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Investigating the Synergistic Effect of Temperature and pH Dynamics on Biogas Yield from Lignocellulosic Biomass Codigested with Cow dung

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Authors' contributions

This work was carried out in collaboration among all authors. All authors have significantly contributed to the development and writing of this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The study investigated biogas production from control, pre-treated and blended waste samples, while also examining the interaction effects of ambient temperature (AT), slurry temperature (ST), and pH on the biogas volume generated from the waste samples. Experimental research design was adopted for the study. Nine biodigesters of 32L capacity labelled A-I, control (A-C), pre-treated (D-F) and blended (G-I) waste samples were used for the experiment. The digestion was carried out

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for a period of 35 days using the water displacement method in a laboratory scale bio-digester system. Data analysis was carried out using Excel, SPSS and STATA softwares. The methods used included descriptive statistics, multiple regression and Analysis of Variance (ANOVA). The pH and temperature readings for 35 days ranged from (6.5-8.4), AT (20-29^{0}C), ST (23-38^{0}C). Based on the study's results, there was a significant difference in the volume of biogas generated among the waste samples (F=6.4, SS=659.379, p=0.002, df=314). Specifically, significant differences were observed between the control and pre-treated samples (p=0.01) and between the control and blended samples (p=0.036). The effects of AT and ST (P=0.03) on the volume of biogas were not significant when analyzed individually; however, an interaction effect between AT and ST on biogas yield was observed. Also, pH, influenced the gas production significantly (F=3.954, p=0.021) likely due to its influence on microbial and enzymatic activity. The interaction effect showed that both temperature and pH had a combined effect on volume of biogas produced (p=0.003). These results underscore the importance of temperature and pH control in optimizing biogas production. Improved understanding of these factors could enhance anaerobic digestion processes, thus reducing greenhouse gas emissions, promoting resource efficiency and supporting circular economy principles.

Keywords: Biogas; temperature; pH; anaerobic digestion; renewable energy.

1. INTRODUCTION

The world today faces critical environmental concerns such as climate change, global warming, resource depletion and poor waste management systems, exacerbated by rapid population growth, urbanization and industrialization. Sustainable and environmentally friendly solutions have become an urgent necessity to secure and preserve the wellbeing of both the current and future generations. Non-renewable energy sources such as fossil fuels which dominate the global energy mix are derived from finite resources and thus place a considerable strain on natural reserves. Also, their combustion as a source of energy is among the major contributors to the emission of the greenhouse gases which are harmful to both humans and the environment. Because GHG emissions accelerates climate change, environmental concerns have risen to be one of the major global issues (U.S. Energy Information Administration, 2023). At present, the need for new sources of energy is driven by the global population increase, global energy usage, and increasing oil prices. Anaerobic digestion (AD) is one promising alternative, with the potential to address several environmental challenges. AD is a naturally occurring biological process where organic materials are broken down by microorganisms in the absence of oxygen, producing biogas as a byproduct. This biogas majorly consists of methane and carbon dioxide, it is a renewable source of energy that can be used in generation of electricity and heat, and also for cooking thus eliminating the reliance on fossil fuels. The United Nations' 7th Sustainable Development Goal (SDG) highlights the need for sustainable, clean energy to reduce fossil fuel dependence. Organic waste from green and abattoir sources, if left unmanaged, produces significant solid waste, posing environmental risks. Repurposing these wastes into bioenergy can thus address both the challenges of solid waste management and fossil fuel reduction (Büyük¨ozkan et al. 2018).

It is noteworthy that like many other countries in the world, Nigeria has some fossil fuels subsidies, which means the country sustains high economic losses due to the excessive consumption of fossil fuels. IISD claims that Nigeria actually offers $3.94 billion worth of subsidies for fossil fuels in 2018, and this has been equivalent to 2.4 per cent of Nigeria's GDP and 17 per cent of government's total revenue(Climate scorecard, 2024). The reliance on fossil fuel imports slow Nigeria's transition to a more sustainable and diverse electricity matrix (UNEP Annual Report 2023). It was identified that petroleum and oil takes about half of Nigeria's annual foreign currency. The country has the potential to produce large amounts of energy through Biomass such as agricultural residues, vegetable wastes and leaf litters (Jekayinfa et al. 2020). Biogas, due to its affordability and cleanliness, is emerging as an important fuel option for both domestic and industrial applications (Maritza Macias-Corral, 2008). Large-scale biogas production in developing countries has faced setbacks due to various factors: limited financing, inadequate technical expertise, insufficient awareness about biogas benefits, a lack of understanding of operational systems, absence of pilot studies, and insufficient government support for biogas technologies (James 2001, Agnes 2008). The accumulation of waste in landfills and from livestock production highlights the need for sustainable waste management practices (Ulloa et al. 2004).

Organic wastes with green and lignocellulosic properties rich in cellulose, hemicellulose and carbohydrates offer valuable carbon microbial fermentation in bio-methane production (Gayathri and Vijayaraghavan 2022, Khai et al. 2014). While animal waste provides beneficial bacteria that enhance biological activity, it alone does not yield sufficient biogas. On the other hand, plant derived lignocellulosic waste is non-biodegradable owing to its structural composition and as such it cannot be used as the sole feedstock for biodigesters (Rong 2018). Cost-effective pre-treatment in anaerobic digestion is therefore used to make cellulose assessible for hydrolysis, thereby improving conversion to biogas. The strategic mix of pretreated lignocellulose and animal waste constitute a promising synergy for efficient generation of biogas, a crucial need for renewable energy as well as a key solution to addressing both waste disposal challenges and the demand for renewable energy.

In our rapidly evolving world, the search for sustainable energy sources and effective waste management techniques has never been more crucial. The generation of biogas is a potent answer to both of these problems because it converts organic waste products into clean and sustainable energy. The efficiency and volume of biogas generated through anaerobic digestion are significantly influenced by operational factors such as organic loading rate, hydraulic retention time, total solids content, temperature and pH (Chulalaksananukul et al. 2012). Optimizing these parameters is essential to maximize biogas yield, and this process must be tailored to the specific type of waste and the digestion method employed. Understanding the subtle interactions between temperature and pH in the co-digestion of lignocellulose wastes and cow dung is essential. This interaction determines the effectiveness and success of the anaerobic digestion process. In order to pave the way for environmentally friendly waste-to-energy solutions that benefit society at large as well as the environment, this research lays the stage for a thorough investigation of how temperature and pH interact in the production of biogas from green waste and cow dung. Therefore, the objective of this study was to examine biogas production from control, pre-treated, and blended waste samples. Additionally, the study aimed to evaluate the interaction effects of ambient temperature (AT), slurry temperature (ST), and pH on the biogas volume generated from both non-pre-treated and pre-treated waste samples of cabbage, leaf litter of sandbox co-digested with cow dung.

2. MATERIALS AND METHODS

The materials used for this research work were cow dung, leaf litter of sandbox and cabbage as the samples. The equipment’s used included 32L biodigesters, top loading balances (50kg capacity, "Five goats", model no. Z051599), plastic water baths for soaking the wastes, water troughs, graduated transparent plastic buckets for measuring the volume of gas production, thermometer and JENWAY 3510 digital pH meters.

2.1 Sample Collection

The waste samples used for this study were (cabbage waste, leaf litter of sandbox and cow dung). Cabbage waste used for this study were collected from a vegetable market. The leaf litter of sandbox was obtained from sandbox trees around Nsukka environs and the cow dung was obtained from a cattle market all within Nsukka metropolis in Enugu state, Nigeria. Empty fruit bunches were obtained from an oil mill in Nsukka metropolis and burnt to obtain ash used for pretreatment. A clean container with cover was used for the collection of the waste samples. The biogas production experiment was carried out at the National Centre for Energy Research and Development (NCERD) at the University of Nigeria, Nsukka.

2.2 Biogas Experimental Procedures

The fresh wastes were left to decompose and degrade for four weeks, following that, the wastes were chopped and immersed into a plastic water bath for 10 days to allow microbial degradation by aerobic organisms.

Nine 32L biodigesters (Fig. 1) were used. Six kilograms each of cabbage waste, cow dung, and sandbox leaf litter were weighed and combined with eighteen (18) liters of water in a waste to water ration of 1:3. Empty fruit bunches (EFB) were burned, and the residue collected as ash was used for the pretreatment of the waste. Biodigester A was loaded with cow dung (CD), B with cabbage (CB), and C with sandbox (SB) waste. These waste materials,

Fig. 1. A 32L biodigester

in their unpretreated form, served as the control samples for the study. Three pretreated biodigesters, D (cow dung + ash), E (cabbage + ash), and F (cow dung + ash), were also loaded and pretreated with 0.6% ash, while biodigesters G-I contained a mixture of the three wastes blended in varying proportions of G=20:40:40, H=50:25:25, and I=60:20:20. The substrates were charged into the biodigesters and readings were taken daily. The experiment was carried out for a 35-day retention period.

The contents of the biodigesters was stirred thoroughly to ensure uniform distribution of until a homogeneous mixture was achieved and then left to set for a week prior to charging. The moisture content in the wastes was measured accurately to determine the measurements of wastes to be loaded in the biodigesters. Cow dung was used as inoculum for this study. The quantity of the produced gas was measured by the displacement of an equivalent volume of water expressed in liters(L).

2.3 Physicochemical Analysis of Waste

The amount of ash, moisture, and fiber was determined using the AOAC technique (2012). The quantities of fat, crude nitrogen, and protein were measured using micro-Kjedhal and soxhlet extraction methods described in Pearson (1976). The carbon content was determined using the Walkey and Black (1934) method, whereas the total and volatile solids were determined using the Meynell (1976) method.

2.4 Biochemical Analysis

The pH of the waste in the biodigester was monitored on daily basis using Jenway3510 digital pH meter. Ambient and slurry temperatures were also monitored and recorded daily using liquid in glass thermometer.

2.5 Data Analysis

ANOVA and multiple regression analyses were carried out using IBM SPSS version 22 and STATA version 15 to evaluate the mean differences and interaction effects among the variables under study.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics

As shown in Table 1 sample B showed the highest carbohydrate (1.42) and protein (0.60) content, while Sample A has the highest fat content (0.70) respectively. In contrast, sample E recorded the lowest carbohydrate (0.33) and sample F recorded the lowest fat(0.15) content. These differences can be attributed to the distinct nature and composition of the waste substrates used. The results also indicated that sample A had the highest total solid (T.S) content (3.09), volatile solid(V.S) content (2.63) and the highest calorific value (18,260) .Conversely, sample C had the lowest values for T.S. (0.38) and V.S. (0.31), while sample F presented the lowest calorific content (16,382). These variations are attributed the variation in waste composition and the overall effect of pretreatment on the waste samples.

3.2 Biogas Production Analyses

Fig. 2 illustrates the cumulative biogas production from un-pretreated/control, pretreated and blended waste samples for the 35 days retention period. Amongst the unpretreated/control waste samples (A-C), Sample C produced the highest volume of biogas at 335.4L, while Sample B produced the least, with 292.2L. For the pretreated waste samples(D-F), Sample D generated the highest biogas volume at 417.9L, while Sample E had the lowest at 359.1L. For the waste blends (G-I), Sample H produced the most biogas at 420.9L, whereas Sample G yielded the least with 214.8L.

This suggests that while CD, CB, and SB individually produce notable amounts of biogas, combining these substrates in different proportions can enhance biogas production. In addition, the methane production across biodigester A through I ranged from 22-41% with the highest methane yield produced by biodigester I. Acetic acid concentration varied between 2-13% with biodigester I producing the highest acetic acid content. Lastly, phenol levels ranged from 2-14% with biodigester H producing the highest phenol concentration.

The result from the ANOVA (Table 2) shows significant difference in the volume of gas generated from pretreated and blended waste samples compared to the control waste sample. From the result of the statistical analysis, in comparing the volume of gas generated, there is a significant difference (F=6.4, SS=659.379, p=0.002, df=314) at 5% level of significance in volume of gas produced by the waste samples.

To further examine the significant mean difference in the volume of gas produced between the waste samples, LSD post hoc test as shown in (Table 3) was used for the interclass comparison to explore specific differences between the waste samples and determined which waste sample had significantly different biogas production volumes. From the result there is a mean difference (p=0.001) in the volume of gas produced from control and pretreated waste samples. Furthermore, there is also a significant mean difference(p=0.012) in the volume of gas produced between blended and pretreated waste groups. The result shows that there is a significant mean difference(p=0.036) between the control and blended waste groups. Therefore, In comparing the volume of gas generated, there is a significant difference in the volume of gas generated by the Control-pretreated (p = 0.01), Control-blended (p=0.036) and control-pretreated-blended (F=6.4, SS=659.379, p=0.002, df=314), waste samples.

These mean differences in the volume of gas produced by the different waste groups can be attributed to digester conditions, substrate variability and compositions, weather and climatic environmental conditions during the anaerobic digestion process.

3.2.1 Effect of temperature on biogas production

Based on the result obtained from the study (Table 4), the highest volume of gas biogas produced (8.4L) was obtained on day 2 retention time at an AT temperature of 29^0C and ST temperature of 38^0C.The lowest biogas production of 4.9L was produced on day 20,29,31 and 35 retention times at AT of 29^0C and ST of 37^0C respectively. The temperature obtained for this study was found to be in the mesophilic range of (20-45^0C).Most anaerobic digestion processes occur in the mesophilic temperature range. At this temperature, biogas production is efficient, and the digestion process is relatively stable.

3.2.2 Effect of pH on biogas production

The result obtained from the study as shown in (Table 4) shows that the pH of the slurry ranged from 7.6-7.9 on day 35 retention time, which shows that the medium was alkaline and biogas production starts 24 hours after setting up the biodigester and the biogas increased with increased retention time(days). The highest volume of gas produced (8.4L) was produced at a pH of 7.9 on day11,32,34 and 35 and the lowest of volume of gas 4.9L was produced on day 35 at a pH of 8.4.

The multiple linear regression analysis presented in Table 5 evaluates the effects of ambient temperature, slurry temperature, and pH on biogas production volume. Results indicate that slurry temperature and pH significantly influence biogas volume at confidence levels of [p = 0.05 and p = 0.01], respectively. An R-squared value of 0.237 suggests that these three parameters collectively explain 23% of the variability in

Table 1. Physicochemical characteristics of the feedstock

	Parameters (%)					
Samples	**Carbohydrate**	**Fat**	**Protein**	**Energy Content (Kj/kg)**	**TS**	**VS**
A	0.53	0.70	0.40	18,260	3.09	2.63
B	1.42	0.65	0.60	16,910	0.49	0.33
C	1.24	0.60	0.50	16,600	0.38	0.31
D	1.19	0.30	0.40	18, 024	2.89	2.35
E	0.33	0.20	0.50	16,720	0.68	0.34
F	0.58	0.15	0.40	16,382	1.26	0.36
G	0.92	0.60	0.50	17, 136	0.25	0.21
H	1.08	0.65	0.40	17,360	0.50	0.39
I	0.56	0.65	0.40	17,640	1.19	1.00

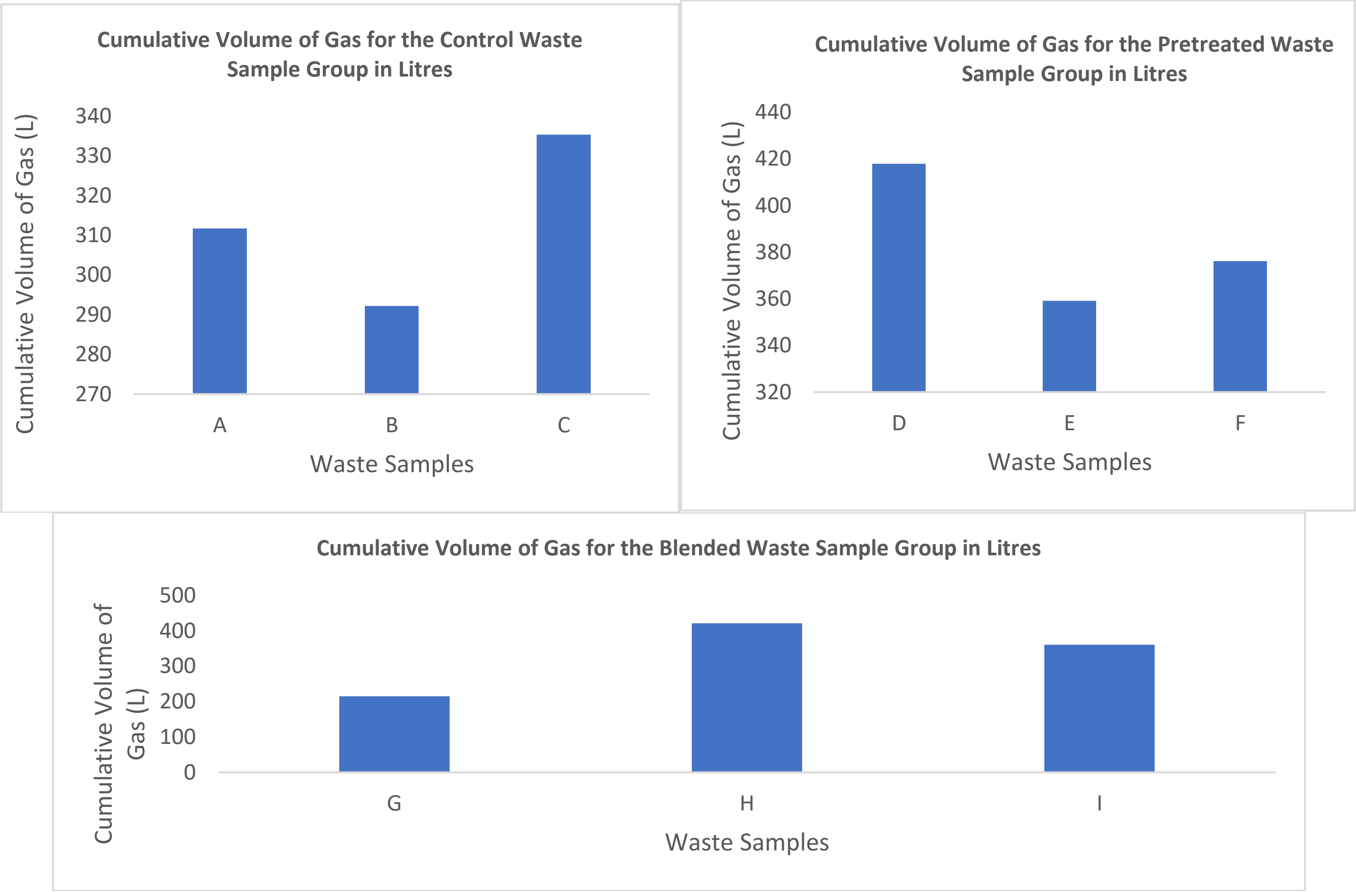


Fig. 2. Cumulative biogas yield for control, pretreated and blended waste samples for 35 days

Table 2. ANOVA Result showing the mean difference in the volume of biogas generated amongst (waste samples)

ANOVA					
Volume of Gas					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.968	2	12.984	6.396	.002
Within Groups	633.411	312	2.030		
Total	659.379	314			

Table 3. ANOVA Least Significant Differences (LSD) Result showing the mean difference in the volume of gas amongst biodigesters(samples)

Multiple Comparisons
Dependent Variable: Volume of Gas
LSD

(I) Samples	(J) Samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Pretreated	-.67905*	.19665	.001	-1.0660	-.2921
	Blended	-.18095	.19665	.036	-.5679	.2060
Pretreated	Control	.67905*	.19665	.001	.2921	1.0660
	Blended	.49810*	.19665	.012	.1112	.8850
Blended	Control	.18095	.19665	.036	-.2060	.5679
	Pretreated	-.49810*	.19665	.012	-.8850	-.1112

**. The mean difference is significant at the 0.05 level*

biogas production. Among the variables, pH shows the most substantial effect on gas volume [p = 0.000] compared to AT [p = 0.081] and ST[p = 0.02]. To further explore the interaction effects of these parameters on biogas volume, additional analyses were conducted, as reflected in Table 5.

Table 6 reveals that ambient temperature (AT) [F = 1.187, P = 0.308] and slurry temperature (ST) [F = 1.502, P = 0.106] individually exceed the benchmark p-value threshold of 0.05, indicating that, when considered independently, AT and ST do not significantly contribute to biogas volume across biodigesters. However, when analyzing the combined effect of AT and ST, a notable mean difference [p = 0.03] in biogas volume was observed, highlighting the importance of their interaction. Specifically, the values [F = 3.954, P = 0.021] confirm that pH significantly impacts gas production through its influence on microbial and enzyme activity. The interaction of ambient and slurry temperatures with pH demonstrates a significant effect [P = 0.003] on biogas production, suggesting a synergistic relationship between pH and temperature that enhances enzyme activity. Enzyme activity is impacted not only by individual factors but also by the interaction of temperature and pH. For example, enzyme function is relatively low under cooler, acidic conditions but significantly improves under warmer, alkaline conditions. This combined influence on enzyme activity is not merely additive but rather synergistic in nature, enhancing the overall biogas yield in this study. Furthermore, the coefficient of determination (R^2) indicates that the variables AT, ST, and pH collectively account for 45% of the variance in biogas volume, underscoring their combined importance in optimizing biogas production.

The Figs. 3-5 shows the interaction effect scatter plots between ambient, slurry temperature and pH on the volume of gas produced which depicts a positive interaction effect between all three parameters on the volume of gas produced. Fig. 6 shows the average readings of AT, ST, and pH throughout a 35-day period. For control waste substrate digestion, the average temperature readings for ambient and slurry temperature ranged between 20 ^{0}C and 29 ^{0}C between day 10 and day 24, whereas slurry temperature fluctuated between 24^{0}C and 36 ^{0}C between day 24 and day 14. The pH value also revealed that the values ranged between 6.4 to 7.7 between day 2, day 23 and 35 respectively. For Pretreated waste substrate, the average temperatures (AT and ST) were recorded within the range of 20 ^{0}C and 29 ^{0}C between day 24 and day 10, whereas ST fluctuated between 24 ^{0}C and 36 ^{0}C between day 24 and day 11.

Table 4. Optimum and minimum parameter values

Samples	Days	AT	Days	ST	Days	pH	Days	Volume of Gas
A	10, 31 and 34	29	35	37	35	7.7	31	5.0
B	10, 31 and 34	29	35	37	35	7.6	20,29,31 and 35	4.9
C	10, 31 and 34	29	35	37	35	7.6	31	5.9
D	10, 31 and 34	29	35	37	32,35	7.9	18	6.2
E	10, 31 and 34	29	35	38	11,32,34,35	7.9	2	8.4
F	10, 31 and 34	29	35	38	32	7.9	2	6.2
G	10, 31 and 34	29	35	38	35	8.4	18	4.0
H	10, 31 and 34	29	35	37	35	8.4	14	6.2
I	10,31 and 34	29	35	37	35	8.4	17,31	4.9

Table 5. Multiple linear regression showing the Effect of AT^{0}C, ST ^{0} C and pH on volume of gas

Volume of Gas	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
AT	.092	.052	1.75	.081	-.011	.195	*
ST	.075	.032	2.33	.02	.012	.138	**
pH	.977	.179	5.46	0.00	.624	1.329	***
Constant	-8.481	1.366	-6.21	0.00	-11.169	-5.793	***

Mean dependent var	3.269	SD dependent var	1.449
R-squared	0.237	Adjusted R Square	0.229
F-test	32.159	Prob > F	0.000
Akaike crit. (AIC)	1049.520	Bayesian crit. (BIC)	1064.531

*** p<.01, ** p<.05, * p<.1

Table 6. ANOVA table Showing the Interaction Effect of AT, ST and pH on Volume of Gas

ANOVA Tests of Between-Subjects Effects (waste samples A-I).
Dependent Variable: Volume of Gas

Source	Type II Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	314.884[a]	94	3.350	1.923	.000
Intercept	3289.917	1	3289.917	1888.791	.000
AT	16.537	8	2.067	1.187	.308
ST	39.232	15	2.615	1.502	.106
pH	13.775	2	6.888	3.954	.021
AT * ST	79.509	38	2.092	1.201	.033
AT * pH	7.490	6	1.248	.717	.012
ST * pH	24.952	10	2.495	1.433	.004
AT * ST * pH	14.457	12	1.205	.692	.003
Error	383.198	220	1.742		
Total	3988.000	315			
Corrected Total	698.083	314			

a. R Squared = .451 (Adjusted R Squared = .217)

The pH value also revealed that the value ranged between 6.6 to 7.9 between day 7 and day 24 and 35 respectively. Finally, the figure also revealed that the value for the blended waste substrates for AT and ST ranged from 20 ^{0}C and 29 ^{0}C between day 24 and day 10, whereas ST fluctuated between 24 ^{0}C and 37 ^{0}C between day 24 and day 10.

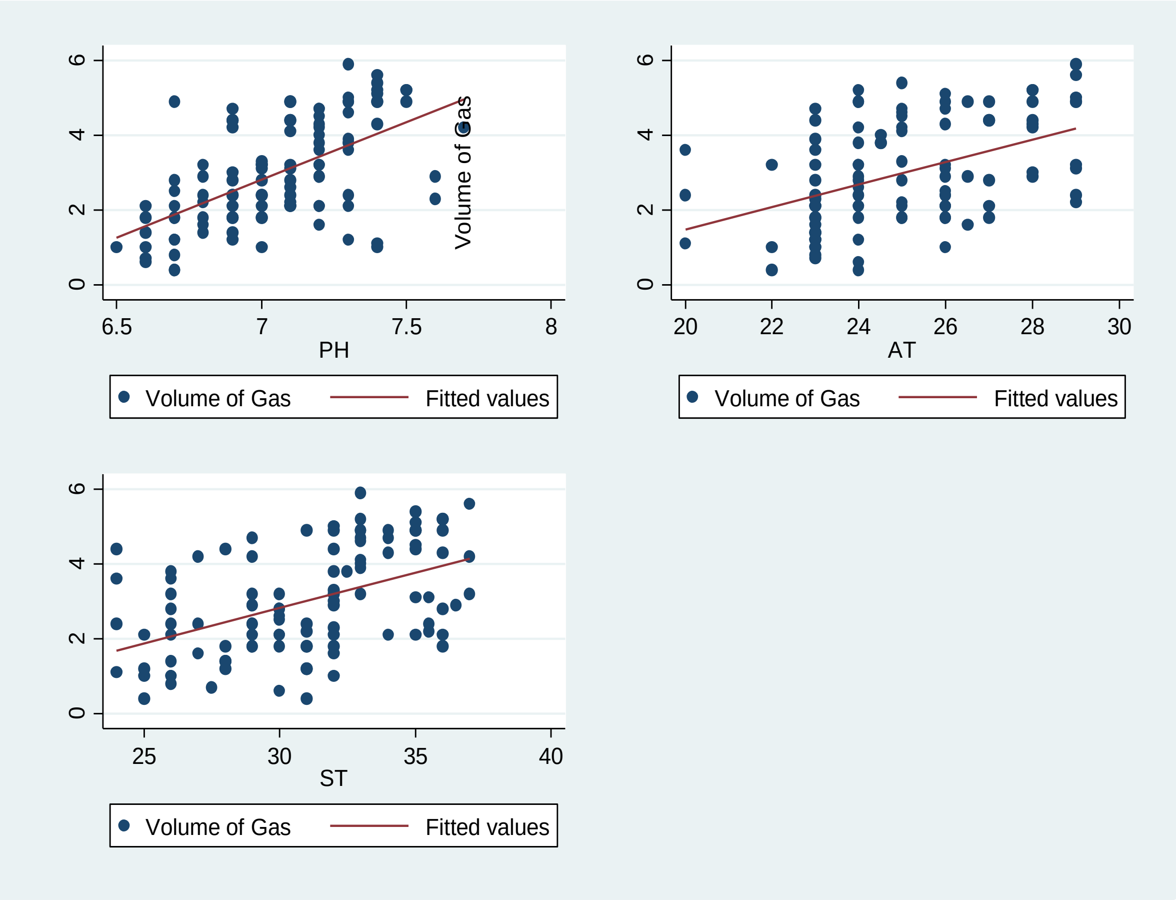


Fig. 3. Scatter Plot diagram showing the interaction effect of parameters on the volume of gas produced for the control waste samples

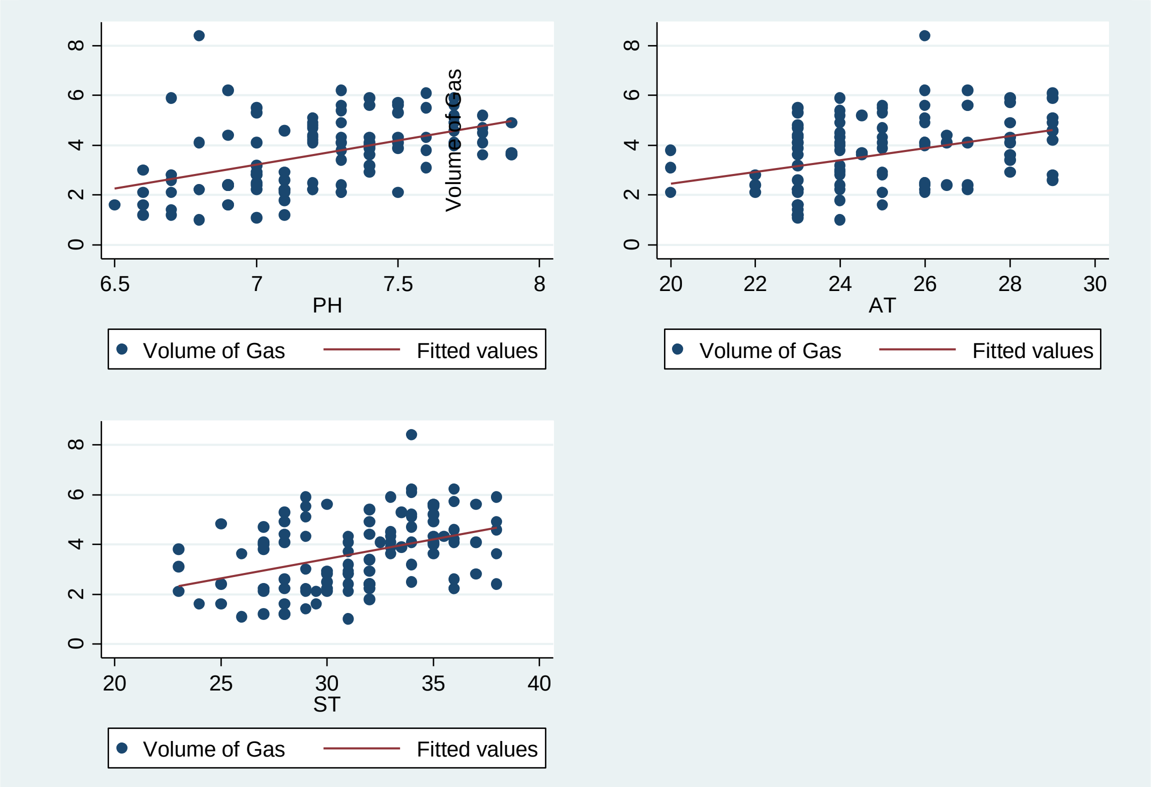


Fig. 4. Scatter plot diagram showing the interaction effect of parameters on the volume of gas produced for the pre-treated waste samples

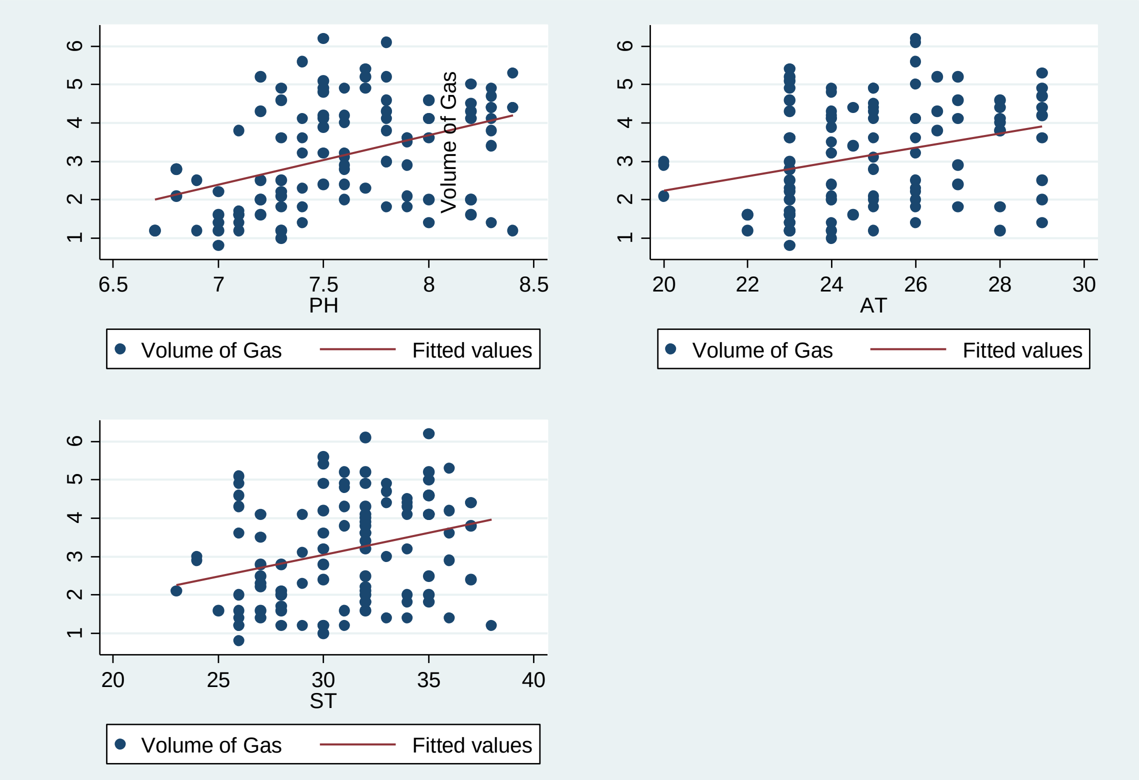
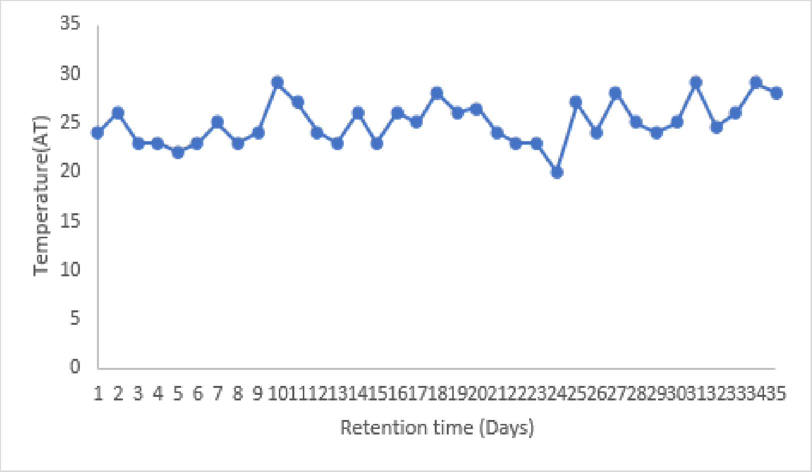
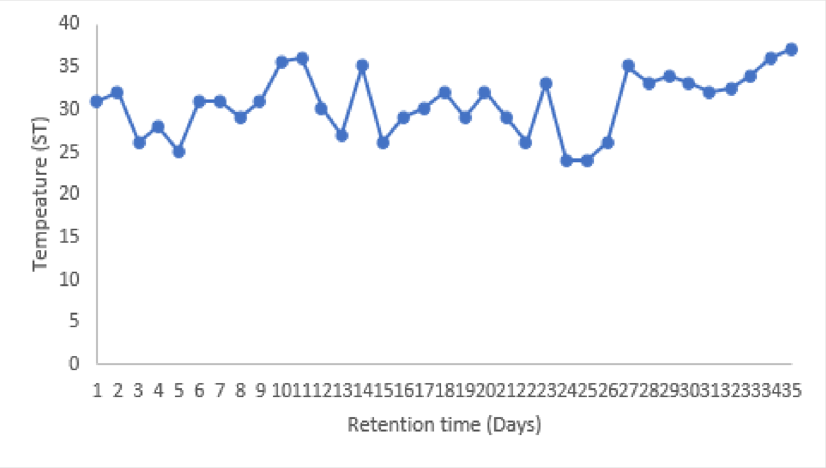
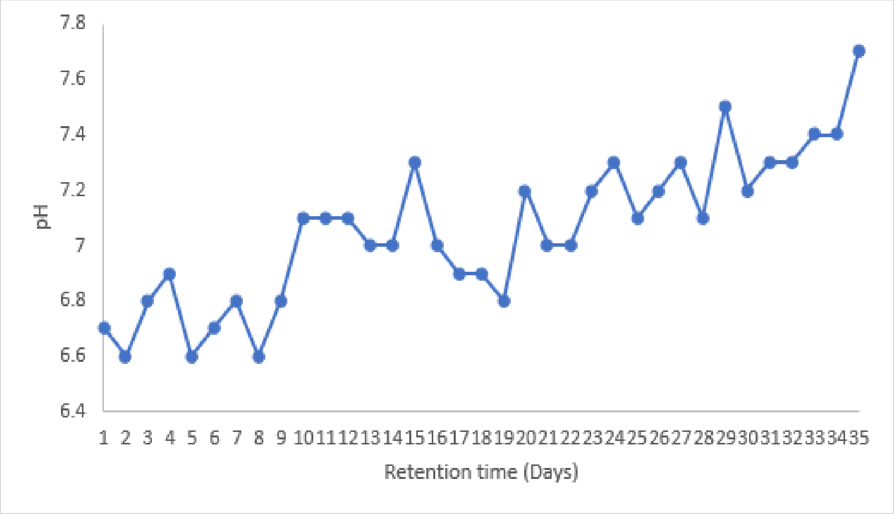
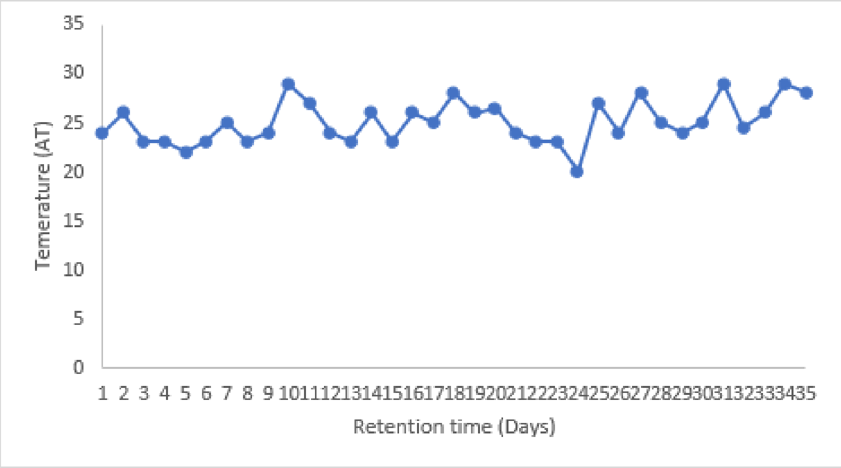


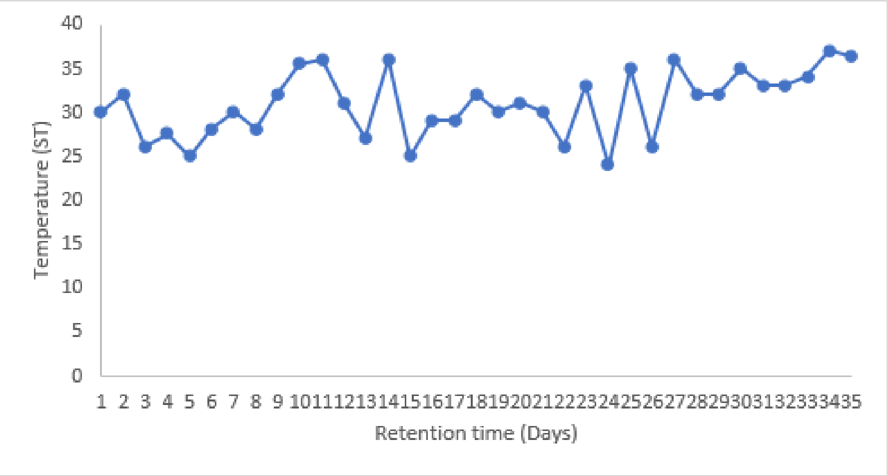
Fig. 5. Scatter plot diagram showing the interaction effect of parameters on the volume of gas produced for the blended waste samples

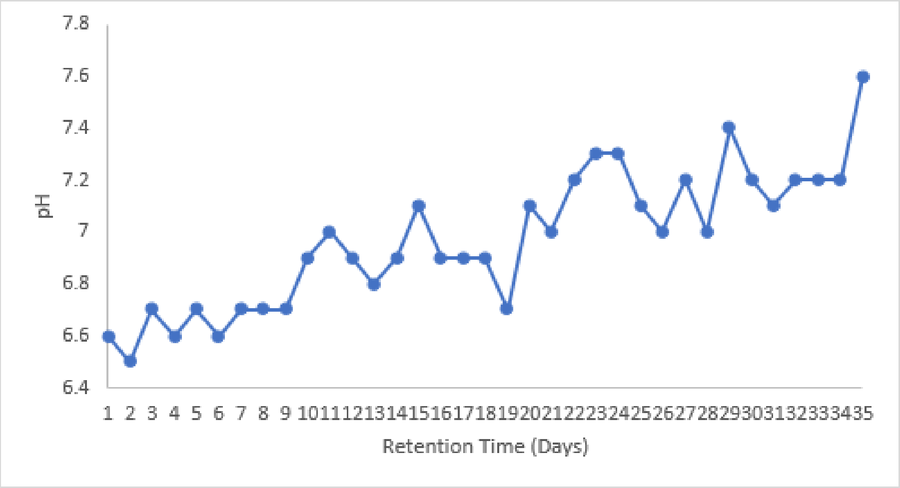

Temperature(AT)
0
5
10
15
20
25
30
35
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Retention time (Days)

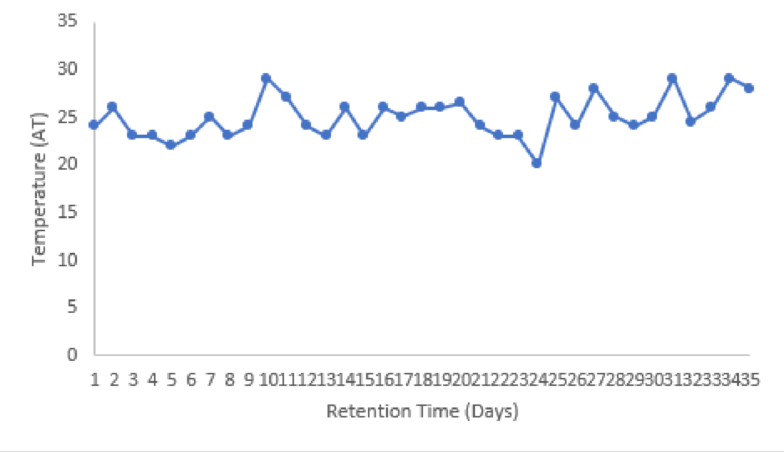

Tempeature (ST)
0
5
10
15
20
25
30
35
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Retention time (Days)


pH
6.4
6.6
6.8
7
7.2
7.4
7.6
7.8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Retention time (Days)


Temerature (AT)
0
5
10
15
20
25
30
35
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Retention time (Days)


Temperature (ST)
0
5
10
15
20
25
30
35
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Retention time (Days)


pH
6.4
6.6
6.8
7
7.2
7.4
7.6
7.8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Retention Time (Days)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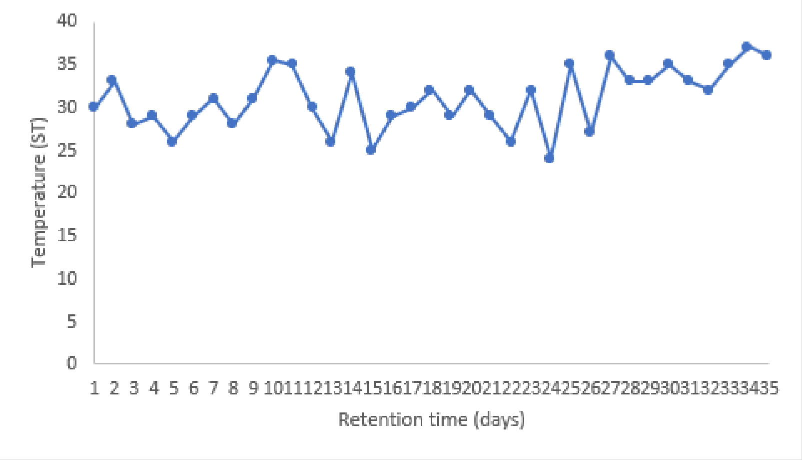
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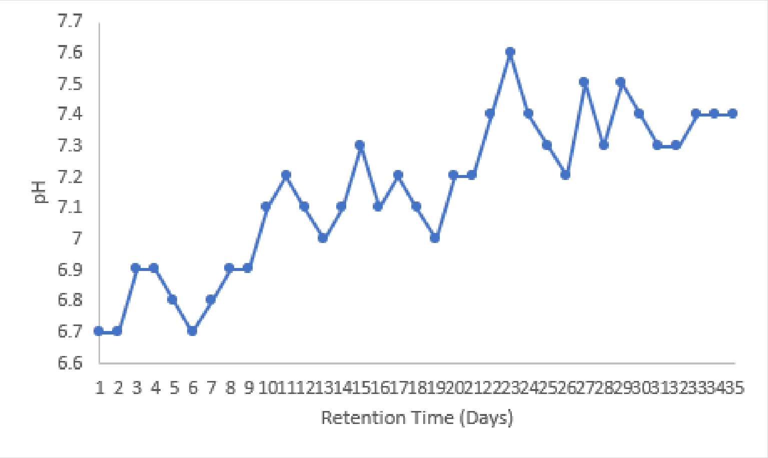
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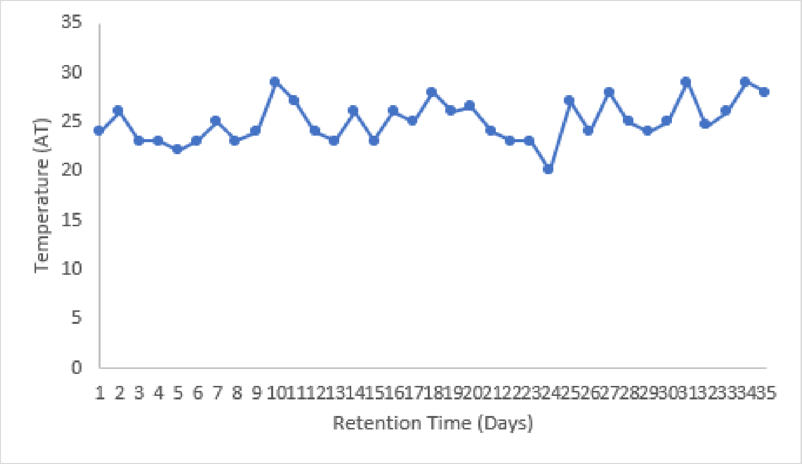
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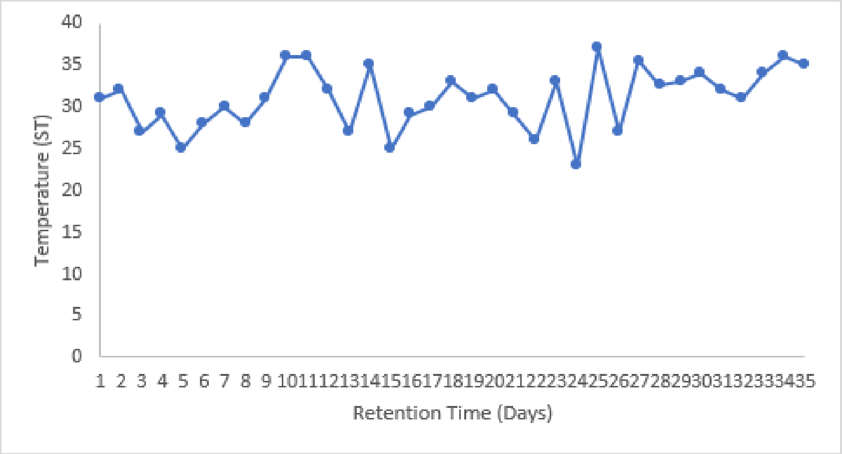
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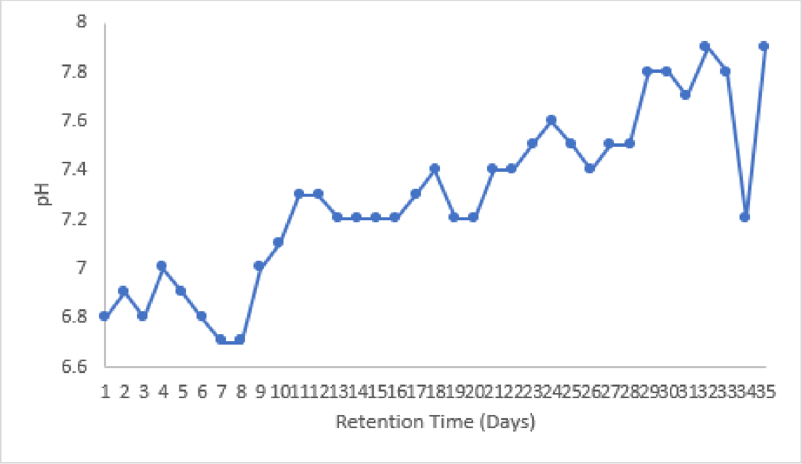
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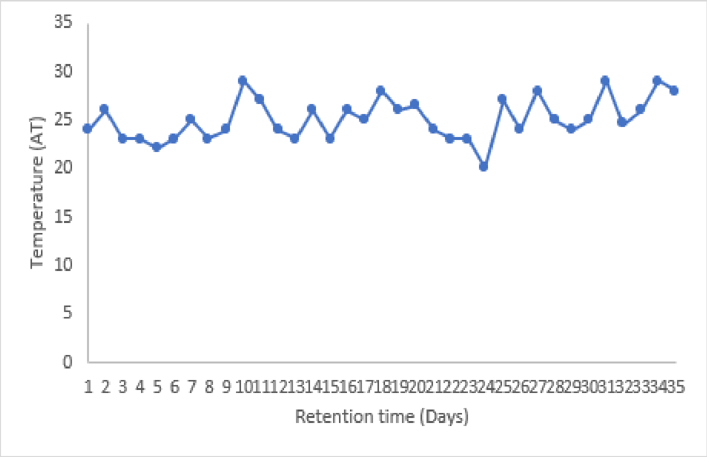
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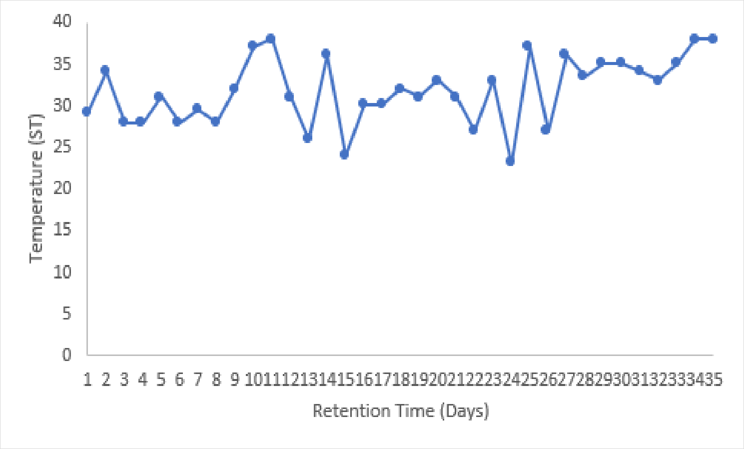
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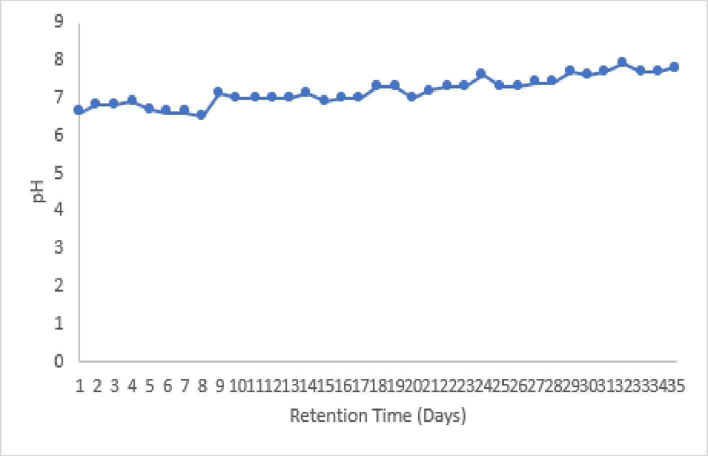
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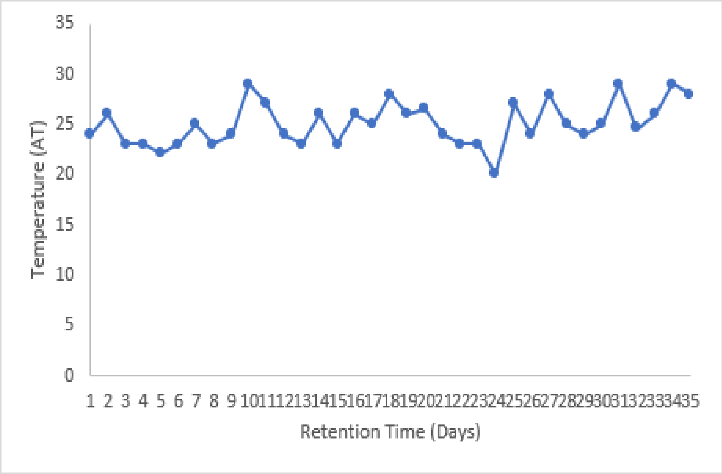
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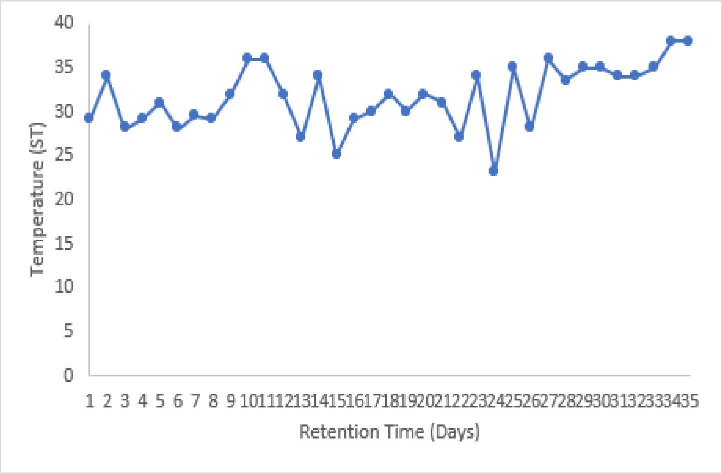
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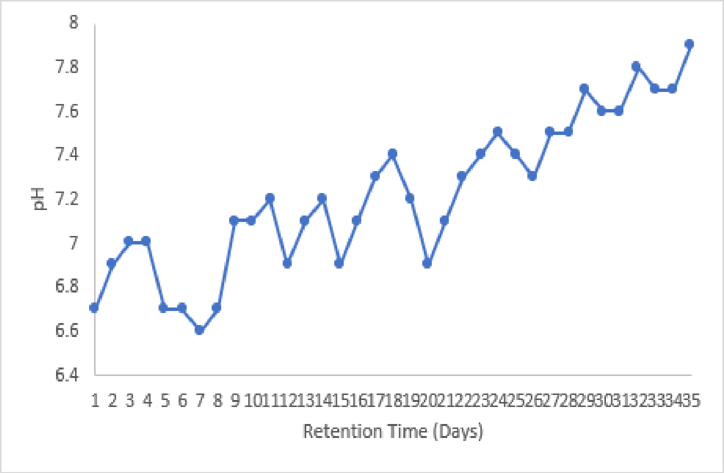
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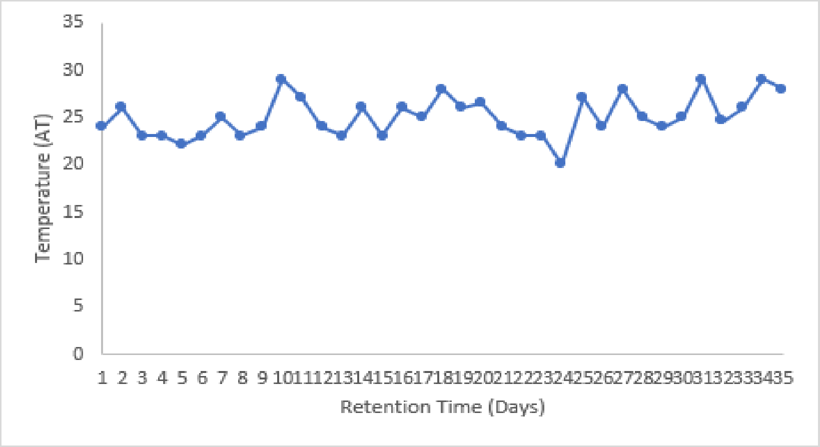
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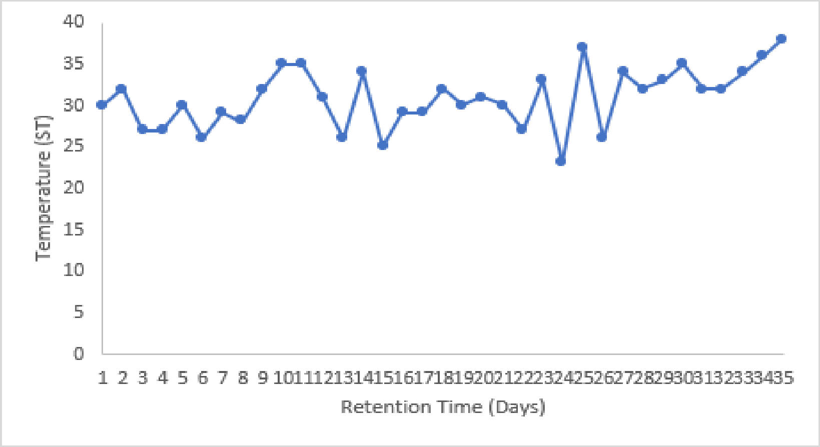
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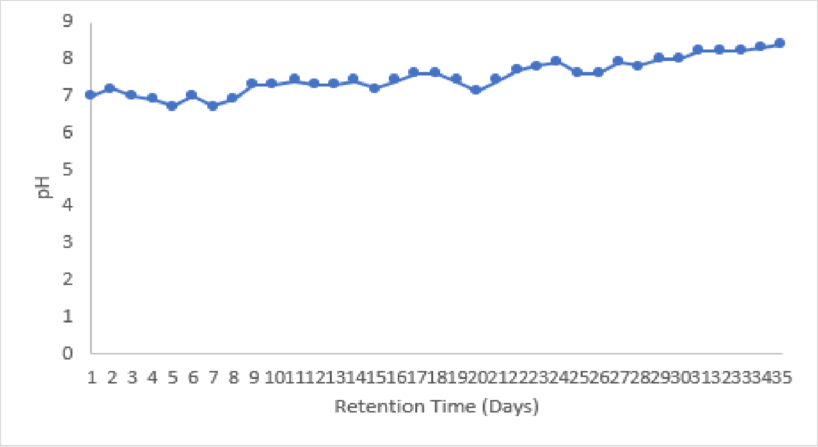
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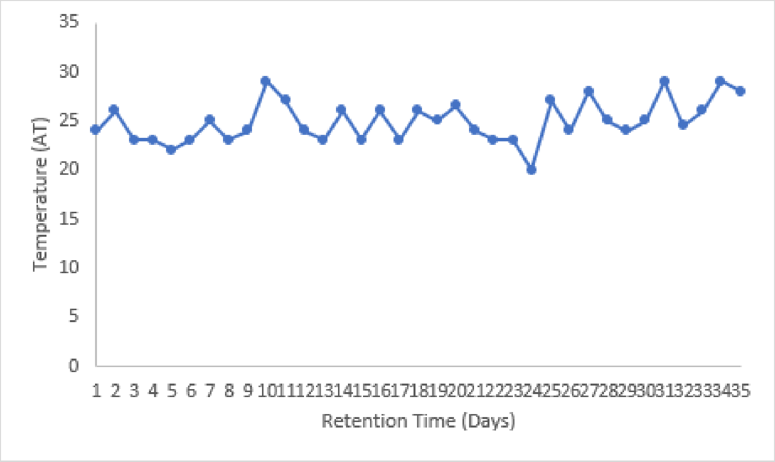
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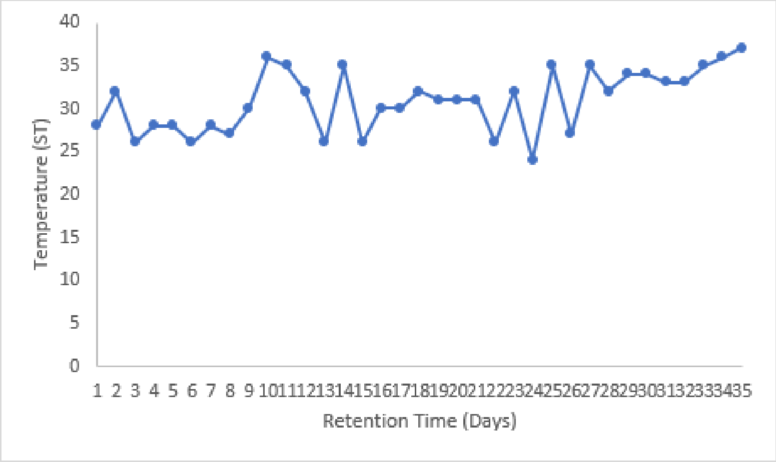
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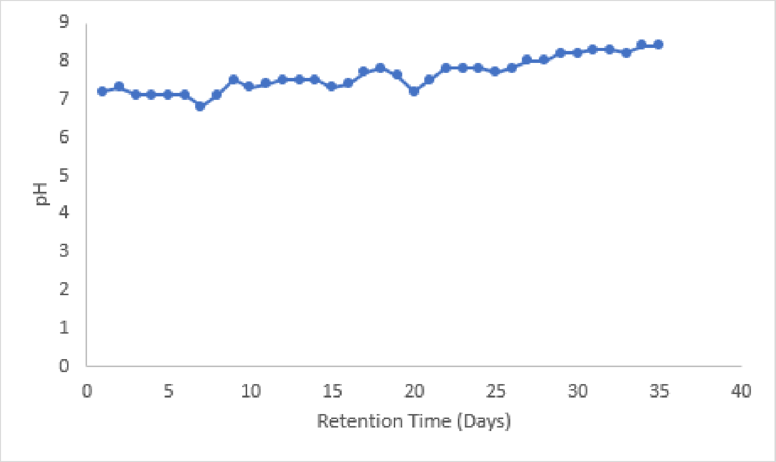
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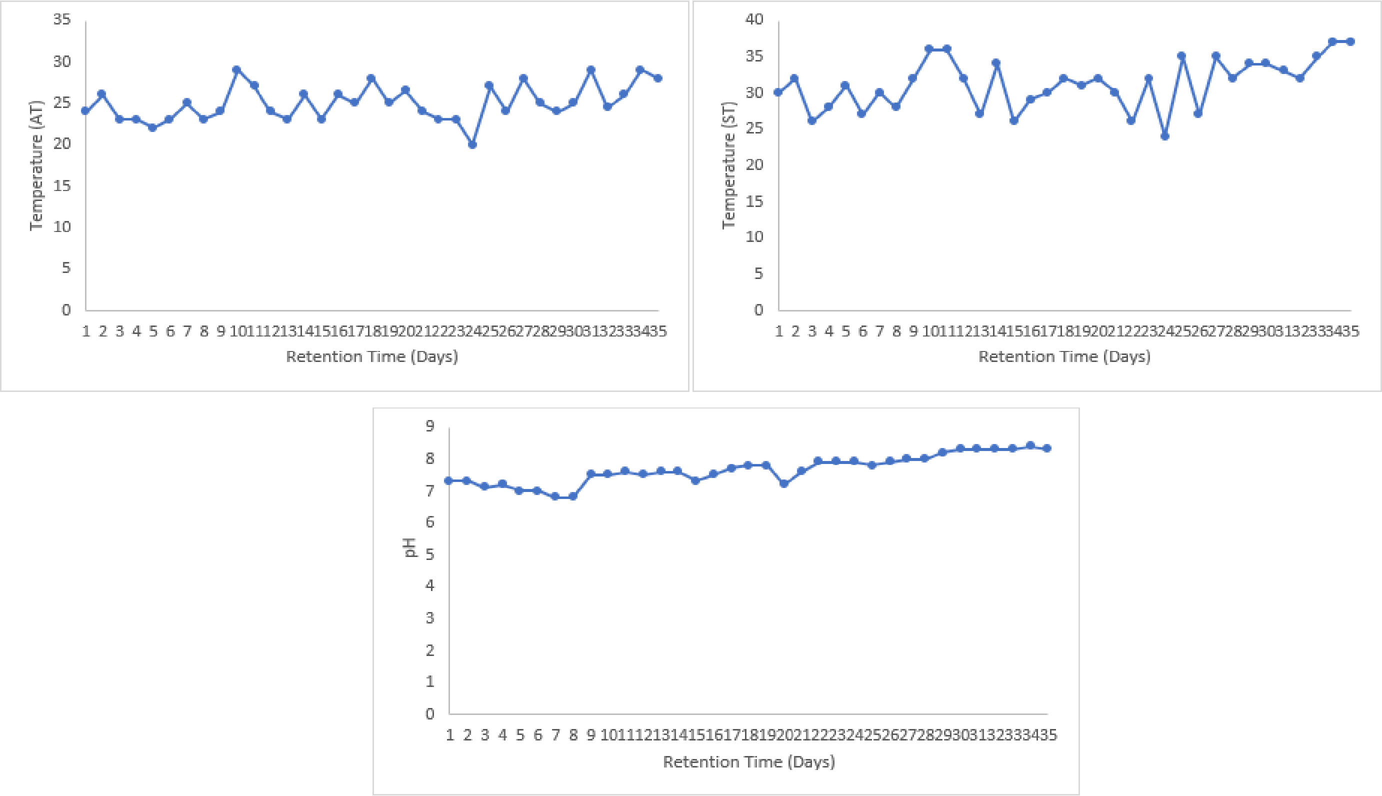

Fig. 6. Graphs showing average temperature and pH readings of biogas production against retention times for the waste samples

The Figures above shows the minimum and maximum values of ATC, STC, and pH throughout a 35-day period. For non-pretreated waste substrate digestion, the min and max temperature values for ambient and slurry temperaranged between 200C and 290C between day 24 and day 10, whereas STC fluctuated between 240C and 360C between day 24 and day 14. The pH value also revealed that the min and max ranged between 6.4 to 7.7 between day 2 and day 23 and 35 respectively. For Pretreated waste substrate, the min and max temperatures (ATC and STC) were recorded within the range of 200C and 290C between day 24 and day 10, whereas STC fluctuated between 240C and 360C between day 24 and day 11. The pH value also revealed that the min and max ranged between 6.6 to 7.9 between day 7 and day 24 and 35 respectively. Finally, the figure also revealed that the min and max value for the blended waste substrate for ATC and STC ranged from 200C and 290C between day 24 and day 10, whereas STC fluctuated between 240C and 370C between day 24 and day 10. The pH value shows that the min and max ranged between 7 to 8.4 between day 7 and day 35 respectively.

The pH value shows that the values ranged between 7 to 8.4 between day 7 and day 35 respectively. The biodigester will operate with greater efficiency if the temperature and pH fluctuations are closely monitored. Methanogens are extremely susceptible to abrupt temperature fluctuations. Consequently, it is best to prevent any abrupt changes in temperature. Over the duration of the retention period, temperatures varied from 20-37 ^{0}C. As a result, when the condition occurs between (25 and 50 ^{0}C), it is mesophilic. The range of pH fluctuation during the digesting phase was between 6.6 and 8.4. pH is one of the key factors impacting anaerobic digestion, nevertheless, monitoring the pH of the anaerobic digestion process, the digester's environment is kept favorable for bacteria, which promotes effective organic matter decomposition and biogas production.

3.3 Discussion

The tables and figures depict results of the volume of gas produced by the waste samples as well as the interaction effect of ambient and slurry temperature on the volume of gas produced from control, pretreated and blended waste substrates of cabbage waste leaf litter of sandbox codigested with cow dung. From the results of the statistical analysis, the significant value of [p=0.002], at 5% level of significance depicts that there is a significant difference in the volume of gas produced from the various waste samples. It also revealed that there is [P=0.01] significant difference between control and pretreated waste samples. The result of the statistics further revealed that [p=0.036], at 5% level of significant depicts that there is a significant difference between control and blended waste groups. Following the presented results, the ANOVA value [P=0.002] shows that there is a significant mean difference amongst the waste samples. These differences can be attributed to digester conditions, substrate compositions, weather and climatic environmental conditions during the anaerobic digestion process, which is in concurrence with the result from study by (Esposito et al. 2012), comparing all of the mixed blended ratios to the control waste samples, analysis of variance (ANOVA) of the cumulative biogas yield means reveals a significant difference ($P \leq 0.05$). The biodigester with BR3 recorded the highest overall biogas production (21.443dm3). The synergistic effects (additional impacts) of the nutritional contents of the combined separate feedstocks may be responsible for the observed rise in the cumulative biogas yield means. Prior research on the co-digestion of several organic substrates has demonstrated a synergistic effect of the combined treatments since the combined mixture's biodegradability was significantly higher than that of the individual substrates when the experiments were conducted individually. Zhong (2011) also investigated biogas generated from corn straw and subjected to 8% sodium hydroxide, 5% ammonia, at room temperature of (15 °C), 4% urea pre-treatments for 20 days prior to anaerobic digestion process and discovered that pre-treatment significantly increased the degradation of hemicellulose, cellulose, and lignin. Furthermore, corn straw treated with NaOH produced biogas at 0.472 m3/Kg VS, which was 207 percent higher than the untreated sample.

Similar research has also shown that co-digestion of food wastes with livestock manure such as cow dung, sewage sludge, or effluent increases biogas generation and methane content, whereas mono-substrate digestion was found to be primarily unstable (Uzodinma et al. 2007). From the study result, there is evidence of a statistically significant difference in the volume of gas produced among the waste samples. This finding suggests that the waste samples used in the study have distinct characteristics or composition that influence and enhance biogas production. The rate of biogas production is significantly influenced by temperature variations as well as other factors including pH, total solids, substrate combinations, carbon to nitrogen ratio etc.

3.3.1 Effect of pH on biogas production

pH is one of the most important parameters controlling microbial activity and, thus, biogas yields in anaerobic digestion. In the study, there was variability in pH level; this was especially during the early digestion phase. This can be attributed to the high volatile solid content where the activity of the acid producing bacteria prevalent in producing VFAs and other acids to be used by the methanogenic bacteria in producing the biogas. A temporary rise in the pH was observed following a decline, possibly due to ammonia production during protein breakdown. Ammonia, a base, combines with carbon dioxide and water to form ammonium bicarbonate, a natural pH buffer, suggesting high acetogenic and hydrolytic activity. This is due to the highly acidic nature of the reaction mixture along with the presence of lignocellulose which hinders the production of flammable biogas as noted by (Olanrewaju 2018).The study recorded the initial pH values ranging from 6.5 to 8.4 a shift from the recommended pH of 6.0 – 7.0 as espoused by (Nnabuchi & Ukpai 2012) who opined that slightly higher initial pH of 7 enhances the yields of biogas. The production of methane was poor at low pH because the activity of methanogens is inhibited leading to low yields of methane. Over time, accumulating free fatty acids further decreased pH levels, particularly as gas production began to decline.

3.3.2 Effect of temperature on biogas production

During the 35 days of the anaerobic digestion process, biogas production started within 24 hours of charging the biodigesters as corroborated by the findings of (Huan et al. 1982) who affirmed that biodigester charging initiates biogas production within 24 hours. Therefore, the biogas yield in biodigester E was observed to be highest yielding 8.4 litre when the ambient and slurry temperatures were 29 ^{0}C and 38 ^{0}C respectively followed by biodigesters D and F yielding 6.2 litre of biogas. Previous studies by (Gollakota and Meher 1988, Owamah 2019) indicate that at across various loading rates the biogas production is higher at 37°C than at 30°C, thus, both ambient and slurry temperature play a significant part in biogas production. The ambient and slurry temperatures recorded were between 20°C and 29°C and between 23°C and 38°C respectively which is in contrast to (Budiyono et al. 2010)'s work that maintained controlled co-digestion conditions at a stable 37 ±1°C. This variation reveals that environmental factors affect the biodigester wall heat exchange, either absorbing or dissipating heat depending on the environmental temperature. The range of temperature used in this study is in the mesophilic range (20–45°C), which is found appropriate by (Kavuma 2013) for optimum biogas production.

3.3.3 Interaction effects of temperature and pH on biogas production

Throughout the 35-day retention period, biogas yields varied significantly due to digester conditions, substrate composition, and ambient climatic factors. Biogas production started on day 1 for all the substrates including control, pre-treated and blended waste substrates, but the cumulative biogas yields differed with the type of substrate and pre-treatment methods. For the single substrate biodigesters A, B and C, the cumulative biogas production was observed 103.9 L, 97.4 L and 111.8 L, respectively. While Biodigesters D, E and F, which has undergone pre-treatment yielded 139.3L, 119.7L and 125.4L respectively from blended substrate biodigesters G, H and I gave 71.6L, 140.3L and 120.2L. Of all these biodigesters, biodigester H produced the highest total biogas of 140.3L.

While operating within the mesophilic range, the methane generated in the biogas was low, probably due to the low pH level in the reactor. They further indicate that methanogenic activity is inhibited at pH values < 6.3 and > 7.8 while the most favourable range for this process is 6.8 – 7.6 as reported by (Gerardi 2003). The results showed that there was a strong interaction between the temperature of ambient (AT) and slurry (ST) on the volume of the produced gas, and the temperature and pH showed a strong interactive effect on the microbial and enzymatic activity. Consequently, this study demonstrates that both temperature and pH are critical in optimizing the biogas yield through their correlated influence on enzyme and microbial efficiency.

This result is in variance with study carried out (Ojikutu and Osokoya 2014). He revealed that the kind of food waste had a significant (P<0.05) impact on the substrate's temperature and pH but no significant (P>0.05) impact on the production of biogas. According to his study's findings, even though the mixed treatment produced the most biogas (8016.67 ml/day), each type of food waste produced the same amount (P>0.05). Fish waste produced the least, with a daily average volume of 1090 ml.

4. CONCLUSION

This study demonstrated that the co-digestion of cabbage waste, leaf litter of sandbox and cow manure effectively increased biogas production. The findings of this study also revealed that the volume of biogas produced by cabbage waste and leaf litter of sandbox was significantly increased by adding adjuncts such as cow manure and ash pretreatment as well as blending the waste substrates. The study's findings highlight the significant interaction effect between temperature and pH in biogas production from lignocellulosic wastes and cow manure during anaerobic digestion emphasizing the need to maintain ideal conditions of the digestive parameters (AT,ST and Ph) to maximize the waste-to-energy processes.

This research is pivotal in the advancement of sustainable biogas production from waste materials that promotes greenhouse gas emission reduction, energy conservation and waste reduction. The study also highlights the importance of organic wastes transformation into biofuels thereby supporting the optimization and efficiency of biogas systems. Biogas as a renewable energy source offers a viable solution to meet the world's ever-increasing energy demand sustainably fostering a healthier and cleaner environment. The study underscores the benefits of sustainable energy recovery by assisting the advancement and dissemination of scientific knowledge and successful biogas production whilst promoting renewable energy solutions and environmental sustainability.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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