

Response Surface Methodology Application for the Optimization of Biogas Yield from an Anaerobic Co-Digestion Process

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Abstract

This study carried out Bg anaerobic Cd. RSM was employed to investigate optimum experimental conditions for Bg yield. Input parameters were PD substrates combination, either with WH or GS. Using six different identical 25 L cylindrical digesters, 6 substrate combinations experimental setups were carried out, for an incubation time of 22 days, under ambient T of 33 °C and pH of 6.7. The setups digesters were: 25% PD:75% WH (1); 50% PD: 50% WH (2); 75% PD:25% WH (3); 25% PD:75% GS (4); 50% PD: 50% GS (5); and 75% PD:25% GS (6). From experimental observations, Bg yield started on day 5, for setups 1, 2, 4 and 5, followed by setup 3 on day 6. Setup 6 was the last digester to produce Bg, on day 7. Setup 2 recorded the highest Bg yield (75 cm³/day), while setup 6 had the lowest value yield (48 cm³/day). Furthermore, additional setups (7 and 8) were carried out for determining Bg characteristics, and evaluating the effect of a pre-activated active slurry on its yields. Optimum Bg yield (75 cm³/day) was attained by 50% PD: 50% WH. This result validates Bg production through substrates Cd, and the employment of optimization tools, in order to obtain ideal process parameters. Furthermore, quadratic model developed by RSM was highly reliable and reproducible, while the predicted values were close to the experimental ones.

Keywords: AD; additives; Bg; Cd; optimization; RSM.

Introduction*

With the increasing demand for a cleaner and safer environment, Bg technology has been the target of numerous scholars [1-2].

There are vast deposits of municipal waste in numerous parts of the world. Nigeria, in particular, is yet to offer a suitable alternative to the predominant practice of burning these wastes in the open air [3-7].

* The abbreviations list is in pages 217.

Additionally, the energy demand is ever increasing with the scaling high population, since African countries that still are behind in power generation need to explore the conversion of vast deposits of organic wastes into Bg [8-9].

AD is a Bg technology that converts organic matter into useable energy (gas), via the action of bacteria that serve as the main degrading agent. In AD, organic matter is broken down (degraded) by enzymes and bacteria, in an O-free environment [10-12].

AD end product is only called Bg, when the obtain gaseous fuel constituents include 50-75% CH₄, 25-45% CO₂, an insignificant trace of water content, within the range from 2 to 7%, and other gases, such as O, H, NH₃ and N [13].

Previous studies have shown that grasses are rich in energy substrates, and highly effective in greenhouse gas control [14-15].

Many other organic matters suitable for Bg production have also been exploited, with some recorded success. PD was found to have low C/N ratio and high NH₃ [17-18], which is a setback.

Thus, Cd of PD with other high energy substrates, suitable to AD for CH₄ yield, was recommended by [19-20].

Substrates Cd was investigated in various works, and reported to produce high biodegradation and bio CH₄ yield. In anaerobic Bg production, fermentation is the splitting of a substrate into more fragments, oxides and compounds, according to [10].

Co-generation of PD and OP, along with other additives, was considered by [21]. Co-generation of CSH and CD for Bg yield lasted 45 days. Obtained results were 193 and 33 mL/g VS, for CSH and CD mono-digestion, respectively. Maximum Bg obtained at a 75:25 ratio for CSH to CD was 186 mL/g [22]. Optimum Bg production, using PD and CP, was studied. Five different substrate Ct were considered, and the results showed that 75% PD:25% CP gave the highest Bg of 24.6 L/g VS, at a C/N ratio of 23.4, which was attributed to micro-organisms synergy in the mixture [23].

WWS and olive pomace Cd were used for Bg yield. Cd yield was 0.21 L CH₄/g. CH₄ yield increased by 17-31% [24].

Co-generation of WWS and fish waste or garden grass caused a 75% increase in the former Ct, and enhanced CH₄ yield by 1.9. There was an increase of 25% in CH₄, and the addition of 50% grass gave the production rate and final product of 1.5 and 1.7, respectively [25].

Cd of PD with WS and MG, at a blending ratio of 70:30 and 50:50, produced 330.1 and 340.1 N/kg VS, respectively, and gave an average increased yield 1.14 and 1.13 times higher than that of the individual plants [26]. Another good method for improving AD is process optimization [26-28].

The use of optimization tools, such as RSM, helps to determine the process parameters, and reduces the tedious numbers of experiments [29].

RSM and artificial neural network were used to optimize the process parameters of [30].

RSM was used to optimize the process parameters in bio-H generation from the Cd of CS WWS with BD. Under optimal conditions, obtained results showed that H generation peaked at 1787 mL/L [31].

The present study aimed at modelling and optimizing AD operating parameters, in order to enhance Bg yields from optimized parameters in PD, GS and OP anaerobic Cd. Also, the model equation will aid researchers to produce Bg without wasting time and money, and reduce the bottleneck in performing rigorous experimental runs.

Materials and methods

Materials collection

The substrates selected for the anaerobic Bg production in this study were PD, WH and GS. PD and GS substrates were obtained in a dry state, free of impurities, within Oghara area of Delta State, Nigeria, while WH was obtained from Ethiope River, in Delta state. None of the substrates was subjected to any form of chemical treatment.

Process optimization

In the experimental design of this work, RSM aided in the quantification of the controllable input parameters relationship with the obtained results. [32] and [33] stated that RSM is used in data for creating approximation models based on physical experimented observation, thus reducing the number of experimental runs needed to provide enough information for statistically acceptable results. In these six experimental setups, categorical and numerical factors were considered using one experimental design factor in RSM. The feedstock combinations for the six setups were: 25% PD: 75% WH; 50% PD:50% WH; 75% PD:25% WH; 25% PD:75% GS; 50% PD:50% GS; and 75% PD:25% GS, from 7 to 22 days.

Statistical data analysis

The statistical tool employed in analyzing the performance of the developed model was ANOVA. The interaction between the process parameters and the response of different regression models developed for the five substrates combinations was investigated. R^2 represents the quality of the fitted polynomial quality, while F-test was employed to check the statistical significance, using Design Expert version 6. Finally, the model terms were considered using P-value, at a 95% confidence level.

Experimental setup and AD

In this study, 6 AD setups were simultaneously carried out in six identical 500 cm³ cylindrical digesters, for easy stirring, and incorporated with slurry inlet, gas collection and thermometer ports. The substrate combination for the six setups was: 25% PD: 75% WH; 50% PD: 50% WH; 75% PD: 25% WH; 25% PD: 75% GS; 50% PD: 50% GS; and 75% PD: 25% GS. Each digester contained 40 g of the respective substrate combinations, along with 400 cm² water, while the slurry was occupied 75% of each digester, which tended to provide enough space for Bg production.

Furthermore, two additional setups were carried out, to determine the generated burning gas characteristics, and also to observe the time taken by the digester that received an activated slurry obtained from another functioning one. Setup 7 determined the yielded burning gas characteristics. It contained 10 g PD, 200 g WH

and 1500 cm³ water, which were agitated inside a reagent bottle. In setup 8, time taken to start Bg production by an activated slurry digester was evaluated. 30 g PD, 70 g WH, 400 cm³ slurry and 500 cm³ tap water were weighed inside a 1250 cm³ capacity plastic container. The setup was exposed to ambient T (29 to 32 °C).

Results and discussion

Bg production by anaerobic fermentation of WH and domestic waste

Set-up 1 represented Bg production with PD and WH (Fig. 1). Bg yield began after day 5, due to microorganisms inaction. Likewise, in the inactive period, aerobic bacteria consumed O available within the digester. Thus, acid formation began after all O was used up. The first gas produced was C (IV) oxide but, as fermentation proceeded, more substrates were formed and digested by active anaerobes that were enough for CH₄ production. Produced CH₄ increased steadily before Bg yield peaking, on day 11. Subsequently, a gradual decrease in production was recorded. The decrease in Bg yield was due to substrates total conversion into CH₄. At this point, the substrate available for bacteria to digest decreased, as well as C or N content availability [10, 34]. Therefore, the decline in gas yield finished once C and N were consumed.

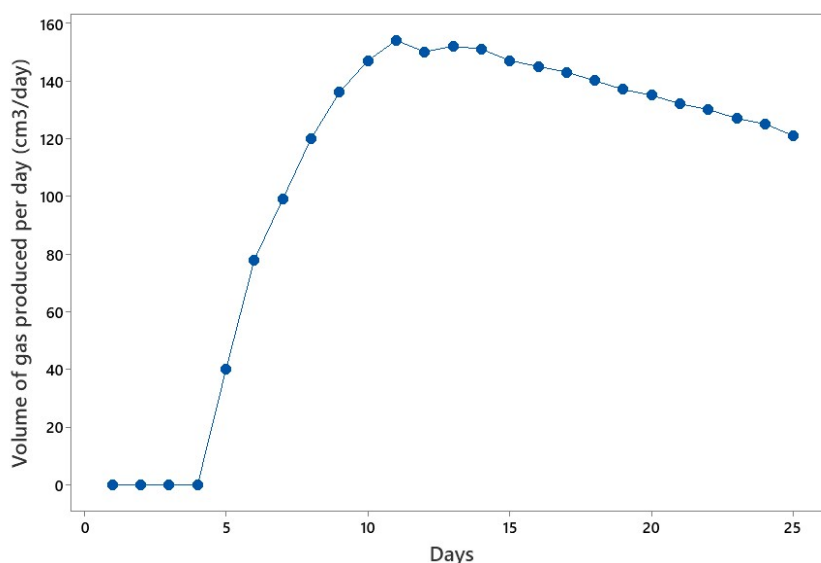


Figure 1: Daily Bg yield from setup 1 (25 PD: 75 WH).

Required ratios for optimum production

In this work, experimental substrate combinations of PD with organic wastes, such as WH and GS, were considered for optimum Bg yield. The results for Bg generated on a daily basis are presented in Fig. 2. In Bg production, the substrate combination of 50% PD: 50% WH was 1 day slower than GS samples. This was due to the high cellulose content in GS, and also to the weak presence of bacterial digesters, which settled the sample before producing Bg. The earliest Bg production was achieved by setups 2 (50% PD: 50% WH), 1 (25% PD: 75% WH) and 4 (25% PD: 75% GS), which began on day 5. This revealed that WH and GS chemical compositions were similar. The highest C/N ratio was obtained by setup 2 (50% PD: 50 % WH), with a Bg volume of 76 cm³/day, while the

lowest one was recorded for setup 6 (75% PD: 25% GS) (49 cm³/day). The bacteria responsible for the anaerobic process consumed about 30 times more C than N. At favorable Bg yield conditions, a C/N ratio of 30:1 is acceptable for any plant raw material [35]. A higher ratio than that will result in more leftover C content, after N has been consumed, which will starve bacteria in the element [36-37]. This, in turn, will lead to N restoration in the mixture, slowing the process. Exceeding N may remain at the digestion end, which happens when C has been consumed. Thus, selecting the right C/N ratio will prevent CH₄ content loss [38].

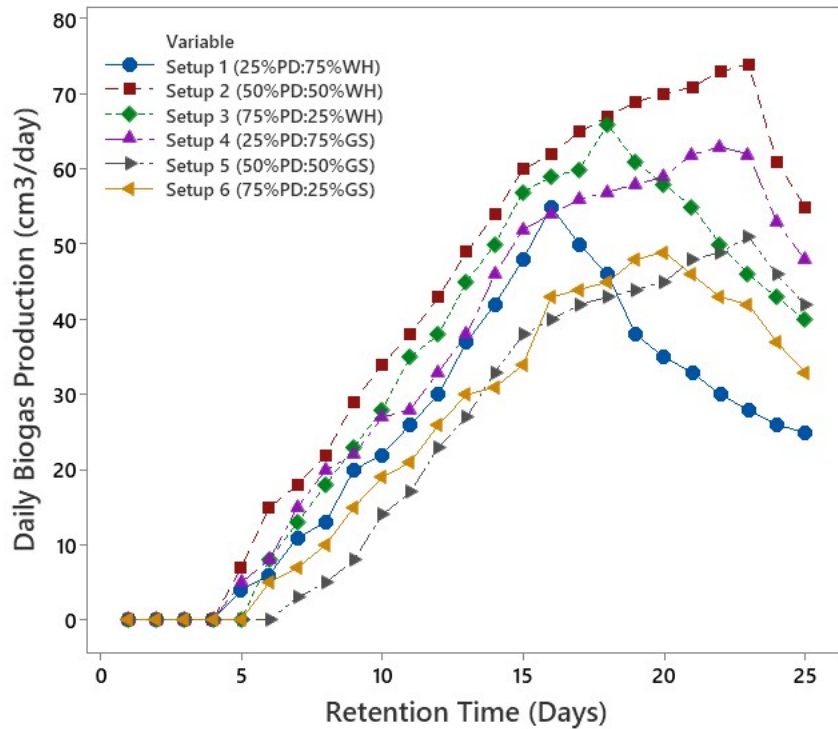


Figure 2: Graph of Bg volume produced on a daily basis by the substrate combinations of PD, either with WH or GS.

Results for burning characteristics determination

Observations from the experiment to obtain the produced Bg burning characteristics are shown in Table 3. With a Bunsen burner and a match, the produced Bg was ignited (Table 1). The early yielded Bg did not combust, since it was predominantly C (IV) oxide, because bacteria that were able to form CH₄ were not fully active yet. Hence, the acid phase was predominant in the digester. Later in fermentation, more CH₄ was formed, which aided Bg tendency to burn. At maximum capacity, as methanogens digested the substrate produced by bacteria that generated acid, CH₄ aided Bg combustion. There was blue and smokeless flame, and no soot deposition, which depicts CH₄ characteristics.

Table 1: Experimental results for yielded Bg burning characteristic.

Days	Ignition test
1 - 5	No combustion
6 - 8	Partial combustion
9 - 22	Complete combustion

Results for gas volume in a restarted digester

Results from experimental setup 7 containing a pre-activated slurry, which came from another Bg-yielding digester, are presented in Fig. 3. Bg yield started on day 3, while that of setup 1 began on day 5, since it lacked a pre-activated digester (Fig. 2). Furthermore, Bg production in setup 7 peaked earlier. This indicates that using slurry from a preceding digester to set up a new one will aid earlier Bg yield, because microbes required for the digestion process are active. However, the time lag was due to O₂ use by aerobic bacteria within the digester. By day 3, O₂ was already consumed, and Bg production started fully, due to bacteria abundance in the setup. Furthermore, already established bacteria fed on the substrate, which accelerated O₂ consume. This explains the increase in Bg yield, since the substrate bacteria population grew. Therefore, there were bacteria to digest the substrate and release more Bg in the process.

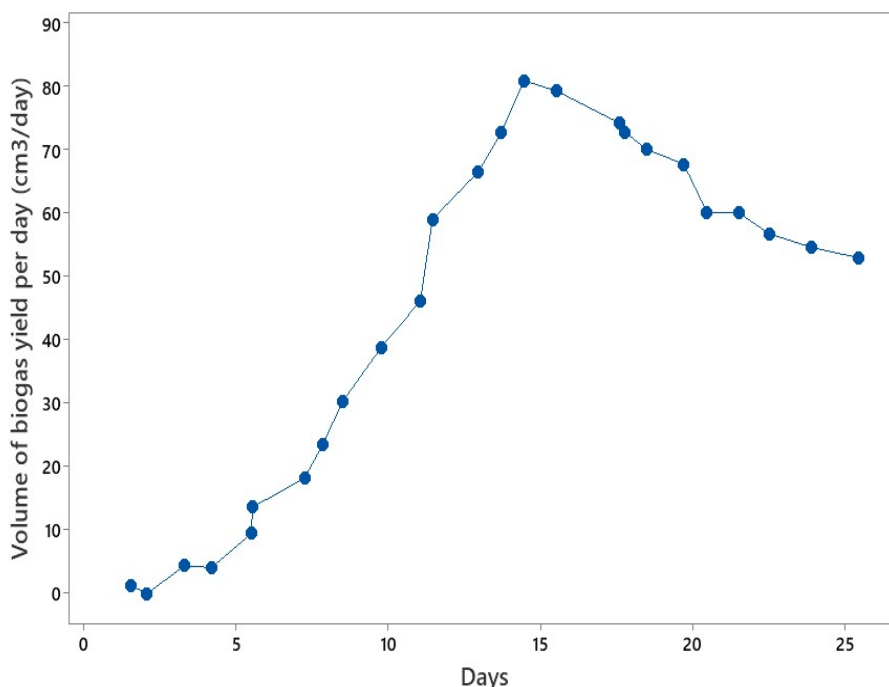


Figure 3: Volume of Bg yield per day in the restarted digester.

RSM modelling

ANOVA was employed to investigate Bg yield using the quadratic model, which was deemed fit for the optimization. Table 2 summarizes Bg yield, for 32 runs, from the various substrates combinations. The criteria for accepting regression (quadratic) model relied mainly on F- and P-values (42), where the former would compare the developed regression mean square value to the residual mean square. According to Hossain et al. (2017), a developed model can be considered to be reliable and reproducible when F- and P-values are high and low, respectively.

Experimental data consisting of one numerical factor (days) and one categorical factor (substrate Ct), with Bg yield, are shown in Table 5 (32 runs). Data were

analyzed using multiple regression techniques, for developing a RSM model. A cubic model was developed and tested for accuracy, using R² value. ANOVA was performed on the data, in order to determine the significance level of the substrates mixed in different ratios, at p<0.05 (95%).

Table 2: Experimental data showing substrates variables and Bg yield.

STD	Run	Setup	Factor 1 A: day	Factor 2 B: substrate Ct(%)	Response 1 Bg yield (cm ³)
27	1	4	10	25 PD:75 GS	27
23	2	3	22	75 PD:25 WH	57
19	3	6	10	75 PD:25 GS	36
5	4	1	14	25 PD:75 WH	39
10	5	2	7	50 PD:50 WH	6
3	6	1	10	25 PD:75 WH	19
2	7	1	7	25 PD:75 WH	0
31	8	4	22	25 PD:75 GS	51
24	9	1	22	25 PD:75 WH	57
20	10	5	14	50 PD:50 GS	52
1	11	1	7	25 PD:75 WH	0
13	12	2	14	50 PD:50 WH	56
30	13	4	18	25 PD:75 GS	48
4	14	1	14	25 PD:75 WH	39
26	15	4	7	25 PD:75 GS	0
29	16	4	14	25 PD:75 GS	41
6	17	1	18	25 PD:75 WH	47
15	18	2	22	50 PD:50 WH	76
9	19	2	7	50 PD:50 WH	6
22	20	1	18	25 PD:75 WH	61
16	21	2	22	50 PD:50 WH	75
18	22	3	7	75 PD:25 WH	9
14	23	2	18	50 PD:50 WH	63
12	24	2	14	50 PD:50 WH	56
17	25	1	7	25 PD:75 WH	9
8	26	1	22	25 PD:75 WH	41
28	27	4	14	25 PD:75 GS	41
32	28	6	22	75 PD:25 GS	51
11	29	2	10	50 PD:50 WH	39
21	30	3	14	75 PD:25 WH	62
7	31	1	22	25 PD:75 WH	41
25	32	4	7	25 PD:75 GS	0

In ANOVA analysis on Bg yield (Table 3), the model F-value of 556.24 was considerable high, thus indicating the model significance. There was only a 0.01% chance that a F-value model this large could occur due to noise. Prob > F-values lower than 0.0500 indicate that model terms were significant.

Table 3: ANOVA for response surface quadratic model.

Source	Sum of squares	DF	Mean square	F-value	Prob > F	Significance
Model	14681.62	8	1835.202	556.2392	<0.0001	significant
A (days)	11001.39	1	11001.39	3334.458	<0.0001	Significant
B (Ct)	1370.75	3	456.9167	138.4888	<0.0001	Significant
A2	2193.422	1	2193.422	664.8135	<0.0001	Significant
AB	116.0556	3	38.68519	11.72526	<0.0001	significant
Residual	75.88399	23	3.299304			
Lack of fit	75.88399	11	6.898544			
Pure error	0	12	0			
Cor total	14757.5	31				

In this case, A, B, A2 and AB were significant model terms. Values greater than 0.1000 indicate that the model terms were not significant. Factor A, which is time (days), had the highest F-value of 3334.45 and, thereby, was the most significant on Bg yield. The substrate Ct was significant, with an F-value of 138.48. The model evaluation results shown in Table 4 had a R² value of 0.9948.

Table 4: Model estimation result.

STD	1.816399	R ²	0.994858
Mean	36.625	Adj. R ²	0.993069
CV	4.95945	Pred. R ²	0.990848
Press	135.0647	Adeq. precision	68.28444

The predicted R² of 0.9908 is reasonable agrees with the adjusted R² of 0.9931. Adequated precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The model ratio of 68.284 indicates an adequate signal. This model can be used to navigate the design space. This statistical result shows that the selected model performance was accurate.

Quadratic model equations developed for the substrates Ct were expressed as follows:

[A] For substrate Ct 25 PD:75 WH

$$\text{Bg yield} = -71.89273 + 12.40749 \text{ day} - 0.32976 \times \text{day}^2 \quad (1)$$

[B] For substrate Ct 50 PD:50 WH

$$\text{Bg yield} = -69.64088 + 13.35564 \times \text{day} - 0.32976 \times \text{day}^2 \quad (2)$$

[C] For substrate Ct 75 PD:25 WH

$$\text{Bg yield} = -63.88810 + 12.77786 \times \text{day} - 0.32976 \times \text{day}^2 \quad (3)$$

[D] For substrate Ct 25 PD:75 GS

$$\text{Bg yield} = -74.85662 + 12.89638 \times \text{day} - 0.32976 \times \text{day}^2 \quad (4)$$

The plots in Figs. 4a-b show the experimental variables with no outliers. The experimental runs align with the straight line, which indicates model accuracy.

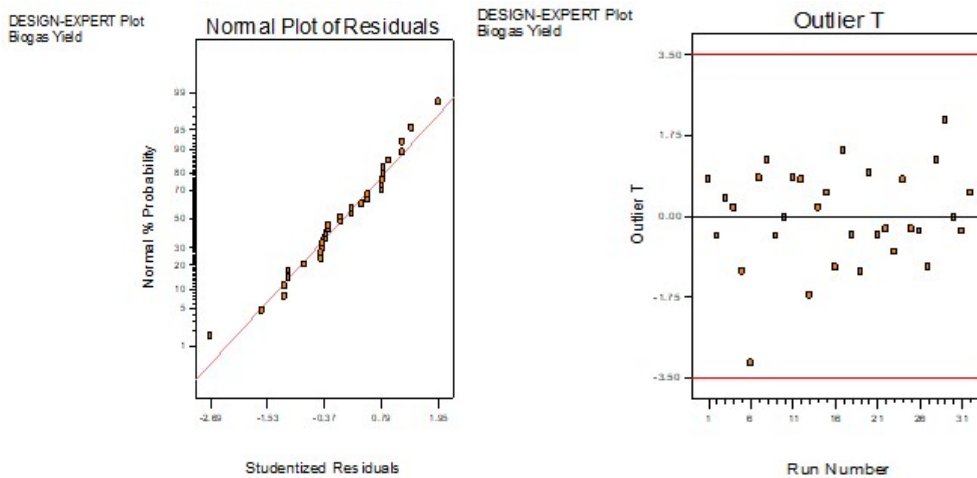


Figure 4: Diagnostic plots of design variables: (a) studentized residuals and (b) run number.

Fig. 5 shows that data points were aligned with the straight line, which indicates how precisely Bg yield was modelled. Predicted R^2 value for the developed model was 0.9908, i.e., 99.1% reliability of the empirical model developed for calculating Bg yield.

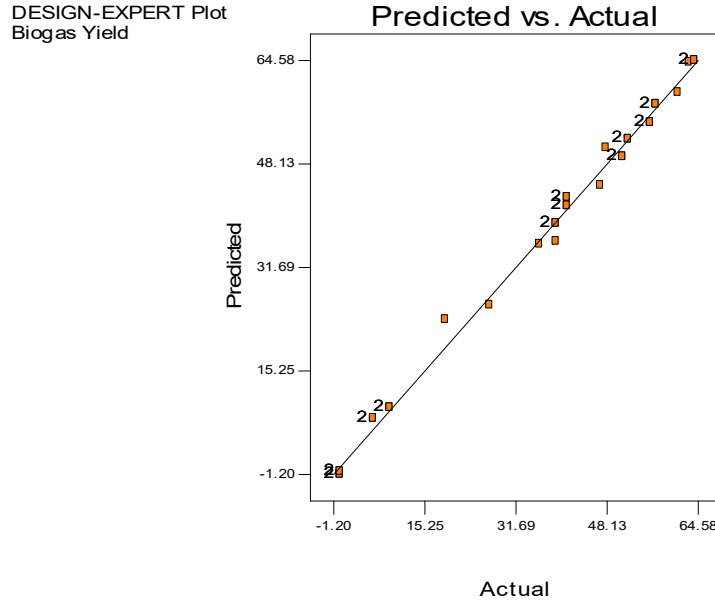


Figure 5: Plot of actual against predicted data.

Effect of variables on Bg yield

The effect of time on Bg yield volume is shown in Fig. 6. Produced Bg volume was low from day 7 to 14. Bg yield of 47 cm³ was achieved on day 18, and it decreased on day 22, for 25% PD: 75% WH (setup 1).

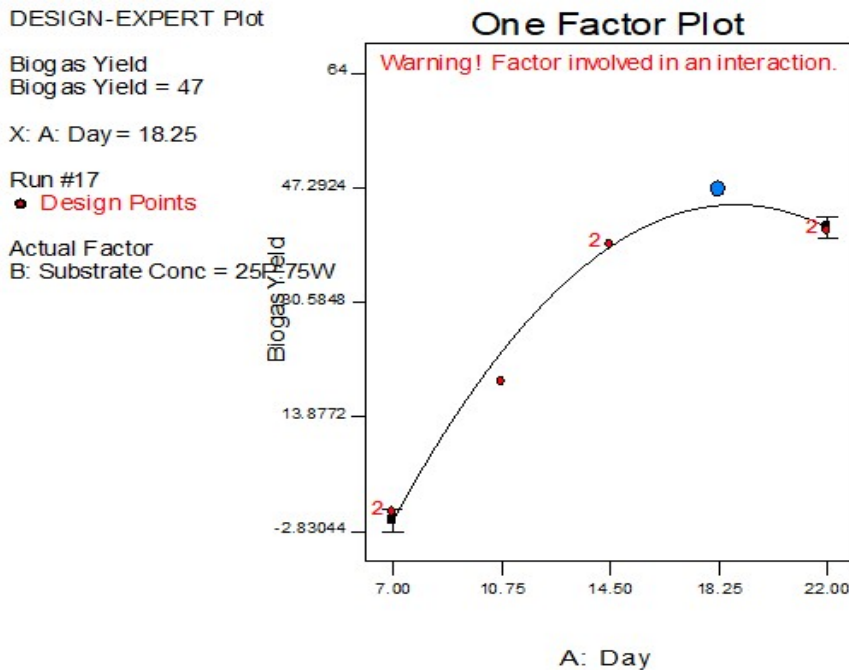


Figure 6: Plot of time (days) against Bg yield.

The substrates Ct was plotted against Bg yield, for determining their effectiveness, as shown in Fig. 7. Substrate formulation of 50% PD: 50% WH gave the highest yield of 75.56 cm³, followed by 75% PD: 75% WH. The lowest Bg yield (setup 6) was produced by 75% PD: 25% GS. All Bg yields started being recorded in day 18.

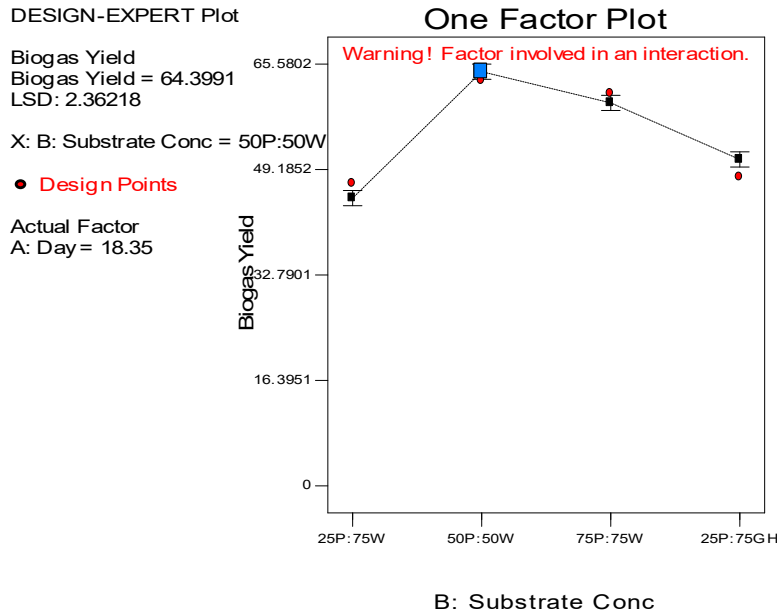


Figure 7: Plot of substrate Ct against Bg yield.

Fig. 8 shows the synergetic effect of time (days) and substrate Ct on Bg yield. 50% PD: 50% WH had Bg highest yield, on day 22, while 25% PD: 75% WH attained the lowest. On day 22, yielded Bg decreased for all the other substrates formulation, while, for 50 PD:50 WH (setup 2), it increased.

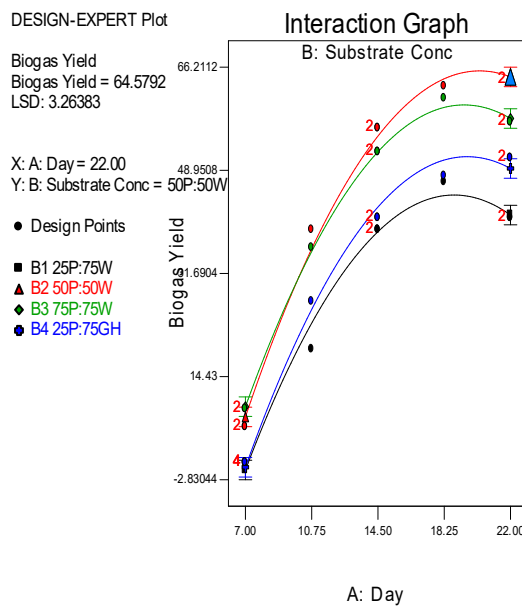


Figure 8: Plots of time (days) and substrates Ct on Bg yield.

Optimization of process variables

Numerical optimization was performed on experimental data, in order to determine optimum Bg yield with corresponding variables values. The time (days) and the substrate Ct were set in range, while Bg yield was maximized. Optimization solutions are expressed in Table 5.

Table 5: Numerical solutions for Bg yield optimization.

Number	Day	Substrate Ct(%)	Bg yield(%)	Desirability	
1	20.97	50 PD:50 WH	75.4195	1	
2	20.51	50 PD:50 WH	75.5665	1	
3	20.66	50 PD:50 WH	75.5331	1	
4	21.03	50 PD:50 WH	75.39	1	
5	19.41	50 PD:50 WH	75.3561	1	
6	19.97	50 PD:50 WH	75.5627	1	Selected
7	21.85	50 PD:50 WH	74.7433	1	
8	21.79	50 PD:50 WH	74.804	1	
9	19.38	75 PD:25 WH	63.8941	0.935845	
10	19.56	25 PD:75 GS	51.2324	0.800507	
11	18.81	25 PD:75 WH	44.8177	0.700277	

Eight out of eleven solutions (50% PD: 50% WH) had a desirability of 1, which indicates the best performance of the numerical optimization process. The optimum Bg yield of 75.56 cm³ was produced after 19-20 days. It was selected, since it had a desirability of 1, as shown in Fig. 9. This, therefore, implies that optimum Bg yield can be achieved using 50 PD% :50% WH, during 19-20 days.

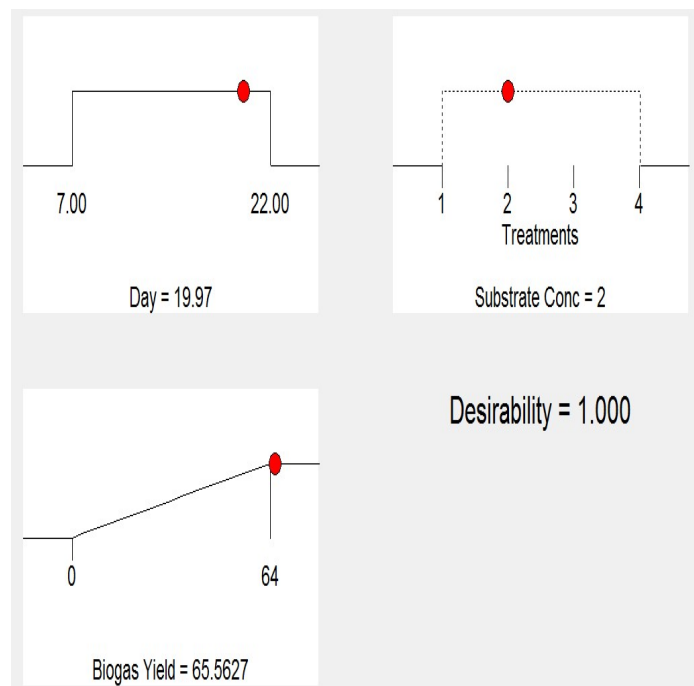


Figure 9: Ramps showing optimum Bg yield and optimal input variables.

Table 6 compares anaerobic Cd previous investigations with Cd carried out herein.

Table 6: Anaerobic Cd from different feed stocks in previous investigations vs. the present study.

S/N	Ref.	Results
1	[12]	The study evaluated Bg production from Cd of powered SD, CC and GS, using mesophilic bacteria, in the T range from 19 to 48 °C. Digester A (5 (w/w) SD:GS with 12 kg H ₂ O) had peak cumulative Bg yields of 1386 cm ³ . Digester B (SD, CC and GS, in a ratio of 1:1:1, with 15 kg H ₂ O) cumulative Bg production was 2811 cm ³ .
2	[39]	The study optimized bio-H yield from Cd of BD, CS and WWS, using RSM. Peak bio-H production under optimal conditions was 1787 mL H ₂ /L.
3	[40]	The study utilized a modified Gompertz model to predict kinetic typical parameters of the anaerobic Cd process, in order to obtain CD and HD best combination, for optimum Bg production. Digester D (25% CD:75% HD) had the peak daily Bg yield of 13.8 L/g VS, at production rate λ , and shortest lag phase (λ) of 0.69 L/g VS, after 5.20 days, respectively, closely followed by C (50% CD:50% HD), B (75% CD: 25% HD) and A (100% CD), while E (100% HD) had the lowest Bg production.
4	[41]	The authors investigated Bg yields from anaerobic Cd of CC, peanut husks, coffee shell, sawdust and sugar cane bagasse. The results showed that maximum Bg yield was 126.0 Nm ³ CH ₄ ton /residue.
5	[42]	The paper generated a mathematical model and optimized Bg yields from Cd of PD, CD and sugar beet root waste. Results showed that variations in C/N ratio, pH and digestion time influenced Bg production. Also, the maximum cumulative Bg yield was in the range from 105.3 to 357.1 mL/g VS.
6	[43]	The study investigated Cd optimum combination of PD with LCS biomass samples, such as WH and MS. The study depicted that Cd of PD and LCS gave a better yield of Bg and bio-CH ₄ than their mono-digestion.
7	[44]	The paper utilized RSM to optimize the feeding combination C:N ratio for enhanced Bg production from the Cd of WS, chicken manure and dairy manure. An optimum Bg product was attained at C:N ratio of 27.2:1, when the DM/CM combination was 40.3:59.7.
8	This study	This study employed RSM to optimize anaerobic Cd typical parameters, using PD, WH and GS. The results showed that 50% PD with 50% GS had the highest Bg production. Furthermore, the quadratic model developed by RSM was found highly reliable and reproducible, while the model-predicted values were in close range to the experimental values.

Conclusion

Anaerobic Bg production via Cd of PD combined with GS and WH, was established in this work. RSM application was considered for developing a quadratic model, in order to optimize the process parameters. The optimized experimental parameters were validated using Design-Expert 6.0. Furthermore, ANOVA was used to validate the developed model, considering F- and p-values. Six substrate combinations, labeled setups 1-6, were prepared. It was observed that maximum Bg was obtained in setup 2, which contained 50% each of PD and WH. Setup 4 recorded the lowest Bg yield. Setup 6 presented yielded gas characteristics that recorded no combustion from days 1 to 5, while partial combustion was recorded from days 5 to 7, and perfect combustion from days 8 to the end of the experiment. The effect of an already activated slurry was shown in setup 7, of which Bg yield started on day 3, while that of the other substrates combinations begun on day 5. Bg yield peaked on day 13, while its gradual decrease occurred on day 17. Hence, Cd different results were due to the use of the substrates varied Ct and combinations in the digesters. Finally, it was found that is advisable to initiate a digester with a pre-activated slurry.

Authors' contributions

U. J. Efetobor: conceived the theory, formulation, and methodology; sourced and analyzed the data; prepared and edited the manuscript. **A. O. Onokwai:** conceived the theory, formulation, and methodology; sourced and analyzed the data; prepared and edited the manuscript; handled the submission and review processes. **E. Onokpite:** conceived the theory, formulation, and methodology; sourced and analyzed the data; prepared and edited the manuscript. **U. C. Okonkwo:** conceived the theory, formulation, and methodology; sourced and analyzed the data. The results were discussed and commented on by all the authors.

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Conflicts of interest

The authors declare no conflict of interest.

Abbreviations

AD: anaerobic digestion
ANOVA: analysis of variance
BD: buffalo dung
Bg: biogas
CC: corn cob
Cd: co-digestion
CD: cow dung
CH₄: methane
CP: cassava peels
CS: cassava starch
CSH: cotton seed hull
Ct: concentration
CV: coefficient of variation
DF: degree of freedom
GS: groundnut shell
HD: horse droppings
LCS: lignocellulose
MG: meadow grass
N: Newton
OP: orange peeling
P: probability
PD: poultry droppings
R²: correlation coefficient
RSM: response surface methodology
SD: sheep dung
STD: standard deviation
WH: water hyacinth
WS: wheat straw
WWS: wastewater sludge
VS: volatile solid

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