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The Influence of Biocatalytic Plant Extracts on Biogas Production from Kitchen Wastes at Cryo-mesophilic Temperature Regimes

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Abstract: Radicalization in waste-to-energy systems are on the rise to meet human energy demands. Biogas generation from kitchen wastes is one such scheme, though affected by poor yields and methane levels at low temperatures. In this research, biocatalytic extracts with fermentative properties were hereby assessed on their potential to fasten these processes and increase the biogas yield at ambient temperatures. The variations in kitchen waste substrate anaerobic parameters and elemental composition as well as biogas yields and methane levels were monitored in a 28-day retention period. Three 40-liter batch and unstirred bio-digesters containing biocatalysts *Terminalia b., Acanthaceae spp*. and a control setup were used. The results indicated rapid saccharification rates in the samples with additives. *Terminalia b*. additives exhibited high volatile solids hydrolysis rate of 98.3% followed by *Acanthaceae spp*. (50.8%) and control sample (29.4%). Similar trends were observed in organic carbon reduction as the levels of nitrogen, phosphorus and sulfur linearly increased. The biocatalysts did not affect substrate pH, volatile fatty acids and alkalinity levels. *Terminalia b.* sample produced 2.32 folds higher while *Acanthaceae spp*. sample produced 1.375 folds higher than the control sample. *Terminalia b.* methane levels were highest (45.475±0.922%) followed by the control sample (41.750±1.401) and *Acanthaceae spp.* sample (39.275±0.263%) after 28-day retention period at 19.5±0.5°C. Use of these biocatalysts in biofuel synthesis can thus optimize biogas production leading to greener economies.

Keywords: Kitchen Waste, Biogas, Biocatalysts, Low Temperature

1. Introduction

Biogas production is gradually seeing the light in many countries worldwide. Earlier on, biogas production was reserved for a few developed countries but this trend is changing steadily. This is due to inconsistent fossil fuel prices and environmental pollution as a result of these fuels. Additionally, these fuel sources are non-renewable and are quickly getting depleted. It is therefore crucial to indulge into biofuel production. Biogas is one of the most sustainable biofuels due to its efficiencies of production, affordability, easy to operate and compatibility with most biomasses [1].

One of the most viable biomass sources abundant in both rural and urban areas is kitchen wastes. People living in towns generate tons of organic kitchen wastes daily. These wastes are as a result of peels generated during the process of preparing meals or left-over foods. The waste is collected and dumped into municipal dumpsites which is later on gathered and dumped in landfills. Both municipal dumpsites and landfills are an eyesore to any community. The fate of such landfills is gradual anaerobic and aerobic degradation to

produce odorful biomass products such as ammonia, siloxanes sulfide gases [2]. These odors attract pathogens responsible for tragic diseases such as cholera [3]. On the other hand, water reservoirs created by these wastes are perfect breeding sites for mosquito responsible for malaria [4].

In rural areas, kitchen wastes can either be fed to cattle or used as farm manure. However, increase in population and devolution of resources and government workers from urban areas to rural areas has led to settlement of non-agriculturalists in rural areas. Old people also prefer to settle in rural areas albeit being unable to indulge into farm activities. Such people have kitchen wastes which are dumped and accumulate to breed pathogens. When such biomass is rained on, pollutants in it leach into water bodies to cause water pollution. It is thus pertinent to convert these wastes into biogas energy for domestic heating and lighting purposes.

Kitchen waste substrate is known to contain many organic compounds including carbohydrates, proteins, fats and minerals [5]. Decomposition of such matter can lead to a variety of compounds, some useful, others not depending on several factors. Some of these factors include the amount of air present (aerobic or anaerobic), temperature, pH, presence of biogenic microorganisms and mineral components amongst others [6-8]. Unlike animal dung used in biogas production, the composition analysis of kitchen waste is not properly rationalized for quantitative and qualitative yields of biogas. The ratio of carbohydrates to proteins is not ideal for optimal biogas yields. In most instances, the proteins are quite high leading to production of a lot of ammonia and sulfide gases which are incombustible and odorful.

Most biogas digesters are fabricated to ensure proper sealage for anaerobic environment and high temperature. However, the temperature of the digester is difficult to control and temperature jackets as well as other provisions are used in most commercial biogas plants. Biogas production at low temperatures (cryo-mesophilic temperature) is quite poor and full of contaminants [9, 10]. This is because methane-producing bacteria perform optimally at higher temperature [10]. Kitchen wastes also have quite low pH values which are not conducive for methanogenic bacteria. In such low temperatures and pH values, degradation of the biomass to produce biogas is also low. Additives are thus supplemented to aid in the process. Unfortunately, most commercial additives are quite expensive and out of reach to rural and small-scale biogas investors.

Biocatalytic plant extracts have over time enjoyed populace in traditional setups, especially in Kenya. These extracts were used to hasten domestic fermentation processes. *Terminalia b.* leaves extracts were natively used amongst the Aandia community found in the slopes of Mt Kenya to fasten fermentation of porridge and milk as well as saccharification of wheat bran (cellulose) in preparation of their traditional alcohol. On the other hand, *Acanthaceae spp.* bark extracts have continuously been used by the Maasai community of Kenya to catalyze fermentation of their local alcohol. This study aimed at exploiting the potentials of these two indigenous extracts in hastening biogas production using kitchen wastes at low temperature (cryo-mesophilic) conditions. The change in key parameters of biogas substrate was closely monitored over the entire retention period.

2. Materials and Methods

2.1. Design of Experiment

Terminalia b. and *Acanthaceae spp.* extracts were obtained by solvent extraction method using water. The kitchen waste substrate was analyzed for physical-chemical parameters and anerobic digestion parameters before loading into 40-liter batch bio-digesters. Three setups were used; two for the additives and a control setup (all exposed at the same conditions). Biogas yields were monitored daily against the environmental temperature while methane composition and substrate analysis were done weekly for a 28-day retention period.

Bio-digester fabrication, analysis and monitoring was done at Maasai Mara university, Narok, Kenya while characterization for conjugation of the extracts, biogas composition and some anaerobic digester analysis was done at Taita Taveta university, Voi, Kenya.

2.2. Materials

All reagents used were laboratory and analytical grade. All reagents were sourced from Sigma-Aldrich.

A pH meter (Hanna G-114), and biogas test-kit (Multitec-545) were used.

2.3. Biogas Substrate Used

The kitchen waste used was characterized to have an average pH of 6.300 ± 0.001 and electrical conductivity value of $1.293\ \pm0.002mS$. The total solids content was $11.556\ \pm0.669g/L$ against a volatile solids content of $11.283\pm0.008g/L$ implying most of the solids were actually volatile and not fixed solids. This showed that the substrate had a lot of organic matter. The total suspended solids were $7.253\ \pm0.672g/L$ while the total dissolved solids were $3.904\ \pm0.003g/L$. Alkalinity levels went up to $0.900\ \pm0.132mg/L$, while volatile fatty acids averaged $14.580\pm0.811mg/L$ implying that the substrate had a lot of volatile acids thus acidic. The average FOS/TAC value was 1.240 ± 0.020. Elemental composition was; dissolved oxygen ($8.500\ \pm0.476\%$), organic carbon ($21.600\ \pm2.400g/L$), nitrogen ($3.067\ \pm0.540g/L$) and phosphorus content of $0.780\pm0.020g/L$.

2.4. Methods

2.4.1. Extraction of Biocatalysts

Terminalia b. fresh leaves were squeezed and 5ml of the resulting crude extracts soaked in distilled water to make

100ml solution. The solution was left to macerate completely for 24 hours at room temperature away from direct light. The mixture was then serially filtered using Whatman no. 42 filter papers and the resulting solution preserved. For *Acanthaceae spp.,* the barks of these samples were ground to fine powder. 5g of these powders were soaked in 100ml distilled water and the procedure done for *Terminalia b*. repeated.

2.4.2. Characterization of Samples

1) *pH, conductivity and dissolved oxygen*

pH, electrical conductivity and dissolved oxygen were measured using a pH meter, conductivity meter and oxygen meter respectively.

2) *Total solids and volatile solids content*

100.0 ml of sample solution was weighed, M_1 and placed in an oven conditioned at 105°C for 6 hours before removing, cooling (in a desiccator) and reweighing. The new mass was recorded as M_2.

$$\%TS = \frac{M2}{M1} \times 100\% \quad (1)$$

100.0 ml of another sample solution was also weighed, N_1 and placed in an oven conditioned at 540°C for 1 hour before removing, cooling and reweighing. The new mass was recorded as N_2.

$$\%VS = \frac{N2}{N1} \times 100\% \quad (2)$$

3) *Sample alkalinity*

A raw sample was distilled in water (1:1) and the distillate titrated against standard 0.05N H_2SO_4 solution up to pH 4.0. The volume of sample solution used was used to determine the concentration of Alkalinity in the sample.

4) *Sample volatile acids*

A raw sample was distilled in water (1:1) and the distillate titrated against standard 0.1N NaOH solution up to pH 8.3. The volume of sample solution used was used to determine the concentration of VFAs in the sample.

5) *Total dissolved solids and total suspended solids*

For these procedures, sample concoction that had not been serially filtered were used. For the total suspended solids, the mass of 100.0ml extract solution was weighed. The solution was then passed through a pre-weighed Whatman #41 filter paper. The used filter paper was then dried in an oven at 105°C for 1 hour, cooled in a desiccator before reweighing the filter paper and solution again. The difference in weight of the filter paper is the Total Suspended Solids (TSS).

Total dissolved solids were obtained by subtracting total suspended solids from the total solids' values.

6) *Nitrogen determination by Kjehdahls' method*

Digestion; 1.00g of ground sample was digested using concentrated hydrochloric acid, potassium sulfate, anhydrous copper sulfate and sulfuric acid. Boiling chips were added to regulate the vigorous reaction.

Distillation; 85ml of 20% HCl acid was added together with antifoam. The mixture was distilled to collect about enough distillate.

Titration; The excess acid was titrated against 1N NaOH solution. Methyl orange indicator was used. A reagent blank (B) was also titrated.

7) *Organic Carbon determination by Walkley-Black method*

A weighed ground sample (1.00g) was treated with 90.0ml potassium dichromate solution and 10.0ml concentrated sulfuric acid. The mixture was gently swirled and left at room temperature in a fume hood for 16-18 hours and then 100.0ml triple-distilled water added to the mixture. The excess of dichromate was back-titrated with the standard ferrous ammonium sulfate. Blank titration of the acidic dichromate with ferrous ammonium sulfate solution was performed at the beginning of the batch analysis.

$$\text{Organic carbon } (\%) = \frac{(B-S)\times 0.0006}{m} \times 100 \quad (3)$$

Where B is the volume of ferrous solution used in the blank titration, S is the volume of ferrous solution used in the sample titration, m is the mass of the sample in gr used in the analysis. No correction factor was applied to the OC content calculation.

8) *Total Phosphorus analysis*

A filter paper was weighed and stored in a desiccator. A pre-weighed sample (W_1) was dissolved in distilled water and a different filter paper used to filter the mixture. Magnesium sulfate solution was added to the filtrate followed by dilute ammonia solution slowly while stirring. A white precipitate was formed and the mixture allowed to stand at room temperature for 15 minutes. The precipitate was then quantitatively transferred to the pre-weighed filter paper and washed with water and 95% ethanol. The precipitate was then spread on a watch glass for 8 hours and dried in the oven at 100°C for 1 hour. The precipitate was again cooled for 15 minutes before reweighing, W_2. The percentage total phosphorus was then calculated as shown below:

$$\text{Total Phosphorus } (\%) = \frac{(W1-W2)}{W1} \times 100 \quad (4)$$

9) *Total Sulfur Content by Barium Chloride Gravimetric Method*

The method is according to Abe and Yasushi [11, 12].

1.0g of sample (W) was added onto 50.0 ml of potassium hydroxide/ethanol mixture as V_1. The mixture was then heated to boil. Thereafter, 250ml of distilled water was added to the mixture (V_2) and filtered. 50.0ml of water and 5.0ml of hydrogen peroxide was added onto the filtrate solution before re-heating for 15-20 minutes. After cooling, 2 drops of phenolphthalein indicator were added then 1M HCl acid added until the color changes again. 50.0ml of 1M HCl acid solution was then added and the mixture boiled for 5 minutes. 6.0ml of saturated barium chloride solution was added and the mixture filtered using a pre-weighed filter paper. The contents

were then ignited at above 800°C using a pre-weighed crucible and mass change recorded as A.

$$\text{Total sulfur (\%)} = \frac{(AX0.343)\times W\ X\ V2}{V1} \times 100 \qquad (5)$$

2.4.3. Biogas Methane Composition Analysis

Biogas methane composition was monitored using a biogas test kit, Multitec-545.

2.5. Data Analysis

Biogas digester parameters such as temperature and pH readings that were taken daily and other substrate, biogas and bio-slurry data were subjected to statistical analysis. Statistical tools used include mean and median to test the appearance of the data sets while standard deviation and variance monitored the spread of the data. Correlation and regression to check on relation between the analysis were also done. f-test was used to check the significance in the variances, with 14 degrees of freedom and 95% confidence level being adopted. Statistical packages used include Microsoft Excel, and OriginLab applications.

3. Results and Discussions

3.1. Monitoring Change in Biogas Substrate Parameters Over the Retention Period

Most parameters of biogas substrate in an anaerobic digester gradually changes as digestion proceeds [13]. Over time, elemental composition of the substrate such as dissolved oxygen, carbon, hydrogen and nitrogen and sulfur from protein substrate also change [14]. These changes have an effect on the gas produced as well as the quality of bio-slurry formed [14]. It is thus essential to monitor changes in substrate parameters and composition over the given retention period.

3.1.1. Substrate Total Solids (TS) and Volatile Solids (VS) Content

For both total solids and volatile solids, there was a general decrease in these values over time. The digester with *Terminalia b.* extracts had a steeper gradient in change of total solids implying higher degradation rates. Table 1 below summarizes the solid content change in the biogas substrate over time.

Table 1. *Change in total solids and volatile solids in biogas substrate over time.*

Samples	Parameter (g/L)	Sample days				
		Day 1	Day 7	Day 14	Day 21	Day 28
Control	TS	11.157±0.669	8.492±0.314	5.663±0.451	5.198±0.333	4.653±0.616
	VS	11.083±0.787	7.956±0.881	5.106±0.661	4.508±0.791	4.312±0.669
Terminalia b.	TS	12.322±0.317	5.129±0.022	4.751±0.394	3.985±0.612	3.664±0.217
	VS	12.294±0.991	4.597±1.013	4.313±0.788	3.845±0.799	3.552±0.813
Acanthaceae spp.	TS	12.361 ±3.056	8.786±0.527	7.026±0.821	6.522±0.142	6.435±0.899
	VS	8.737±1.012	8.634±0.129	6.666±0.189	5.182±0.623	4.892±0.677

As a rule of thumb, a good biocatalyst should be able to enhance fast biomass saccharification at ambient conditions. From table 1 above, *Acanthaceae spp.* sample had the lowest TS/VS ratio of at the onset of anaerobic digestion implying presence of a lot of inorganic matter. It is therefore not surprising that the rate of reduction in TS in this sample was quite low. Over time, biomass degradation lead to reduction in total solids in biogas slurry [15]. Reduction in total solids content was highest in the *Terminalia b*. sample followed by the control and lastly the *Acanthaceae spp.* sample. In reference to the control setup, the *Terminalia b.* sample hydrolyzed kitchen waste biomass to 70.3% compared to 58.5% total solids in control sample within 28 days. Yat *et al.,* (2008) states that quick hydrolysis and saccharification of biomass is essential for conversion of waste biomass to energy [16]. Gumisiriza *et al.,* (2017) used salts to hydrolyze kitchen waste biomass to biofuels at thermophilic temperatures [17]. Most salt catalysts and enzymes used in biomass hydrolysis operate at thermophilic temperatures for enhanced degradability of strong cellulosic bonds [18]. The high conjugation effect of *Terminalia b*. extracts was attributable to the enhanced saccharification rates. Fast degradation of biomass lead to quick conversion of these compounds to biogas. The patterns of reduction in volatile solids were similar to those of the total solids. *Acanthaceae spp*. extracts had fewer volatile solids (more fixed solids) which are difficult to hydrolyze thus had less saccharification rates.

3.1.2. Substrate Total Suspended Solids (TSS) and Total Dissolved Solids (TDS)

The correlation of TSS and TDS in biogas substrate is fundamental in predicting the composition of bio-slurry as well as expected biogas quantity. The variation in TDS and TSS values in the biogas samples over the retention period are summarized in table 2 below.

Table 2. *Change in total suspended and total dissolved solids in biogas substrate over time.*

Samples	Parameter (g/L)	Sample days				
		Day 1	Day 7	Day 14	Day 21	Day 28
Control	TDS	3.904±0.003	2.098±0.173	2.883±0.232	2.493±0.211	2.356±0.052
	TSS	3.252±0.672	6.394±0.210	2.780 ± 0.142	2.705±0.211	2.297±0.152
Terminalia b.	TDS	4.976±0.002	3.003±0.425	3.043±0.413	2.614±0.311	2.416±0.002
	TSS	7.689±0.284	2.126±0.113	1.662±0.115	1.371±0.315	0.125±0.415
Acanthaceae spp.	TDS	4.047±0.003	3.016±0.333	3.119±0.613	3.017±0.212	2.346±0.001
	TSS	8.314±3.058	5.770 ± 0.511	3.907±0.211	3.505±0.512	4.089±0.615

The values of TSS and TDS decreased with decreasing value of total solids. This is because more solid content was progressively being converted to biogas without further replenish of the organic load [15]. The TDS and TSS values of the additives decreased by larger margins compared to the control sample. The TSS value of *Terminalia b*. on retention day-28 was extremely low and significantly different from the rest ($p \leq 0.05$, $n = 14$). Increased TSS implies high organic load which is associated to more biogas yields [19]. More TDS values implies more inorganic matrix and therefore better fertilizer quality Al-Wabel *et al.,* (2018), *Terminalia b.* sample showed the largest degradation of these solids i.e 51.4% reduction for TDS and 98.3% for TSS compared to 42.0% for TDS and 50.8% for TSS in the *Acanthaceae spp*. sample [20]. The control sample solids reduced by only 39.6% for TDS and 29.4% for TSS. These deviations in reduction of dissolved and suspended solids in biomass imply that the additives were effective in degradation of the kitchen waste to energy. The high hydrolysis rates experienced in the additive samples call for shorter organic loading rates in order not to starve the anaerobic archea [21]. On the other hand, bio-digesters with these additives optimize on biomass substrate loaded for optimal energy production.

3.1.3. Substrate pH

Sample pH values fluctuated throughout the retention period. The highest pH achieved was 7.10 ± 0.022 fot the *Terminalia b.* extract on the 14th retention day. Anaerobic conditions in a biogas digester induce fermentation of organic matter [22]. The resultant products have varying pH values depending on the preceding steps [23]. After the 14th retention day sample pH decreased as illustrated in figure 1 below.

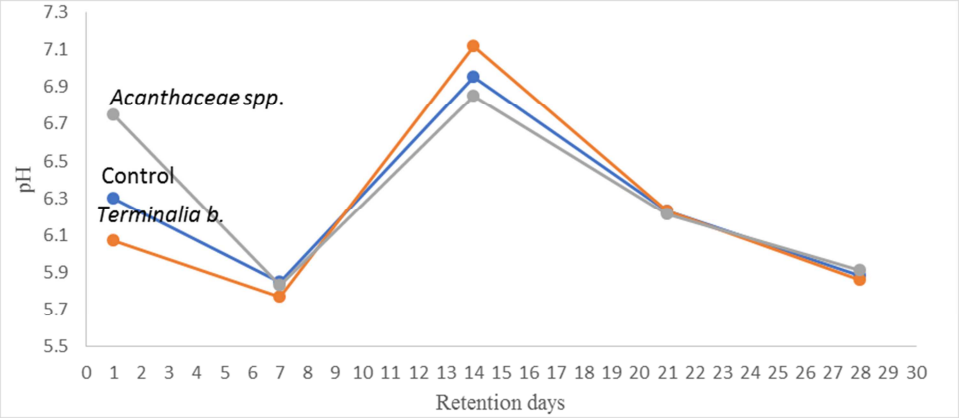

Figure 1. *Variation in sample pH over the retention period.*

The initial pH values of all the samples was slightly acidic due to presence of volatile acids. The pH decreased after the first week due to preceding anaerobic digester reactions which lead to increased acidity. Hydrolysis, acidogenesis and acetogenesis reactions in biogas digesters all lead to acidic products [24]. The pH increased on the 14th retention day due to the fourth process of biogas production (methanogenesis) which require neutral pH. Thereafter, since the organic load was not added (the digesters were in batch mode), biomass that had not fully undergone above steps began the second cycle of reactions [25]. This led to decreasing pH values. These variation in pH cycles in a biogas system are normal and have been used as an indicator to determine the status of biogas production [26]. It is however worth noting that these cycles did not limit continuous biogas production. Use of batch digestors in the experiments limited the organic loading rate therefore limited entry of process buffers responsible for controlling pH drifts [27]. Use of more inoculum is a sure means to boost the pH due to production of bicarbonate buffers by methanogenic bacteria [21].

3.1.4. Substrate Volatile Fatty Acids (VFAs), Alkalinity (ALK) and VFA/ALK Ratios

Biomass substrate from kitchen waste has a lot of carbohydrates which contain volatile acids [28]. Enough inoculum should thus be added to increase the alkalinity levels and adjust pH to neutral. The alkalinity and volatile acids levels of the substrate over the retention period are summarized in table 3 below.

Table 3. *Change in substrate volatile acids and alkalinity during biogas retention period.*

Samples		Sample VFAs (g/L), ALK (g/L) and VFAs/ALK ratios				
		Day 1	Day 7	Day 14	Day 21	Day 28
Control	VFAs	14.580±0.180	14.289±1.250	13.812±0.413	12.785±0.665	11.364±0.649
	ALK	0.900±0.132	0.298±0.001	0.336±0.000	0.342±0.012	0.422±0.068
	VFA/ALK	16.200±1.385	47.949±12.134	41.107±1.881	37.383±2.577	26.929±2.314
Terminalia b.	VFAs	12.667±0.257	11.905±1.152	11.816±0.662	10.417±0.525	9.315±0.662
	ALK	0.5167±0.029	0.412±0.228	0.384±0.089	0.486±0.098	0.558±0.075
	VFA/ALK	24.512±1.00	28.896±2.314	30.771±2.301	21.434±0.698	16.694±0.459

Samples		Sample VFAs (g/L), ALK (g/L) and VFAs/ALK ratios				
		Day 1	Day 7	Day 14	Day 21	Day 28
Acanthaceae spp.	VFAs	11.440 ±0.080	11.965±0.613	11.862±0.773	10.016±0.448	9.259±0.000
	ALK	0.467±0.058	0.384±0.089	0.412±0.128	0.422±0.068	0.480±0.000
	VFA/ALK	24.497±1.212	31.159±0.989	28.791±1.322	23.735±1.001	19.290±0.884

VFA/ALK values were low at the onset of digestion. Thereafter, as the pH levels reduced volatile acids increased and VFA/ALK ratios increased significantly before reducing continuously over the retention period. The control sample had large fluctuations in VFA/ALK ratios. Instability in volatile acids and alkalinity minimize acetogenic and methanogenic bacteria leading to less biogas output and low methane level [29]. *Terminalia b.* sample was the most consistent in VFA/ALK ratios and consecutively had the highest biogas yields and methane levels. Fluctuations in volatile acids and alkalinity levels in biogas systems have continuously been used to monitor methane production [30]. Increased levels of non-dissociative VFAs in the control sample and homeostasis are attributable to inconsistent pH drifts. Such drifts cause an uncomfortable environment for methanogenic bacteria leading to low biogas production.

3.1.5. Substrate Organic Carbon

Organic carbon represents the load that bacteria digest to produce biogas [31]. Organic carbon content in the biogas samples reduced linearly over retention period since the system was in batch mode. The samples with additives carbon content decreased by a larger margin compared to the control sample as seen in figure 2 below.

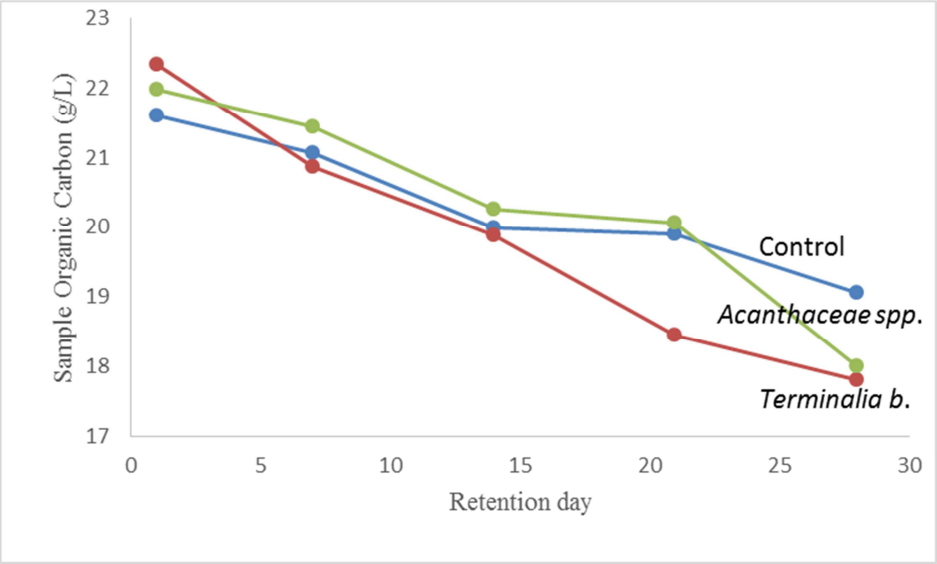

Figure 2. *Variation in organic carbon over retention time.*

The organic carbon content of the control sample on retention day-28 ($19.06\,0 \pm 0.000 g/L$) was found to be significantly different from that of *Terminalia b.* ($17.800 \pm 0.698 g/L$) and *Acanthaceae spp.* ($18.000 \pm 2.400 g/L$) ($p \leq 0.05$, n = 14). This indicates that the rate of biomass hydrolysis in the control sample was lower than that of the additives. Previous studies have shown that carbon levels gradually reduce in a biogas digester as the element is continually being converted into methane and carbon dioxide [32]. Decreased carbon content of the kitchen waste substrate is directly attributable to the rate of VS reduction. As seen earlier on, the samples with additives, especially *Terminalia b.* were able to degrade the biomass by 98.3%. The *Acanthaceae spp.* sample reduced the VS by 50.8% while the control sample could only manage 29.4% within the same period and conditions. Rapid reduction in carbon content requires regular organic loading into the bio-digesters.

3.1.6. Substrate Nitrogen, Phosphorus and Sulfur Variation

The concentrations of all the samples analyzed were found to increase over the retention period. Degradation of kitchen waste substrate by anaerobic bacteria led to breakdown of large biomass samples thus freeing the nutrients available [33]. Nitrogen, phosphorus and sulfur are key components of these nutrients. The variations in nitrogen, phosphorus and sulfur content are illustrated in table 4 below.

Table 4. *Variation in nitrogen, phosphorus and sulfur over the retention period.*

Sample	Parameter	Retention period				
		Day 1	Day 7	Day 14	Day 21	Day 28
Control	Nitrogen (g/L)	1.850±0.700	6.183±0.545	11.083±0.867	11.433±0.910	12.367±0.652
	Phosphorus (g/L)	0.780±0.020	1.335±0.025	1.560±0.019	2.305±0.062	1.960±0.088
	Sulfur (g/L)	2.178±0.315	2.435±0.331	2.950±0.000	3.293±0.000	4.842±0.005

Sample	Parameter	Retention period				
		Day 1	Day 7	Day 14	Day 21	Day 28
Terminalia b.	Nitrogen (g/L)	1.633±0.313	9.100±0.632	13.533±0.667	13.767±0.882	14.350±0.350
	Phosphorus (g/L)	0.587±0.023	1.232±0.055	2.420±0.648	2.490±0.171	2.520±0.250
	Sulfur (g/L)	3.463±0.051	1.853±0.023	4.139±0.212	5.381±0.022	6.412±0.150
Acanthaceae spp.	Nitrogen (g/L)	1.633±0.313	7.000±0.585	11.433±1.022	12.367±0.652	13.183±0.435
	Phosphorus (g/L)	0.660±0.000	1.345±0.076	1.525±0.454	1.675±0.112	2.420±0.648
	Sulfur (g/L)	1.852±0.110	2.371±0.012	3.990±0.0120	4.921±0.121	5.612±0.151

Hydrolysis of the kitchen waste biomass led to degradation of proteins present freeing more nitrogen either as elemental nitrogen, ammoniacal nitrogen or anionic nitrates. Chen *et al.,* (2017) portrayed bio-slurry nitrogen to be 2 to 2.7 folds higher than in mineral fertilizer [34]. Use of the two additives caused more degradation of the proteins and therefore these samples had more nitrogen content than their corresponding control sample. Ezekoye *et al.,* (2011) found the total nitrogen concentration to increase from 1.99% to 2.25% in poultry droppings and from 0.49% to 0.80% in cassava peels [35]. The same study found phosphorus levels in poultry droppings increased from 0.31% to 0.90% and from 3.01% to 5.67% in cassava peels during substrate degradation process. The *Terminalia b.* sample showed the largest increment in sulfur across the retention period. The sulfur levels were found to be significantly different in *Terminalia b.* and *Acanthaceae spp.* samples as contrasted to the control setup ($p \geq 0.05$, $n = 14$). This implies that using these biocatalysts enhances fast degradation of the kitchen waste biomass to release sulfur.

3.2. Biogas Yields

Biogas production was in the order *Terminalia b.* (15,861.4ml/gVS), *Acanthaceae spp.* (13,219.6ml/gVS) and control (7,444.8ml/gVS) at 19.5 ±0.5 °C. The sample with *Terminalia b.* extracts produced biogas right from the first day. This sample had the highest biogas production yield (2.32 folds the control sample). *Acanthaceae spp.* sample also produced high biogas volumes (1.375 folds compared to control sample) at the same temperature. The trend of biogas production over the retention period is illustrated in figure 3 below.

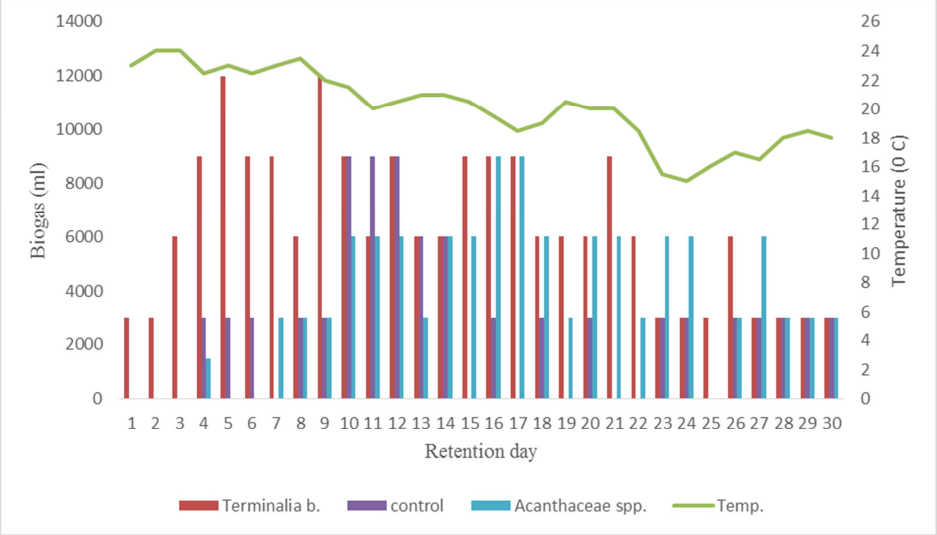

Figure 3. *Variation of biogas volume with temperature over the retention period.*

Production of biogas was proportional to temperature variation. Biogas production was found to increase with increase in temperature. Increased temperature is known to provide optimal survival conditions for anaerobic bacteria. The bacteria thus multiply at higher temperature and convert more biomass into biogas [10]. At higher temperature, glycosidic bonds of the biomass are also weakened and can be easily degraded to biogas. Biogas production in *Terminalia b.* sample was quite high and only declined after retention day-12 due to decreased temperature but maintained a rate of 6500ml biogas/retention day. This value was slightly lower than the 7000ml/retention day achieved using acetate enzymes in a thermophilic biogas digester [36]. Upon commencement of biogas production, *Acanthaceae spp.* sample had consistent biogas yields averaging 3850 ml/retention day. The control sample biogas yields averaged 2800ml/retention day. Biogas production from *Terminalia b.* sample was proven to be significantly different from the rest at 95% confidence level ($n = 14$).

3.3. Methane Levels in Biogas Samples

The quality of good biogas is expressed in terms of the total methane percentage in the gas. Most biogas systems produce between 55% to 75% methane content from raw biogas using animal dung substrate [37]. The situation is different when kitchen waste is used due to increased contaminants as a result of protein breakdown. Biogas

composition was analyzed on a weekly basis for CH_4 gas. Table 5 below summarizes the trend in major gas levels over the 28-day retention period.

Table 5. *Variation in methane levels in raw biogas over 30-day retention period.*

Samples	Methane levels (%)			
	Week 1	Week 2	Week 3	Week 4
Control	14.450±0.968	22.250 ±0.777	29.800 ±2.272	41.750 ± 1.401
Terminalia b.	22.050±0.982	29.050 ±0.173	35.650 ±4.638	45.475±0.922
Acanthaceae spp.	20.825±2.353	23.950 ±0.402	34.450 ±0.436	39.275±0.263

Terminalia b. methane levels were highest (45.475±0.922%) across the entire retention period with the *Acanthaceae spp.* sample having the least methane levels expect on the last retention week (39.275 ±0.263%). *Terminalia b*. methane levels were 1.04 folds higher than the control sample, slightly lower than when pure carbohydrases and proteases enzymes are used (1.72 folds and 1.53 folds respectively) [38]. Methanogenesis process of biogas production is known to begin after about 15-25 days depending on temperature. Lv *et al.,* (2014) found out that accumulation of ammonia in biogas substrate containing a lot of proteins such as kitchen waste cause intoxicates methanogenic bacteria [39]. This leads to poor production of methane in such systems. Methane production is also reduced in low temperature regimes. The findings in this study were lower than those of Gaby *et al.,* (2017) who produced 70% and 69% methane levels from food waste biomass with digesters operated at 55°C and 60°C respectively [40]. Wang *et al.,* (2019) reported methane levels of the range of 25-50% from cow dung manure and wheat straw in biogas digesters operated between 20-25°C (cryo-mesophilic temperatures) [19].

4. Conclusion

The biocatalytic additives used were found to positively affect the rates of kitchen waste substrate parameters and composition as well as biogas yields and methane levels. The rates of biomass saccharification were enhanced in the sample containing *Terminalia b.* and *Acanthaceae spp.* according to reduction rates of total solids, volatile solids, total suspended and dissolved solids. *Terminalia b*. additives exhibited high volatile solids hydrolysis rate of 98.3% followed by *Acanthaceae spp*. (50.8%) and control sample (29.4%). These findings were echoed by the rapid degradation in organic carbon in the same order. Nitrogen, phosphorus and sulfur content linearly increased due to immobilization of these compounds as the biomass was being degraded. The trends of pH and volatile fatty acids/alkalinity were relatively similar in all the experimental setups. Biogas production patterns were in the order of *Terminalia b*. (15,861.4ml/gVS), *Acanthaceae spp*. (13,219.6ml/gVS) and control (7,444.8ml/gVS). This implied that *Terminalia b.* sample produced 2.32 folds higher while *Acanthacae spp*. sample produced 1.375 folds higher than the control sample. *Terminalia b.* methane levels were highest (45.475±0.922%) followed by the control sample (41.750±1.401) and *Acanthaceae spp.* sample (39.275±0.263%) after 28-day retention period at 19.5±0.5°C.

The two biocatalytic additives were thus found to affect biogas production by fast hydrolysis of biomass, pH stabilization and increased biogas production. Determination of variations crucial biogas parameters from kitchen waste substrate and how use of biocatalysts affects the parameters is quite important. The additives not only affect the solid and hydraulic retention period, but also the amount of biogas expected and its quality.

Conflicts of Interest

The authors declare to have no conflicts of interest whatsoever.

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No funding was received for this study.

Data Availability Statement

All data used in this study is within the manuscript and any supplementary sheets attached.

Authors Contribution

"Conceptualization, B. C, A. O, J. M and M. M.; Methodology, B. C.; Software, M. M.; Validation, B. C., A. O., J. M. and M. M.; Formal Analysis, M. M.; Investigation, B. C.; Resources, A. O. and J. M.; Data Curation, M. M.; Writing – Original Draft Preparation, B. C.; Writing – Review & Editing, B. C.; Visualization, B. C.; Supervision, A. O., J. M. and M. M.; Project Administration, A. O.; Funding Acquisition."

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