}$$

Where,
$z_2 - z_1$=weight of wet sample
$z_3 - z_1$=weight of the sample after drying @ 105 °C
$z_4 - z_1$=weight of sample after drying @ 550 °C

2.6.3. Determination of carbon

The first step was the weighing of an empty and dry porcelain crucible (Z_1). A 10 g well-mixed organic waste sample with a determined moisture content was placed in the crucible and its weight was determined again (Z_2). The sample was then heated progressively with temperature set at intervals of 100 °C up to reach 550 °C. It remained at the final temperature of 550 °C for about 8 h. The crucible containing the white ash after being subjected to high heat was brought out from the furnace and cooled down in a desiccator before being subjected to weighing (Z_3). All measurements were conducted in triplicate.

The % Ash, % Organic matter and % Carbon were calculated as follows:

$$\% \text{ Ash in waste Sample} = \frac{z_3 - z_1}{z_2 - z_1} \times 100 \quad (3)$$

Where Z_1 = Weight of the empty dry crucible
Z2 = Weight of the dry crucible containing organic waste sample
Z3 = Weight of the dry crucible containing organic waste sample following ignition

$$\% \text{ Organic matter in waste Sample} = 100 - \% \text{ Ash} \quad (4)$$

$$\% \text{ Carbon in waste Sample} = \frac{\% \textit{ Organic matter in waste Sample}}{1.729} \quad (5)$$

Where 1.729 is the conversion factor

2.6.4. Determination of total nitrogen

The analytical procedure employed a Markham steam distillation apparatus with ammonia-free distilled water. It involved passing steam through the system for half an hour during which time, two portions of steam bank samples were collected each having a volume equaling to 50 ml that were titrated against N/70 'HCl'. Thereafter, 5mls of digestion mixture was transferred into the reaction vessel and then 10 ml 40 % sodium hydroxide added. Steam was immediately driven into this solution over 5mls 1 % boric acid containing mixed indicator (4 drops). Distillation continued for two more minutes where upon green color change observed in the pH indicator marked termination of this process; finally, collected distillate was titrated using N/70 HCl solution till pink colour was observed. The volume of standard HCl used was recorded. Reagent blanks were digested for a blank determination followed by distillation and titration with N/70 HCl. To obtain the corrected volume of N/70 HCl, subtract the volume of N/70 HCl required for the blank from the reading of the microburette.

2.6.5. Determination of C/N ratio

C:N ratios were calculated according to [51], considering the carbon content to be 50 % of the organic matter (total dried mass minus ash content multiplied by 0.5). The substrate composition utilized in the treatments comprised a mixture of 30 gs of fiber and 15 gs of agar per liter of distilled water. This formulation enabled the attainment of desired carbon-to-nitrogen (C:N) ratios.

2.6.6. Determination of other physico-chemical parameters of the substrate

Pallintest Photometer (Model 7500PHOT) used for chemical analysis of substrate samples (P, S, K, Mg, Ca, Fe, Cu, Zn, Al, Mn). Calibration: absorbance 0.5, wavelength 450 nm. Tests conducted on substrates and inoculum before anaerobic digestion. Before starting the anaerobic digestion process, chemical analyses were conducted to quantify these elements and nutrients. The photometer was calibrated following the standard procedures, set to an absorbance of 0.5, and adjusted to a wavelength of 450 nm prior to sample analysis. These tests were carried out on the substrates and inoculum of the digestions. All chemical evaluations were performed at the Environmental Engineering and Soil Mechanics/Geotechnics laboratories of Landmark University, Omu-Aran, Nigeria.

2.7. Daily monitoring of gas

To evaluate the efficacy of anaerobic treatment, gas monitoring and parameter evaluation were performed. This comprised of daily weight-based measurements of gas generation, as well as observation and chemical analysis of digestates. This was done daily with a measuring scale, and the pH was measured in the morning and evening to get an average value with a pH meter. Gas chromatography equipment was used to characterize the gases [52].

2.8. Data and statistical analysis

The data were analyzed using Design Expert software version 13.0. One-way ANOVA was used to confirm the results at a 0.05 significance level. Descriptive statistics were carried out, and the results were reported as averages with standard errors (SE).

3. Results and discussion

3.1. Characterization of un-treated and thermo-alkaline pre-treated substrate

The modified samples' micrographs demonstrate appropriate interaction between the *G. sepium* and the thermos-alkaline modifier. The Fig. 3 displays a SEM micrograph made using a SEM machine at 250 magnifications of the *G. sepium* samples before and after treatment. The mesopore voids in the treated *G. sepium* tend to be larger than those in the untreated *G. sepium*, which indicates that the thermos-alkaline modifier was able to penetrate into more voids, increasing the degradability of the substrate, as can be seen from the aforementioned SEM results. The result correlates with a similar study conducted by [36].

3.2. physio-chemical analyses of un-treated and thermo-alkaline pre-treated S. bicolor and pig dung

The outcomes of the chemical examination of the structural parameters performed on the thermo-alkaline-treated and untreated substrates utilized in the digestions are reported in Table 3. When compared to the results from the untreated experiment, the thermo-alkaline pre-treatment studies showed an increase in the concentrations of the structural elements, particularly those of cellulose, hemicelluloses, and a constant value for klason lignin. The concentration of cellulose reduced by 1.0 after the thermo-alkaline pre-treatment, whereas the content of hemicellulose decreased by 6.0 g. The result obtaion is better/higher than a study conducted by [53–55]. Comparing the treated and untreated *G. sepium*, the untreated *G. sepium's* C/N ratio was higher, ranging from 7:1 to 8:1. Iron, zinc, aluminum, copper, BOD, COD, as well as T.alkalinity, T.nitrogen, T.phosphate, T.carbon, potassium, sulphate, calcium, magnesium, and manganese well as total solids, volatile solid all showed an increase after pre-treatment as shown in Table 3.

The thermo-alkaline pre-treatment in this study led to the reduction of hemicelluloses and this eventually led to higher biogas yield in the pretreated experiment, with values of 3 ± 4.20 and 9 for pretreated and untreated respectively. These findings are consistent with earlier research that used a variety of pre-treatment techniques, including the alkaline thermo-mechanical, and thermos-alkaline [31]. However, Pig dung had the highest VS content; the VS content of the three substrates varied little. In the same vein, the nitrogen content of the substrates varied slightly. Aluminum levels were highest in Pig Dung (0.33 mg/L).

Furthermore, for the mineral elements Magnesium, Copper, Potassium, and Zinc, the greatest v values were recorded (32 ± 2.17, 2.15 ± 0.42, 4.2 ± 0.20, and 11.6 ± 0.11mg/L, respectively). This is most likely owing to the presence of these metals in Pig feed, as various elements are added to such feeds during manufacture.

3.3. Biogas generated from G. sepium co-digested with pig-dung

Gas production in the pretreated experiment started on day 3 at mesophilic temperature and continued to rise steadily until day 30, in contrast to the non-pretreated experiment where production began on day 5 and peaked on day 28. The observed delay in biogas generation may be attributed to factors like microbial acclimatization, substrate characteristics, and the acidic conditions created during the initial stages of anaerobic digestion, specifically enzymatic hydrolysis and acidogenesis, which result in a slightly acidic pH. Initially, acid accumulation hinders microbial growth due to the microorganisms' inability

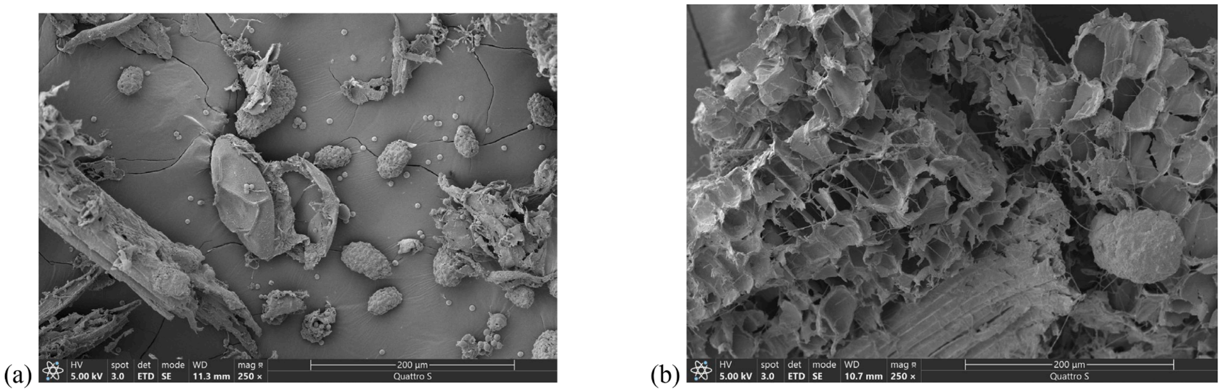

Fig. 3. SEM Micrographs of *G. sepium* at 250x mag before (a) and after treatment at 500x mag (b).

Table 3
Physiochemical properties of selected wastes before digestion.

S/N	Parameters	Pig Dung	Untreated *G. Sepium*	Treated *G. Sepium*
1	pH	7.90 ± 1.02	7.84 ± 1.31	7.65 ± 0.51
2	Moisture content (%)	90.44 ± 2.12	168 ± 5.70	88.75 ± 1.01
3	T Alkalinity	305 ± 12.60	19.6 ± 2.11	23.5 ± 43.50
4	T. Nitrogen	20.3 ± 3.66	5 ± 0.60	24.5 ± 4.50
5	T. Phosphate	2.08 ± 0.11	6 ± 1.30	2.35 ± 0.33
6	T. Carbon	248.6 ± 6.54	9 ± 0.50	198.7 ± 33.20
7	Potassium	4.2 ± 0.20	1.41 ± 0.29	4.3 ± 0.70
8	Phosphate	1.36 ± 0.31	137.3 ± 2.71	1.2 ± 0.12
9	Sulphate	56 ± 3.15	2.9 ± 0.18	60 ± 9.00
10	Calcium	44 ± 5.32	0.35 ± 0.21	50 ± 12.20
11	Magnesium	32 ± 2.17	42 ± 5.23	40 ± 2.12
12	Manganese	0.028 ± 0.30	36 ± 6.54	0.032 ± 0.0042
13	Iron	3.68 ± 0.24	2.8 ± 0.13	3.8 ± 0.60
14	Zinc	11.6 ± 0.11	0.019 ± 0.01	12 ± 1.32
15	Aluminum	0.33 ± 0.04	1.8 ± 0.25	0.36 ± 0.04
16	Copper	2.15 ± 0.42	6.2 ± 2.71	2.3 ± 0.323
17	BOD	234 ± 10.20	0.23 ± 0.12	76 ± 6.00
18	COD	974 ± 30.54	85.6 ± 8.47	390 ± 32.00
19	Total Solids	29.9 ± 13.33	26.17 ± 7.23	34.1 ± 21.20
20	Fixed Solids	18.5 ± 4.23	73.83 ± 7.21	20.6 ± 6.11
21	Volatile Solids	84.2 ± 12.41	73.83 ± 7.21	79.4 ± 14.10
22	C/N	12:1	7:1	8:1
23	HM		9	3 ± 4.20
24	Cellulose		4	3 ± 1.00

to catabolize it. However, as the methanogenic stage starts later, the pH increases, turning the substrate alkaline, and marking the commencement of methane generation.

After 30 days, the pretreated experiments showed a decline in biogas production, which ultimately ceased, likely caused by the reduced activity of biogas-producing microorganisms due to the limited availability of nutrients. Fig. 4 explains the potential of the expected value of the biogas yield to the actual value of gas yield. The total yield of biogas is subsequently affected by the mix ratio. The generated gas was observed to rise with an increase in the mix ratio. The total gas produced reduced when the mix ratio is increased to 1:3.5.

The total gas production observed in this experiment is on par with the yields reported from other prominent substrates used for biogas generation, including abbatior waste, corn chaff, Carica papayas and poultry droppings [32,36,56]. With higher biogas production compared to maize silage and lemon grass, the combination of *G. sepium* and pig dung emerges as a potential substrate for efficient biogas generation.

Compared to similar experimental runs, pre-treatment at 80 °C for 70 min was used and 1:1.55 produced the highest daily biogas yield of 0.0764m^3/kg as shown in Fig. 4. The production of biogas began in digester 1:1.55 on the fifth day of digestion, whereas it did so gradually in experiment 1:1 between the fifth and sixth days as shown in Fig. 4. The highest biogas yield, as seen in Fig. 4, produced at the highest retention time, despite the increase being minimal. The output shows that (1:3.5) has a detrimental impact on biogas output.

During the initial digestion phase (days 1–4), biogas production rates are low, possibly due to the presence of oxygen trapped in the digester at the start of the process.

The cumulative gas yield data for three distinct ratio setups; 1:3.5′, 1:1.55′, and 1:1′ are shown in Fig. 4 at various time points (days). The cumulative gas yield, which is typically expressed in conventional quantities like cubic meters or cubic feet, represents the total volume of gas generated up until a specific day.

3.4. Process parameter from S. bicolor co-digested with pig-dung

Optimal biogas production is facilitated by microorganisms operating within a narrow pH range of 6.5 to 7.5. pH values outside this range are detrimental to the survival and metabolic activity of these microorganisms. Notably, the pH of digesters co-digesting pig dung with *G. sepium* fluctuated between 6.5 and 8.5 throughout the digestion period, as illustrated in Fig. 5. The biomass pH remained relatively stable across different ratios. Average pH values were 7.50 (1:1.55), 7.7 (1:1), and 7.2 (1:1.35).

The pH value of the substrates may influence AD performance when it is too alkaline or acidic. The pH of the digestions in this investigation

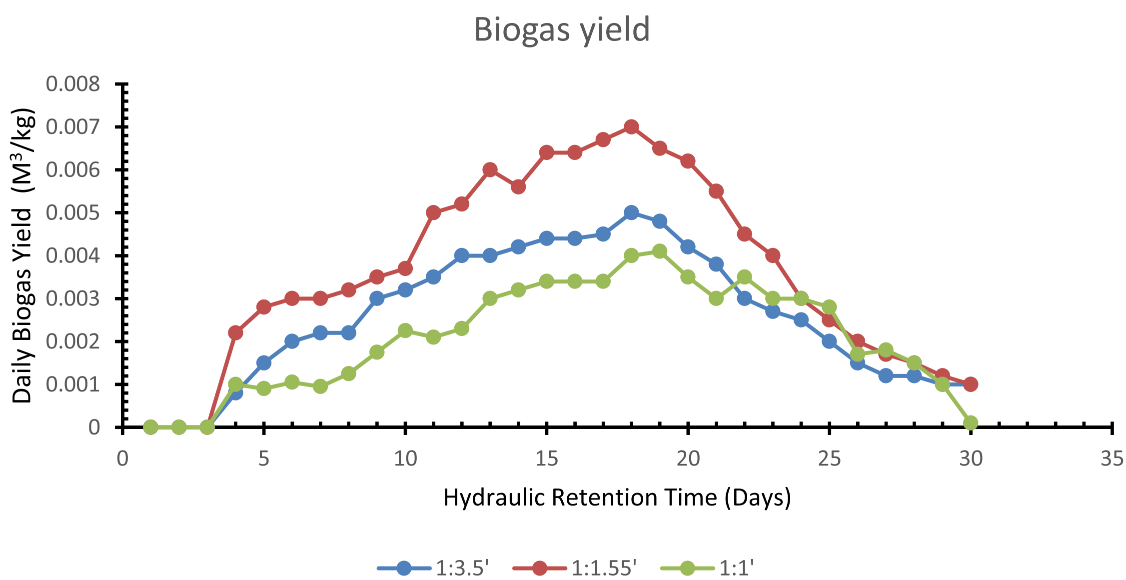

Fig. 4. Daily gas generated in the anaerobic degradation of the three samples of pig dung co-digested with *G. sepium*.

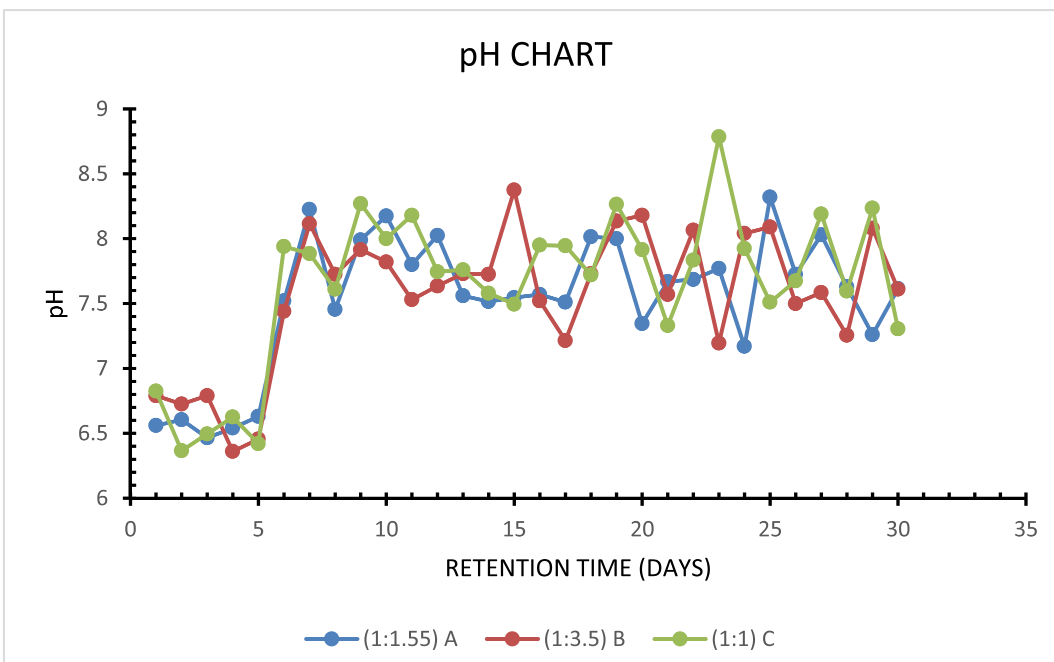

Fig. 5. pH chart samples of pig dung co-digested with *G. sepium*.

fluctuated between the acidic and alkaline range, ranging from 5.99 to 8.82 for pig dung co-digested with *G. sepium* as shown in Fig. 5. Though the pH initially fell into the somewhat acidic range in the first five days of digestion throughout the three digestions, it quickly returned to the alkaline range and remained there throughout the digestion period.

Similarly, throughout the AD period, the temperature of all reactors remained consistent within the mesophilic range.

The pH readings show the biogas production system's acidity or alkalinity at various times during the 30-day retention period. The pH scale runs from 0 to 14, with 7 denoting neutrality, values above 7 denoting alkalinity, and values below 7 denoting acidity.

These average pH levels fall within the range needed for effective anaerobic digestion. This result is justified by [52]. The digester with ratio 1:1 produced the highest pH reading of 8.86.

3.5. Optimization of G. sepium co-digested with pig-dung

Fig. 6 displays the most desirable variable values to produced optimum result. It is clear that at the inoculum to subtrate mix ratio of 1:1.55 and peak HRT, the cumulative biogas volume is at its largest. The optimal values identified for the enhancement of methane yield were retention time of about 29.66 days, mix ratio of 0.65 and temperature of approximately 27.33 °C. This optimal combination produces methane of 0.0767 m^3 thus attaining a desirability index of 1.0 which indicates that the best possible conditions within the tested range have indeed been achieved.

0.65 mix ratio indicates that an optimal of *Gliricidia sepium* and pig dung was recorded, which seeks to optimize the Carbon to Nitrogen (C/N) ratio that supports vigorous microbiological processes. Oladejo et al. [31] reported that enhancing the composition of substrate with animal

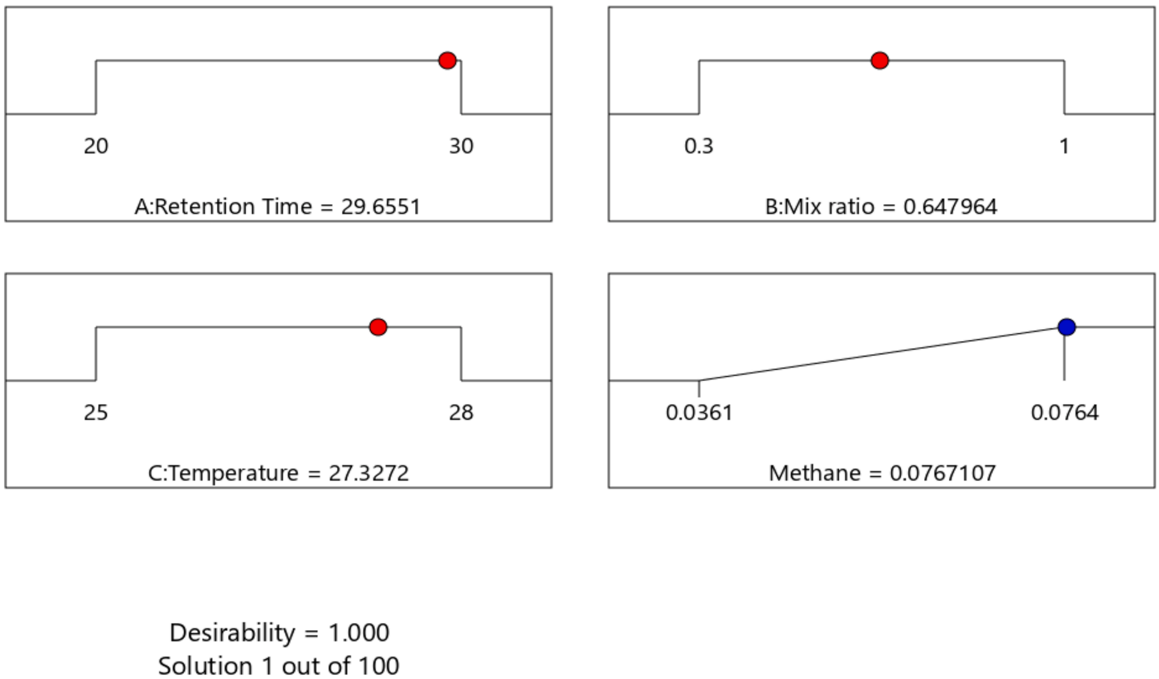

Fig. 6. Most desirable variable values to produce optimum result.

manure in biogas production leads to improved biogas yields as a result of favorable nutrient equilibrium.

The retention period of 29.66 days contributes to the accumulated findings reaches from different studies undertaken on the process of biogas production. Longer retention time promotes the digestion of the more complicated organic waste substrates thus increasing the biogas production maximally reported by Wang et al. [57]. Respective retention periods do not yield enough biogas or economic benefits. Therefore, 30 days is a better period with respect to this setup mixing ratio. A temperature of 27.33 °C is considered to be within the mesophilic range (25–40 °C) which is the most suitable temperature range for most biogas anaerobic digestion systems. The mesophilic temperature ranges encourage steady state operation of the microbes and hence low energy requirements and the possibility of using various organic

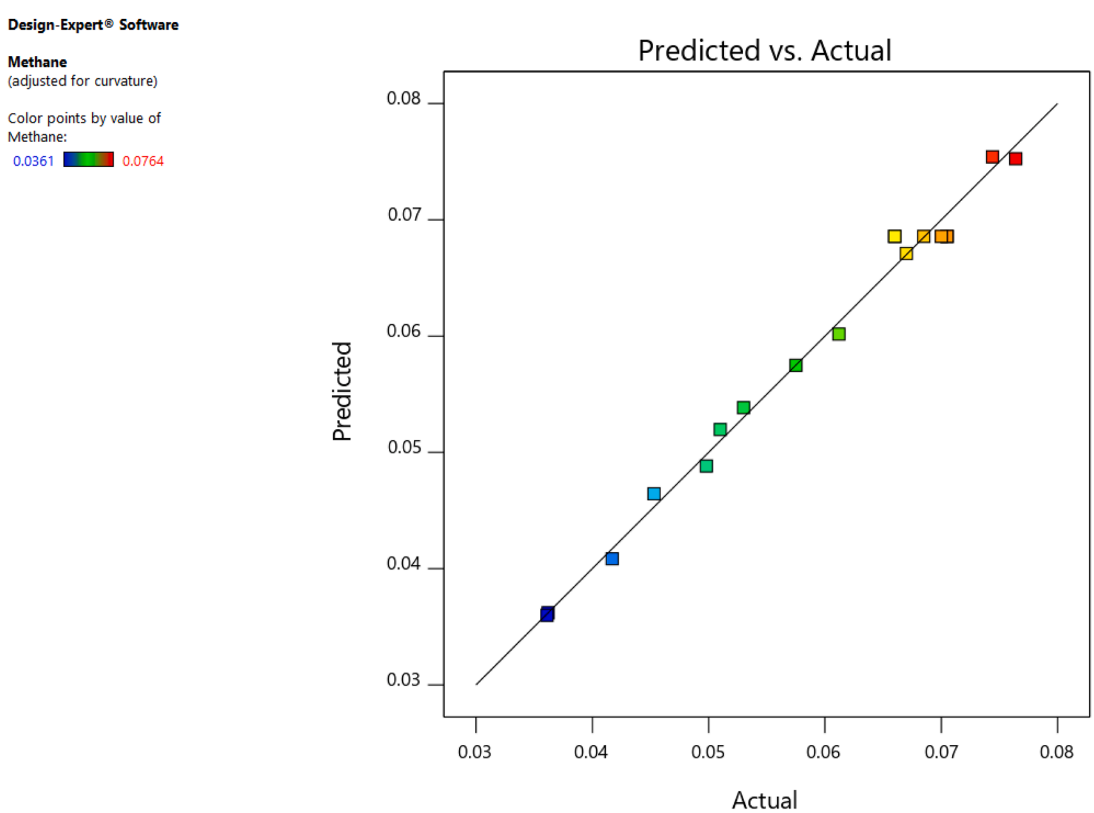

Fig. 7. Plot of predicted response against actual biogas yields of *G. sepium* Co-Digested with Pig-Dung.

substrates. This particular range has been recommended and empirically proven to be the best for biogas generation. According to Zhang et al. [58], mesophilic range temperatures support the growth and activity of microbes at low energy levels which improves the economy and efficiency of the process.

Expected against observed biogas yield values is shown in Fig. 7. Almost all data points lie on the line of fitness or very close to it. This further supports the high R^2 that this model possesses.

The graph depicting the predicted and actual biogas yield in the co-digestion of *Gliricidia sepium* and pig dung avouches the relevance of the prediction model that was used in estimating methane yield across varying conditions. The compactness of the data points along the line of equality (45°) indicates that the model is good in predicting the actual biogas yield. The RSM model, illustrated by the graph, indicates that parameters like retention time, mix ratio, and temperature were appropriately selected to improve methane production. This supports other research on biogas production which emphasizes the need to control these parameters very well to maximize biogas production.

The high correlation between predicted and observed values in this study reiterates the usefulness of RSM in optimizing biogas production parameters. Previous research has confirmed that the yields of methane generated through co-digestion reinforced with mixing ratios of specific ingredients, temperatures and retention times are as seen in this study.

Table 4
Process variables and biogas yield of *G. sepium* Co-Digested with Pig-Dung from RSM.

Run	Retention Time	Mix ratio	Temp.	Methane
1	30	1	25	0.051
2	20	1	25	0.0361
3	20	1	28	0.0417
4	25	0.65	26.5	0.0705
5	30	0.3	28	0.067
6	25	0.65	26.5	0.066
7	25	0.65	29.02269	0.0744
8	25	0.65	26.5	0.0685
9	25	0.061373	26.5	
10	30	0.3	25	0.053
11	25	0.65	23.97731	0.0612
12	20	0.3	28	0.0498
13	25	0.65	26.5	0.07
14	25	0.65	26.5	0.066
15	25	0.65	26.5	0.0705
16	20	0.3	25	0.0362
17	30	1	28	0.0575
18	25	1.238627	26.5	
19	16.59104	0.65	26.5	0.0453
20	33.40896	0.65	26.5	0.0764

$$CBY(M^3) = +0.101517 + 0.011245A - 0.0076625B + 0.00302576C - 0.00548597A^2 - 0.0384239B^2$$

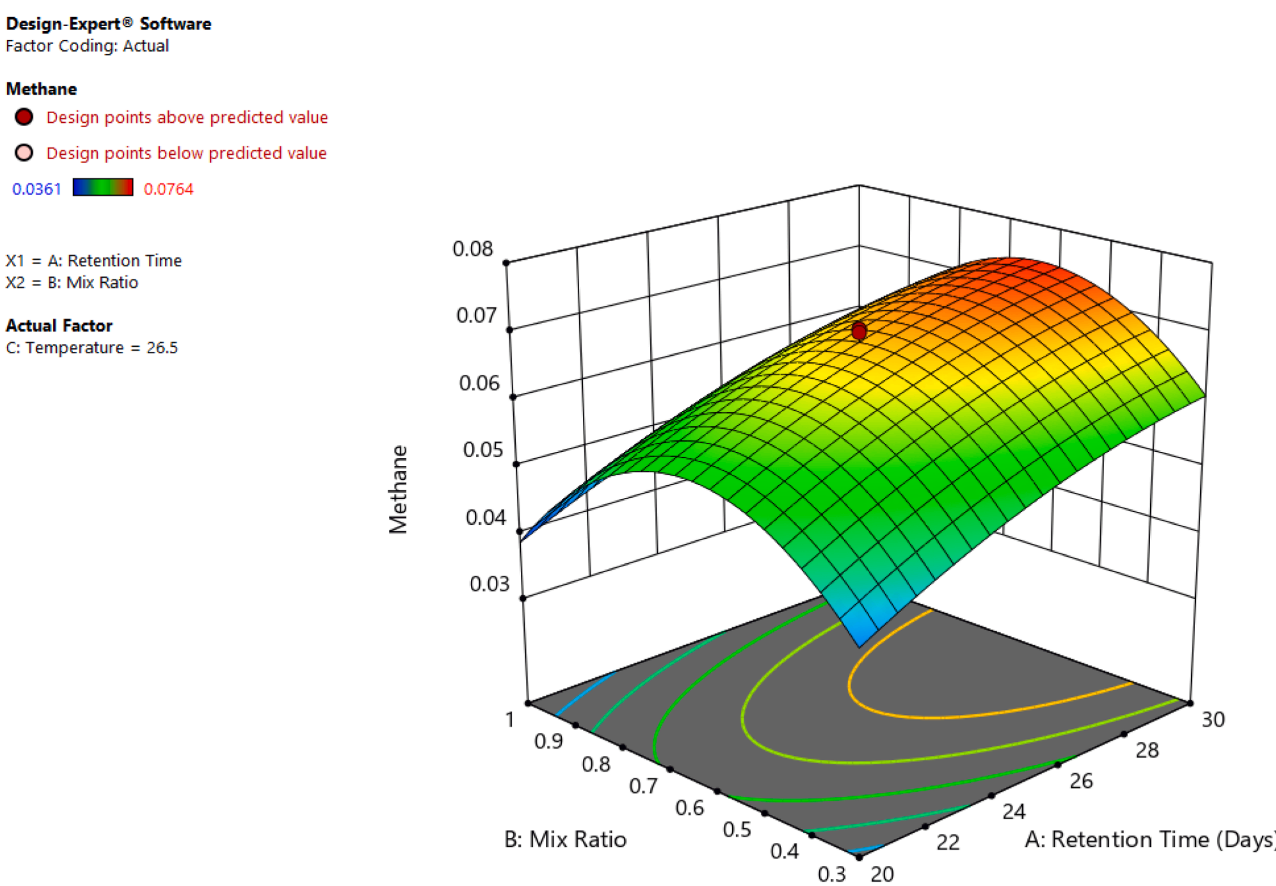

Fig. 8. Plot of predicted response against actual biogas yields.

where CBY is the cumulative biogas yield, A is the mixing ratio of substrate to inoculum, B is the hydraulic retention time and C is temperature. This equation provides a basis for predicting the outcomes associated with specific levels of each factor.

3.6. 3-D plots of relationship between process parameters of biogas from G. sepium co-digested with pig dung

The interaction between cumulative biogas volume, mixing ratio, and hydraulic retention time (HRT) is visualized in the three-dimensional plot presented in Fig. 8. Statistical analysis via ANOVA (Table 4) revealed a significant influence of the mixing ratio on overall biogas production. This correlates with a similar study by Iweka et al. [32]. The relationship between mixing ratio and biogas yield exhibited an optimal threshold. Increases in the mixing ratio initially enhanced biogas production, but a ratio of 1:3.5 and above resulted in a significant decline in cumulative biogas yield. In contrast, hydraulic retention time (HRT) demonstrated a positive correlation with cumulative biogas volume.

As demonstrated on the contour plot, the biogas output from *G. sepium* and pig dung can be maximized with adequate mix ratio and retention time adjustment in recent studies, have pointed out the essence of these factors in biogas optimization. This is further corroborated by Potdukhe et al. [59], Ebner et al. [60], Li et al. [61], Khan et al. [62] who have shown the merits of co-digestion and optimization of the processes for enhanced renewable energy production.

The retention time has a major effect on total biogas production. Research results show that longer retention gives enough time for organic materials to be fully decomposed which results in higher volumes of biogas produced [59]. In this figure, the most suitable retention period is 29 days, which corresponds with the research conducted by Ebner et al. [60] who recorded the greatest methane generation with equivalent co-digestion processes in the same period. When retention times are increased beyond normal, the activity of microorganisms increases enabling them to fully degrade complex structures of biomass.

The mix ratio of *G. sepium* to pig dung, shown here to peak at a ratio of approximately 0.65, significantly impacts the cumulative methane yield. Optimal mix ratios balance carbon and nitrogen levels, enhancing the C/N ratio suitable for anaerobic digestion, as reported by recent studies on co-digestion [61]. The balance achieved with a 0.65 mix ratio here ensures an adequate nutrient profile for microbes, similar to results obtained in studies focusing on lignocellulosic biomass co-digested with animal manure.

Retention Time averages around 25 days, ranging from about 16.6 to 33.4 days. Mix Ratio has an average of 0.65 (proportion of *G. sepium* to pig dung), with values spanning from about 0.06 to 1.24. The average temperature is around 26.5 °C and typically varies between 24 °C and 29 °C. Methane Yield has a mean value of 0.059 with minimum and maximum values of 0.036 and 0.076, respectively. This indicates that the parameter control was quite effective, looking at the effects of retention time, mix ratio and temperature on methane production. In recent works, RSM has been employed to enhance biogas production parameters with different substrates with an emphasis on the right mix ratio, retention time and temperature to optimize the yield.

Response Surface Methodology (RSM) has been utilized to adjust the interaction of these variables, especially for the purpose of optimizing biogas production. RSM was successfully employed by Li et al. [61] to optimize temperature, substrate ratio, and pH resulting in a harvest of methane production at an increase of up to 25 % when all the parameters were varied within a narrow set of optimum range. RSM maintains its competitive advantage as a tool as it reduces the number of experiments to be conducted whilst offering strong solution finding capabilities.

The impact of the mixing ratio, HRT and Temperature on the biogas yield are shown in Figs. 9 and 10, respectively. Optimization of biogas production is achieved through the simultaneous increase of mixing ratio and hydraulic retention time (HRT). Temperature exhibits no statistically significant effect on biogas production rate.

The contour plot provided aligns with recent literature, reinforcing that both temperature and mix ratio are significant factors in

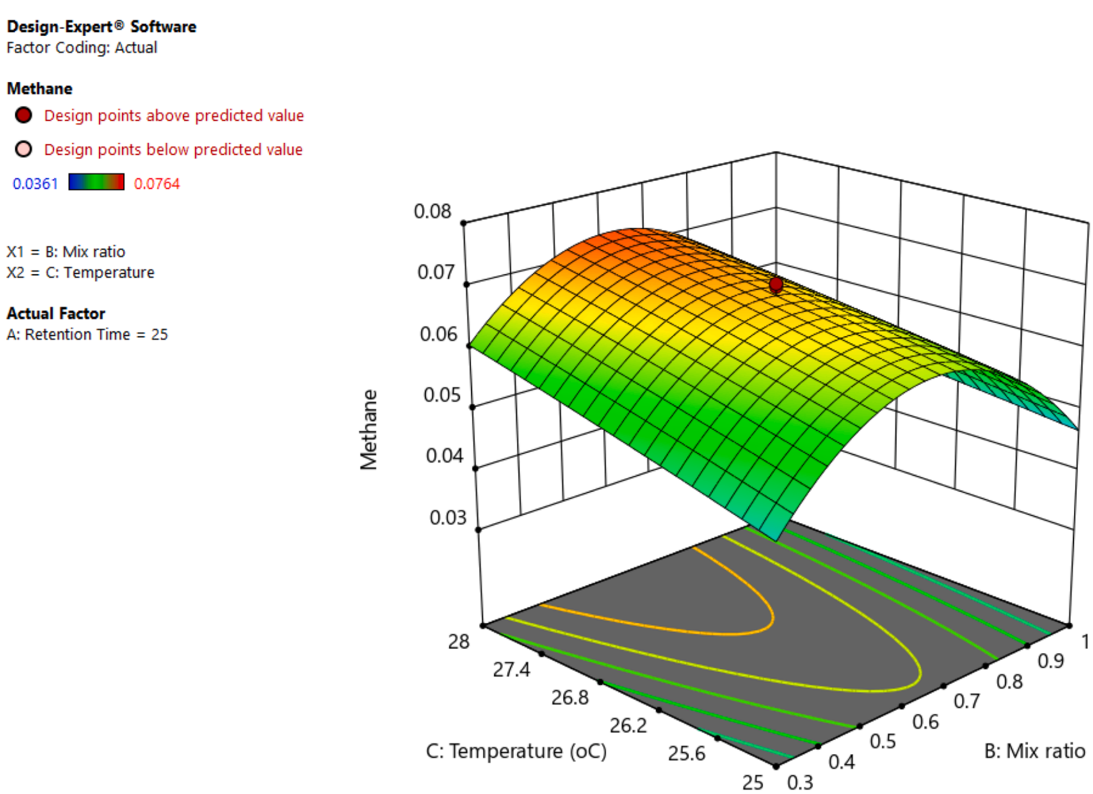

Fig. 9. Contour plot of cumulative biogas yield of *G. sepium* Co-Digested with Pig Dung temperature against and mix ratio.

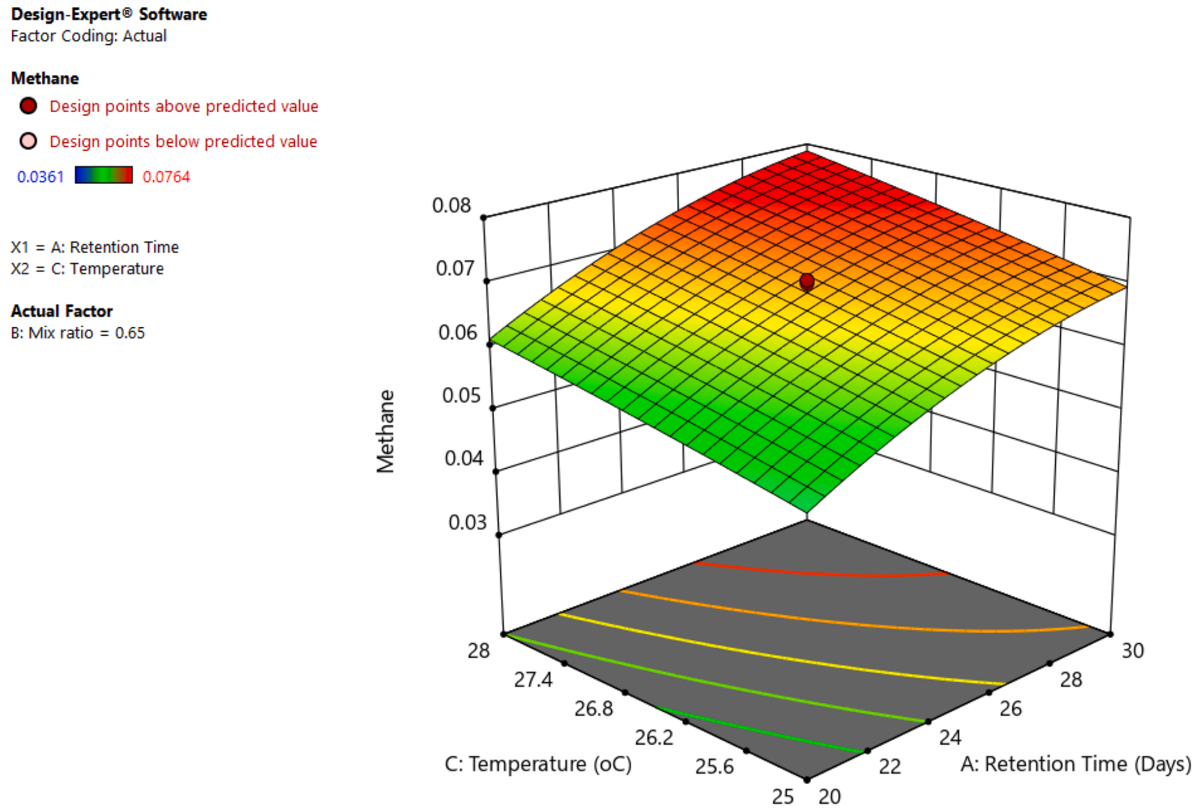

Fig. 10. Contour plot of cumulative biogas yield of *G. sepium* co-digested with pig dung temperature against and HRT.

maximizing biogas yield are shown in Figs. 9. The increasing trend in methane yield with temperature and the nuanced effect of mix ratio indicate the importance of finding optimal operating conditions. Recent studies, such as those by Yadav et al. [63] and Potdukhe et al. [59], highlight the importance of co-digestion and temperature control to achieve higher biogas productivity.

The contour plot analyzed is consistent with recent literature, further proving that both temperature and the mixing ratio are important parameters to maximize biogas production. The increasing methane yield with temperature and the controlling effect of mix ratio suggest the need for optimal conditions to be adopted. In the recent studies by Yadav et al. [63] and Potdukhe et al. [59], the authors point out the significance of co-digestion and temperature variation for enhanced biogas production. Recent studies emphasize the relative importance of temperature on microbial activity in digestion under anaerobic conditions. Conditions with the optimal range of mesophilic and thermophilic temperatures have been shown to promote enzyme activity and subsequently the amount of biogas produced. The study by Kafle and Chen [64] also revealed that there was a significant increase in biogas yield with increasing temperatures within the mesophilic range. This corresponds well with the biogas yield improvement with temperature as illustrated in the plot above. Corresponding trends were reported by Yadav et al. [63] who reported that increasing temperatures led to improved substrate degradation as well as methane production.

There have been several investigations on the effect of different mix ratios on methane yield. According to El Mashad et al. [65], the optimal mix ratio aims at ensuring maximum nutrient availability while reducing the negative effects including ammonia inhibition. The contour plot here shows an optimal mix ratio range where methane yield is maximized, consistent with findings that balancing carbon-rich and nitrogen-rich substrates is important.

Table 6
Fit statistics of predicted biogas model of *G. sepium* co-digested with pig dung.

Std. Dev.	0.0030	R^2	0.9928
Mean	0.0802	Adjusted R^2	0.9848
C.V. %	3.77	Predicted R^2	0.8966
		Adeq Precision	31.2640

The results presented in Table 5 indicate that model terms A, B, C, A^2, and B^2 are statistically significant. In contrast, terms with p-values exceeding 0.1000 are deemed non-significant. Model reduction may be beneficial when numerous insignificant terms are present, excluding those necessary for hierarchical structure. The coefficient estimates represent the anticipated change in response variable per unit change in factor value, while maintaining constancy of remaining factors. A comparison of Predicted R^2 (0.8966) and Adjusted R^2 (0.9848) in Table 6 reveals satisfactory agreement, with a difference of <0.2.

In an orthogonal design, the intercept represents the overall average response (Table 5). Coefficients adjust this average based on factor settings. VIFs (Variance Inflation Factors) measure factor correlation: 1 indicates orthogonality, while higher values indicate multi-collinearity. VIFs < 10 are generally acceptable.

According to the results of the ANOVA, the mixing ratio clearly

Table 5
Coefficients of model from optimization studies of *G. sepium* co-digested with pig dung.

	Intercept	A	B	C	2A	2B	2C	A^2	B^2	C^2
	0.101517	0.011245	−0.0076625	0.00302576	0.000675	0.000925	−0.0001375	−0.00548598	−0.0384239	−9.42808E-05
p-values		< 0.0001	< 0.0001	0.0060	0.5449	0.4115	0.9007	0.0002	< 0.0001	0.9165

Table 7
Coefficient of coded variables in RSM of *G. sepium* co-digested with pig dung.

Factor	Coefficient Estimate	df	Standard Error	95 % CI Low	95 % CI High	VIF
Intercept	0.1015	1	0.0012	0.0987	0.1044	
A-Retention Time	0.0112	1	0.0008	0.0094	0.0131	1.0000
B-Mix ratio	−0.0077	1	0.0011	−0.0101	−0.0052	1.0000
C-Temperature	0.0030	1	0.0008	0.0011	0.0049	1.0000
AB	0.0007	1	0.0011	−0.0018	0.0031	1.0000
AC	0.0009	1	0.0011	−0.0015	0.0034	1.0000
BC	−0.0001	1	0.0011	−0.0026	0.0023	1.0000
A^2	−0.0055	1	0.0009	−0.0075	−0.0035	1.14
B^2	−0.0384	1	0.0016	−0.0420	−0.0348	1.17
C^2	−0.0001	1	0.0009	−0.0021	0.0019	1.14

influences the cumulative biogas yield as shown in Table 8. It was shown that increasing the mixing ratio increased the biogas yield. Raising the mixing ratio to S/I (1:3.5) has a negative impact on biogas production, resulting in a considerable decrease. Similar to how HRT increases, so does the total volume of biogas

Up until the cumulative biogas yield drops, increasing the mixing ratio and HRT boosts biogas production. The results show that S/I (1:3.5) has a detrimental impact on biogas output. When both components are at their peak, the effect can be the deciding factor.

With an Adeq Precision ratio of 31.264, significantly higher than the recommended 4, this model demonstrates a strong signal-to-noise ratio, ensuring reliable navigation of the design space. These rows were ignored for this analysis: 19, 16.

Factor coding is Coded. Sum of squares is Type III – Partial. The model's F-value (123.42) confirms its significance, with only a 0.01 % chance of occurring by chance. Additionally, p-values < 0.05 indicate significant model terms. Most desirable variable values are; Retention Time: 24.7, Mix Ratio: 0.39, Temperature: 26, Methane: 0.0849704 as shown in Fig. 6. where CBY is the cumulative biogas yield, A is the hydraulic retention time, B is the mixing ratio of substrate to inoculum and C is temperature. This equation can be used to make predictions about the response to a given level of each factor.

The coefficient estimates for coded variables in the response surface methodology (RSM) model for *G. sepium* co-digested with pig dung highlight the influence of various factors on methane production:

The baseline methane production has a coefficient of 0.1015, which is significantly positive and suggests a stable foundation for methane output when the factors are at central levels as shown in Table 7. With a positive coefficient (0.0112) and a relatively narrow confidence interval (0.0094 to 0.0131), retention time shows a strong positive influence on methane yield. This finding aligns with other studies like Li et al. [66], which emphasize that prolonged retention times allow microorganisms more opportunity to digest the substrates, leading to higher biogas production. The mix ratio has a negative coefficient (−0.0077), suggesting that higher proportions of either substrate may reduce methane output unless balanced. This finding corroborates the work of Zhang et al. [67] which noted that mixing ratios that are not proportionate may result in nutrient deficiencies and low C/N ratios, which in turn limits methane production. Additionally, temperature also has a favorable influence (coefficient 0.0030, mesophilic temperature ranges), which means that even higher temperature ranges, can stimulate methane production, as evidenced in recent research conducted by Zhang et al. [68] showing that temperature regulation improves microorganisms activity and digestion in biogas systems. The retention time and Mix

Table 8
ANOVA for Quadratic biogas model (Response 1: Methane) *G. sepium* co-digested with pig dung.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0101	9	0.0011	123.42	< 0.0001	significant
A-Retention Time	0.0017	1	0.0017	189.36	< 0.0001	
B-Mix ratio	0.0005	1	0.0005	51.50	< 0.0001	
C-Temperature	0.0001	1	0.0001	13.71	0.0060	
AB	3.645E-06	1	3.645E-06	0.3997	0.5449	
AC	6.845E-06	1	6.845E-06	0.7506	0.4115	
BC	1.513E-07	1	1.513E-07	0.0166	0.9007	
A^2	0.0004	1	0.0004	39.60	0.0002	
B^2	0.0056	1	0.0056	612.53	< 0.0001	
C^2	1.067E-07	1	1.067E-07	0.0117	0.9165	
Residual	0.0001	8	9.120E-06			
Lack of Fit	0.0001	3	0.0000	62.73	0.0002	significant
Pure Error	1.888E-06	5	3.777E-07			
Cor Total	0.0102	17				

Table 9
Biogas breakdown from *G. sepium* stem co-digested with pig dung.

Peak	RT	Name of Gas	Molecular Formula	Molecular Mass	Peak Area	% Composition
1	19.23	Ethane	C_2H_6	30	4.59	5.43
2	13.09	Oxygen	O_2	32	1.18	1.40
3	14.85	Carbon dioxide	CO_2	44	21.03	24.90
4	41.93	Nitrogen	N_2	28	2.47	2.92
5	25.36	Methane	CH_4	16	49.21	58.26
6	34.12	Carbon monoxide	CO	28	3.42	4.05
7	15.12	Ammonia	NH_3	20	1.28	1.52
8	9.05	Hydrogen	H_2	2	0.85	1.01
9	20.96	Hydrogen Sulphide	H_2S	34	0.44	0.52

Ratio interaction has a positive coefficient which is quite low and non-significant (0.0007), indicating that these two do not quite enhance each other to a great extent. This interaction might suggest that the best ratio for co-digestion of the substrates may change depending on the retention time, similar to Potdukhe et al. [59] who argued on the need to refine interactions of several parameters in a biogas system.

The model as a whole is statistically significant ($p < 0.0001$), suggesting that the variables included provide a meaningful prediction of methane production. With an F-value of 189.36 and $p < 0.0001$, retention time is a highly significant factor, indicating its critical role in enhancing methane yield. The ratio of *G. sepium* to pig dung also significantly influences methane production ($F = 51.5$, $p < 0.0001$), highlighting the importance of optimizing co-substrate proportions in co-digestion processes.

Although significant ($p = 0.006$), temperature has a lower impact compared to retention time and mix ratio, aligning with other studies that emphasize temperature's effect on digestion efficiency but at a potentially secondary level in certain co-digestion setups.

The results are consistent with findings in recent literature where co-digestion strategies have been evaluated to enhance biogas yields. A study by Aragaw et al. [69] demonstrated that co-digestion of organic waste with livestock manure can significantly increase methane production, with retention time and substrate mix ratio as significant factors. In addition, [32] identified retention time and temperature as influential parameters in biogas output, aligning with these ANOVA results on the substantial role of these variables.

Co-digestion with *G. sepium* is a novel approach that provides additional nitrogen, which could lead to a balanced C/N ratio, further optimizing the anaerobic digestion process, as observed by Yadav et al. [63]. This approach of combining plant biomass with animal manure is increasingly recognized for enhancing microbial activity and substrate availability, improving methane yields and stability in biogas production.

3.7. Characterization of biogas generated from G. sepium co-digested with pig-dung

The composition of biogas generated from pig dung co-digested with organic waste typically include several gases, with the primary components being methane (CH_4) and carbon dioxide (CO_2). The percentage of CO_2 and CH_4 in the biogas was sampled and quantified at regular intervals. Results showed composition of 58.26 % methane; 24.90 % carbon dioxide and 0.52 % for hydrogen sulfide for experiments *G. sepium* co digested Pig dung. Table 9 shows the cumulative biogas production.

4. Conclusion

At the end of the study the following conclusions were drawn;

1. Alkaline modification improved agro-waste pore structure and surface properties, enhancing hydrothermal pretreatment efficiency, as revealed by physicochemical and spectroscopic analysis.
2. ANOVA result revealed mixing ratio and hydraulic retention duration impact biogas production. The 1:1.55 substrate-to-inoculum ratio produced the highest biogas yield (0.0764 m^3/kg) among the three ratios tested (1:1, 1:3.5, 1:1.55) for *Gliricidia sepium* and pig dung co-digestion.
3. RSM analysis yielded the following equation;

$$CBY_{G.sepium}(M^3) = +0.101517 + 0.011245A - 0.0076625B + 0.00302576C - 0.00548597A^2 + - 0.0384239B^2$$

4. The model accurately predicts biogas production from anaerobic digestion of pig dung and *Gliricidia sepium*, confirmed by an R^2 value of 0.9928 and other statistical parameters.

Ethical approval

All authors listed in the manuscript have consented to authorship, reviewed and approved the final version, and granted permission for submission and publication.

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Data availability

The dataset are available via Databris repository of Landmark University.

Consent to participate

Not applicable.

CRediT authorship contribution statement

Praise Ejigboye: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Olugbenga Elemile:** Writing – review & editing, Validation, Supervision, Software, Investigation, Conceptualization. **Abu Gana:** Writing – review & editing, Validation, Supervision, Project administration. **Oladipupo Oladejo:** Writing – review & editing, Supervision, Software, Investigation, Conceptualization. **Opeyemi Olajide:** Writing – review & editing, Visualization, Software, Investigation. **Boluwatife Badejoko:** Visualization, Validation, Methodology, Investigation. **Rapuruchukwu Mezue:** Visualization, Validation, Software, Resources, Investigation. **Maureen Gesiye:** Writing – review & editing, Visualization, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors collaborated on the design of this study. Ejigboye Praise Oladapo was responsible for material preparation, data collection, and data analysis. Analysis was conducted by Praise Ejigboye, Calistus Mezue, and Boluwatife Badejoko. The manuscript was reviewed by Olugbenga Elemile, Oladipupo Oladejo and Abu Gana. The first draft was written by Ejigboye Praise Oladapo, with all authors providing input on previous versions. Opeyemi Olajide and Maureen Gesije reviewed and revised the manuscript. All authors approved the final version.

Data availability

Data will be made available on request.

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