Optimization of Bioslurry-Available Plant Nutrients Using \textit{T. brownii} and \textit{Acanthaceae} spp. Biocatalysts

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The plant extracts of \textit{T. brownii} and \textit{Acanthaceae} spp. have been used as biocatalysts by several communities in Kenya to hasten anaerobic digestion. This study aimed at assessing the viability of these two extracts in hastening the availability of plant nutrients from bioslurry at ambient conditions. A controlled research design was followed using uncooked kitchen waste as the substrate for 28 retention days. Changes in bioslurry physicochemical properties and available plant nutrients were monitored every 7 days using wet chemistry and spectroscopic methods. The findings indicated that the two extracts significantly impacted the levels of available plant nutrients in the bioslurry compared to the control samples. \textit{T. brownii} additives significantly increased the levels of lime content, total Kjeldahl nitrogen, total phosphorus, phosphoric acid, sulfur, and soluble silicic acid. On the contrary, \textit{Acanthaceae} spp. additives significantly increased the levels of calcium, potassium, nitrates, total ammoniacal nitrogen, sulfates, and phosphates in the bioslurry samples. The use of these plant extracts thus reduces the time taken while increasing the concentration of available plant nutrients from bioslurry.

1. Introduction

Good agricultural practices trickle down to sufficient water for irrigation, good postharvest mechanisms, and storage practices as well as the use of fertilizers before and during planting seasons. Most fertilizers used are inorganic and are expensive to the majority of the farmers in Africa [1]. The primary goal of fertilizers is to supplement depleted nutrients in the surrounding of a plant. A vast portion of land in the globe has been used for tilling for many years. This has led to a gradual reduction of soil nutrients [2]. Practices such as crop rotation that help sustain soil nutrients for long are not always practicable. Fertilizer addition to a piece of land is thus exponentially increased annually. Demand for more fertilizer has, therefore, grown over time [2, 3]. This has led to the diversification of fertilizer raw material leading to the adoption of organic fertilizers.

Unlike inorganic fertilizers, organic fertilizers do not have definite nutrient concentrations. The viability of organic fertilizer is dependent on many factors such as its source, decomposing period, the consortia that it has been mixed in, and other factors [4, 5]. Albeit having lower essential plant nutrients, this type of fertilizer is preferred due to its quick biodegradability nature [6]. Several inorganic fertilizers leach into the soil deeply due to high concentration and solubility finding their way into water systems. The long-lasting fate of this phenomenon is water pollution. Other inorganic fertilizers used volatilize quickly leading to massive loss of fertilizer to the target site and air pollution [7]. In organic fertilizer, the soluble and volatile nutrients are embedded in a myriad of organic compounds which hold them together [8]. This reduces the chances of both water and air pollution. Organic fertilizer is cheap due to the availability of agricultural residues and animal manure in rural farms. The
preparation of organic manure is also quite easy and occasionally involves mixing consortia of agricultural residues and/or animal manure and allowing for decomposition to occur. Organic fertilizer should, however, be prevented from direct sunlight which can trigger some side reactions [9]. Plants grown by organic manure are preferred since the fertilizer also has antimicrobial activities which kill pests without leaving behind toxic chemicals [10]. Anaerobic digestion of agricultural residues or animal manure using a biogas digester is an excellent method of preparing organic fertilizer. The fertilizer is termed as bioslurry.

Biogas slurry, or bioslurry, is obtained after biogas substrate has completed the required retention time in a biogas digester [11]. The optimum bioslurry tapping point from a biogas digester should be at the onset of gas production [12]. The time taken is known as the solid retention time and can be defined as the period when more than half of the total solids have been converted to volatile solids [13]. Intrinsically, the solid retention time of agricultural residues is long due to the strong nature of glycosidic and amide bonds that have to be broken [14]. More energy is required to break these bonds to free the nutrients in a form that can easily be taken by plant roots [15].

Several traditional African communities used various salts and plant extracts to hasten the breakdown of biomass [15]. The ancient Aandia community found that on the western slopes of Mt Kenya, T. brownii leave extracts were used to hasten saccharification of wheat bran (cellulose) during the preparation of their traditional alcohol. The Maasai community of Kenya has continuously enjoyed hastening the fermentation process during the preparation of their native alcohol using Acanthaceae spp. barks [15]. The aim of this work was to investigate whether the use of these two biocatalysts in biogas systems could optimize the availability of plant nutrients in the bioslurry. The use of biocatalysts in the optimization of available plant nutrients is quite cheap and easy to use.

2. Materials and Methods

2.1. Design of Experiment. Three biodigesters of capacity 40.0 liters operated at mesophilic temperature regime were used to monitor the levels of available nutrients in bioslurry. The digesters were all maintained in batch mode. A 30-day retention period was used. Biogas substrate comprising 67% by weight uncooked kitchen waste and the rest inoculum (cow and goat dung in ratio 1:1) was used. The substrate mixture above (kitchen waste and inoculum) was soaked using distilled water (10% water) for 24 hours prior to loading into the biodigesters. Two of the digesters were dosed with 5% v/v additive (T. brownii and Acanthaceae spp. extracts) while the third biodigester was the control setup. The number of replicates was limited by the study budget. Prior to loading, the kitchen waste substrate was characterized by anaerobic digester parameters. The two additives were also characterized for possible functional groups and absorption peaks by FT-IR (Shimadzu-119; Tokyo, Japan) and UV-VIS (Jenway-6850; Staffordshire, UK), respectively.

Characterizing the bioslurry substrate and monitoring the levels of available nutrients were done at Maasai Mara University, Kenya. Volatile organic salts/total anorganic carbon (VOS/TAC Pronova 2000; Berlin, Germany) analysis tests and quantitative UV-VIS analysis for levels of free ligands in the bioslurry across the retention period were done at Taita Taveta University, Kenya. UV-VIS analysis for levels of nitrates, sulfates, and phosphates on retention day 28 was done at Vaal University, South Africa.

3. Materials

3.1. Biogas Substrate Used. The kitchen waste used was characterized to have an average pH of 6.300 ± 0.001 and an electrical conductivity value of 1.293 ± 0.002 µS. The total solids content was 11.1566 ± 0.668 g/L against a volatile solid content of 11.283 ± 0.008 g/L implying that 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 ± 0.672 g/L while the total dissolved solids were 3.904 ± 0.003 g/L. The average values of alkalinity and volatile fatty acid levels were 0.900 ± 0.132 mg/L and 14.580 ± 0.811 mg/L. The average VOS/TAC value was 1.240 ± 0.020. The elemental composition values were dissolved oxygen of 8.500 ± 0.476%, organic carbon of 21.600 ± 2.400 g/L, nitrogen of 3.067 ± 0.540 g/L, and phosphorus content of 0.780 ± 0.020 g/L.

4. Methods

4.1. Extraction and Characterization of Biocatalysts

4.1.1. Extraction. T. brownii leaves and Acanthaceae spp. barks were collected from Kirinyaga County, Kenya (0.6591°S, 37.3827°E), and Narok county, Kenya (1.1041°S, 36.0893°E), respectively. T. brownii fresh leaves were squeezed, and 5 ml of the resulting crude extracts was soaked in distilled water to make a 100 ml 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 the Whatman no. 42 filter papers and the resulting solution was preserved.

To prepare Acanthaceae spp. extracts, the barks of these samples were ground to a fine powder. 5 g of these powders was soaked in 100 ml distilled water. The mixture was then left to macerate and filtered as was the case with T. brownii.

The two extracts were then subjected to functional group by IR analysis (Shimadzu-119) and absorption peaks analysis by UV-VIS (Jenway-6850).

4.1.2. Bioslurry Analysis for Available Plant Nutrients

(1) pH and Dissolved Oxygen. pH and dissolved oxygen were measured by the use of a pH meter (Hanna G-114; Woonsocket, USA) and oxygen meter (GPro-500; California, USA).

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(2) **Total Solids.** 10.000 g of the sample was weighed, $M_1$ using an analytical balance, and then 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 $M_2$:

$$\% \text{TS} = \frac{M_2 - M_1}{M_1} \times 100\%.$$  \hspace{1cm}(1)

(3) **Nitrogen Determination by Kjeldahl Method.** This method was according to [16]. Total Kjeldahl nitrogen analysis was carried out according to the Kjeldahl standard method. The samples were digested in potassium sulfate, anhydrous copper sulfate, and sulfuric acid. The digestate was then distilled in 20% hydrochloric acid. The distillate was titrated with standard sodium hydroxide solution and the concentration of nitrogen in the sample calculated.

(4) **Organic Carbon Determination by Walkley-Black Method.** Organic carbon in the samples was determined by the Walkley-Black method [17]. A fixed mass of the sample was treated with a potassium dichromate solution followed by concentrated sulfuric acid. The mixture was swirled and left to digest for 16–18 hours in a fume hood. The resulting solution was diluted and back-titrated with standard ferrous ammonium sulfate solution. A blank analysis was also done:

$$\text{Organic carbon (\%) = \left( \frac{(B - S) \times 0.0006}{m} \right) \times 100,}$$  \hspace{1cm}(2)

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, and $m$ is the mass of the sample in grams used in the analysis. No correction factor was applied to the OC content calculation.

(5) **Total Phosphorus Analysis.** A sample was dissolved in water and filtered. The filtrate was reacted with the magnesium sulfate solution before filtering again. The solution was gradually precipitated with standard ammonia solution while stirring. The precipitate formed was quantitatively transferred to a preweighed filter paper. The filter paper and its contents were washed with water and 95% ethanol before spreading on a watch glass for 8 hours and drying in an oven at 100°C for 1 hour. The mixture was thereafter cooled and the mass of residue was obtained.

(6) **Nitrates.** Nitrate standards were prepared by dissolving 8.0 g of salicylic acid in 100 ml of 1M H$_2$SO$_4$ acid then swirling to fully dissolve. 10 ml of this solution was added to 90 ml of the aliquot sample solution. Acidification using 1M HCl was done to minimize interference by other ligands. Absorbance was checked in the range of 270–320 nm.

(7) **Sulfates.** For standard preparation, 10 g of NaCl and 10 ml of conc. HCl acid were added to 40 ml of glycerol solution. A yellowish color was formed. 5 ml of this solution was added to 45 ml of analyte solution and the absorbance was read at 410–430 nm.

(8) **Phosphates.** A conditioning reagent was made by dissolving 1.7081 g of ammonium molybdate and ascorbic acid (5.82 g/300 ml distilled water) in 150 ml warm water. The solution was cooled before diluting to 250 ml. 0.125 g of hydrazine sulfate in 100 ml distilled water was added. Analyte samples were diluted by a factor of 10, and the conditioning reagent was added before measuring the absorbance at 830–860 nm against those of the blank and standards.

(9) **Potassium.** This method is according to [18]. 10 ml of ammonium acetate in acetic acid solution was added to 40 ml of sample solution diluted 40 folds. The ammonium acetate/acetic acid solution was prepared by dissolving 15 g of the salt in 50 ml acetic acid. Absorbance was then read at 260–280 nm.

(10) **Loss On Ignition (LOI).** 1.0 g of sample was put into a preweighed crucible and subjected to high temperature (>540°C) in an oven for 1 hour, cooled in a desiccator, and then reweighed.

(11) **Biofertilizer Alkalinity (Lime Content).** 1.0 g of the sample was dissolved in 100 ml of distilled water in a beaker and thoroughly stirred. The pH of this mixture was then taken, and 1M HCl acid solution was added dropwise to the mixture (with pH probe inserted) until pH 4.3 is attained. The number of drops used was then quantified.

(12) **Total Ammoniacal Nitrogen (TAN) by Formaldehyde Method.** The method is according to [19, 20]. A sample of the bioslurry was digested in hydrochloric acid to completely effervescence. Aluminum chloride and indicator solutions were, thereafter, added before filtering. The potassium hydroxide solution was added to the filtrate solution gradually until the color of the mixture changed. The mixture was then reacted with standard hydrochloric acid and formaldehyde solution before standardizing with sodium hydroxide solution.

For the blank determination, 100 ml of 1M HCl acid and 10.0 ml of formaldehyde acid were used:

$$\% \text{NH}_3 = \left( V_5 - V_B \right) \times C \times f \times \frac{V_1}{V_2} \times \frac{14.007}{W} \times \frac{100}{1000}$$  \hspace{1cm}(3)

where $V_5$ is the sample volume, $V_B$ is the blank volume, $c$ is the concentration of titrant in mol/liter, $f$ is the ammonia factor, $V_1$ is the volume of the sample solution, $V_2$ is the volume of sample solution transferred after filtration, and $W$ is the weight of sample in grams.

(13) **Total Phosphoric Acid (TPA) by Quinoline Gravimetric Analysis.** The method is according to [19, 21]. A sample of the analyte was digested with sulfuric acid, potassium sulfate, and hydrated copper sulfate. Distilled water was added to the mixture after white fumes had cleared the flask. A portion of
this mixture ($V_2$) was mixed with concentrated sulfuric acid and quinomiac solution (prepared using quinoline and sodium molybdate). The mixture was filtered and the residue was ashed onto a preweighed filter paper:

$$(T-P_2O_5)\% = A \times 32.07 \times \frac{100}{1000} \times \frac{V_1}{V_2} \times \frac{1}{W}$$

where $A$ is the mass of precipitate and $W$ is the weight of the sample in grams.

(14) Soluble Silicic Acid by Perchloric Acid Method. The method is according to [19, 21]. 1.0 g of sample was added onto 50.0 ml of conc. HCl acid to allow for effervescence before filtering. The solution obtained is $V_1$. 30.0 ml of the above solution ($V_2$) was added to conc. perchloric acid and heated. After white fumes are observed, the heating flask was covered to prevent fume loss and heating continued for a further 15–20 minutes. The mixture was then cooled for 30 minutes before adding 50 ml 1M HCl acid and reheating (while still covered) at 70–80°C. The mixture was then filtered using a preweighed filter paper and washed thoroughly using 1M HCl acid and hot water solution before drying for 1 hour at 120°C. The filter paper with its contents was then ignited at above 800°C using a preweighed crucible and mass change was recorded as

$$(S-SiO_2)\% = A \times \frac{V_1}{V_2} \times \frac{1}{W} \times 100,$$

where $A$ is the mass of precipitate.

(15) Total Sulfur Content by Barium Chloride Gravimetric Method. The method is according to [20, 22]. 1.0 g of sample ($W$) was added to 50.0 ml of potassium hydroxide/ethanol mixture as $V_1$. The mixture was then heated to boil. Thereafter, 250 ml of distilled water was added to the mixture ($V_2$) and filtered. 50.0 ml of water and 5.0 ml of hydrogen peroxide were added to the filtrate solution before reheating for 15–20 minutes. After cooling, 2 drops of phenolphthalein indicator were added, and then 1M HCl acid was added until the color changed again. 50.0 ml of 1M HCl acid solution was then added and the mixture was boiled for 5 minutes. 6.0 ml of saturated barium chloride solution was added and the mixture was filtered using a preweighed filter paper. The contents were then ignited at above 800°C using a preweighed crucible and mass change was recorded as $A$:

$$T.Sulfur \% = (A \times 0.343) \times \frac{V_2}{V_1} \times 100.$$

4.2. Data Analysis. Data collected was reported as a mean ± standard deviation. 14 degrees of freedom were used. The confidence level used was 95%. Statistical analysis was done using Ms Excel and OriginLab (version 6.5) software.

5. Results and Discussion

5.1. Functional Group Peaks of the Additives. The FT-IR profiles of both extracts were similar toward the blue end but differed in functional group peaks and intensity toward the right of their FT-IR spectra. Carboxylic- OH$_{stretch}$, sp$^3$ C-H, and Amide$_{stretch}$ peaks were present in both additives at almost equal intensity. These peaks indicate the presence of weak carboxylic acids present in the extracts [23]. The carbonyl peak at 1680 cm$^{-1}$ in T. brownii extracts was more pronounced compared to that in Acanthaceae spp. In contrast, the C=O$_{stretch}$ peak at 1035 cm$^{-1}$ in Acanthaceae spp. was more pronounced while T. brownii extract had more fingerprint peaks, key among them being trans =C-H$_{bend}$ and =C-H$_{bend}$ peaks. Of more interest was the C-X$_{bend}$ peak at 560 cm$^{-1}$ in the T. brownii extract. Increased diversity of organic compounds enhances reactivity [24]. The collated FT-IR spectra of the two additives are as shown in Figure 1.

5.2. Absorption Peaks of Additives. Plant extracts are known to have several absorption peaks arising from various organic compounds [25]. The UV-VIS spectra of these additives are in Figure 2.

The extracts’ spectra had more peaks before 300 nm indicating the presence of absorption peaks. T. brownii had peaks at 215 nm and 240 nm illustrating more absorption as a result of $\pi - \pi^*$ transition [26]. Characteristics peaks at 300 nm and 840 nm indicated the presence of isolated carbonyls, nitrates, and phosphates in the extracts [27, 28]. $\lambda_{max}$ at 460 nm indicated trace presence of carotenoids while $\lambda_{max}$ at 595 nm indicated the presence of a more conjugated compound (e.g., a blue dye). An increase in the number of absorption peaks leads to a shift of electron density which destabilizes organic molecules making them prone to electrophilic and nucleophilic attacks [29]. This effect leads to hydrolysis of the biomass which frees more nutrients for plant uptake.

6. Bioslurry Available Fertilizer Analysis

The elemental composition of bioslurry at different retention periods is crucial in defining its potential as an organic fertilizer [30]. The availability of these essential nutrients for reach by plant roots is also important [31]. Several bioslurry parameters and elemental composition of elements and their ions were done at different retention periods and summarized hereinafter.

6.1. Bioslurry pH. The pH value of fertilizer is quite important since different plants produce optimally in different soil pH values [32, 33]. Various soil microorganisms are also affected by pH changes. Table 1 illustrates the changes in bioslurry pH over the retention period used. Changes in pH values in anaerobic systems are largely dictated by the type of biomass present and inoculum used [34]. Kitchen waste residues had high volatile fatty acids and were thus acidic at the beginning of anaerobic digestion. The initial stages of anaerobic digestion (hydrolysis, acidogenesis, and acetogenesis) are controlled by acidic enzymes and end up giving acidic products [35]. The pH of bioslurry was thus quite acidic after day 7. Retention day 14 had a neutral pH due to methanogenesis which occurs in neutral
pH [36]. Sample with *T. brownii* extracts had the highest pH value of 7.120 ± 0.022. This implies that this was the best time to collect its bioslurry. Thereafter, the organic load necessary for methanogenesis declined (since the digesters were in batch mode), and bacteria present started hydrolyzing the unreacted biomass leading to previous anaerobic digestion processes and a gradual decrease in pH [37]. The additives also increased biogas yields from the AD systems. The order of biogas output was *T. brownii* (1586.14 ml/g VS), *Acanthaceae* spp. (1321.96 ml/g VS), and control (744.48 ml/g VS). The methane levels were in the order of *T. brownii* (43.475 ± 0.9215%), control sample (41.750 ± 1.4012%), and *Acanthaceae* spp. (39.275 ± 0.2629%). After 28-day retention time, the *Acanthaceae* spp. sample had more pH value than the other samples (5.910 ± 0.010). This was the most suitable sample for the growth of most plants [38]. There was, however, no significant variation in pH as a result of the use of the two additives at a 95% confidence level (*n* = 14).

### 6.2 Bioslurry Electrical Conductivity (EC)

Variation in EC values as a function of using the two additives is important in assessing the viability of the additives. Table 2 summarizes the changes in EC values over the retention period.

The EC values declined after retention day 7 as volatile acids in the organic load increased. Around the 14th retention day, there was less organic matrix in the ions present which were easily detected leading to higher EC values. An ultimate range of 4.520 ± 0.003 (Control sample) to 5.250 ± 0.027 μS (*Acanthaceae* spp.) was attained which is

### Table 1: changes in pH value of bioslurry.

| Samples          | Day 1     | Day 7     | Day 14    | Day 21    | Day 28    |
|------------------|-----------|-----------|-----------|-----------|-----------|
| Control          | 6.300 ± 0.001 | 5.850 ± 0.001 | 6.950 ± 0.022 | 6.230 ± 0.003 | 5.880 ± 0.010 |
| *T. brownii*     | 6.070 ± 0.000 | 5.770 ± 0.017 | 7.120 ± 0.022 | 6.230 ± 0.017 | 5.860 ± 0.003 |
| *Acanthaceae* spp.| 6.750 ± 0.017 | 5.830 ± 0.010 | 6.850 ± 0.017 | 6.210 ± 0.017 | 5.910 ± 0.010 |

![Figure 1: FT-IR spectra of *T. brownii* (red) and *Acanthaceae* spp. (blue) extracts. Though relatively similar, *T. brownii* extracts showed more functional group peaks.](image1.png)

![Figure 2: UV-VIS analysis of *T. brownii* and *Acanthaceae* spp. extracts. The spectra show more peaks toward the blue end of the spectra.](image2.png)
optimal for solubility of plant nutrients and their uptake by roots [39]. Electrical conductivity values in slurry depict the presence of soluble ions for plant roots to uptake. High EC values imply that the bioslurry is quite soluble and can thus require less water during application to plants.

6.3. Bioslurry Total Solid (TS) and Loss on Ignition (LOI). The content of total solids in a fertilizer sample is crucial in determining its primary mode of application. Table 3 summarizes the TS and LOI variation in the bioslurry over the retention period.

The TS of the samples decreased linearly over the retention period to about one-third of the initial value by the 28th day. Hydrolysis of kitchen waste biomass in the sample with *T. brownii* extract was accelerated by a large margin as evidenced by the reduction in TS within the first week by about half (12.320 ± 0.317 g/L to 5.129 ± 0.022 g/L). The LOI values in *T. brownii* sample were also quickly reduced. The bioslurry from this extract thus had low TS and LOI values making it suitable as a top dress or for foliar application. The TS and LOI values of samples with the *Acanthaceae* spp. extract were quite high right from the onset indicating more particulate levels in this sample. This bioslurry is, therefore, not appropriate for aerial application.

Fertilizer samples with high total solids content require more water if they are to be applied as top-dressing fertilizer [40]. On the contrary, low TS content implies that the fertilizer can easily be applied by top-dressing. High LOI values imply that the bioslurry fertilizer should not be on the soil surface to minimize evaporation. High LOI bioslurry is volatile, and most of it does not end in the target location when applied as a top-dressing fertilizer. Such fertilizer samples require to be planted together with the plant or injected into the soil. The eventual TS and LOI values in all digester samples were significantly different at a 95% confidence level (*n* = 14). This implies that the two types of additives should not be applied in a similar method for optimal absorption by plants.

6.4. Bioslurry Alkalinity (Lime Content). The alkalinity of bioslurry implies its ability to buffer acidic soil. Many plants perform optimally in neutral and slightly basic soil [38, 41]. Table 4 illustrates the variation in alkalinity values in the bioslurry samples.

The control sample had the highest alkalinity level at the onset which drastically reduced after 7 days of the retention period. This can be attributed to precursor reactions that lead to the formation of volatile acids such as acetic, propanoic, and butyric acid [42]. The control sample had thus very little buffering capacity which has direct effects on the bioslurry quality. While the alkalinity levels of *Acanthaceae* spp. samples had little variations, those of the *T. brownii* sample largely fluctuated. *T. brownii* sample had the highest alkalinity level of 0.558 ± 0.075 g/L after 28 days. This value was significantly different from the rest at a 95% confidence level (*n* = 14). Bioslurry alkalinity is primarily contributed to its lime content and has previously been used as an indicator of lime levels [43]. High alkali levels are desired for any bioslurry as the fertilizer can work well in acidic soils.

6.5. Bioslurry Oxygen, Calcium, Carbon, and Potassium Levels. The elemental composition of bioslurry is quite useful as these elements are the required plant nutrients. Elucidation of whether an element is a micro- or macronutrient varies with different types of plants as well as their growth stage [44]. Table 5 summarizes the abundance and variation of these elements in the bioslurry over the retention period.

From the substrate analysis, volatile solids and total suspended solids declined over retention time. Similarly, oxygen and carbon content in the bioslurry of all three samples decreased linearly. The dissolved oxygen at the onset of digestion is gradually converted into other by-products by anaerobic archaea present in the slurry [45]. Similarly, carbon is the main element in biomass for the production of methane. Carbon present as carbohydrates, lipids, or proteins is sequentially converted to low weight products by anaerobic archaea present in the slurry [45]. The oxygen and carbon levels at retention day 28 are thus the lowest in the series. The sample with *T. brownii* extracts had the largest disparity in carbon content after the 28-day retention period due to massive biogas conversion by this sample. This sample had lower bioslurry viability as far as oxygen and carbon are concerned. Wang et al. [47] showed that the use of bioslurry from poultry dung had almost similar carbon content with mineral fertilizer. Available carbon from the bioslurries above was higher than that obtained when using slurry with pure animal manure origin (1.3%) [48].

Unlike oxygen and carbon, calcium and potassium content increased over time. As the organic matrix in the biomass was being converted into biogas, the inorganic ions availability increased as these ions were continuously being freed. This is also justified in the increment of the total dissolved solids (as seen above). *Acanthaceae* spp. sample
which had the highest total solids and total dissolved solids had the highest calcium and potassium levels.

6.6. Bioslurry Nitrogen Content. Being the most vital nutrient for plant growth and optimal production [41], elucidation of nitrogen levels in their various forms in bioslurry is crucial. Since nitrogen is senescence over time from older tissues to newer ones to facilitate the growth of new cells [44], most fertilizers provide nitrogen as a supplement. Total Kjeldahl Nitrogen (TKN), Total Ammoniacal Nitrogen (TAN), and nitrogen as free nitrates were analyzed in all three samples at different retention periods as summarized in Table 6.

The general trend in all three nitrogen forms analyzed was a linear increase in concentration over the retention period. Hydrolysis of the kitchen waste biomass led to degradation of proteins present freeing more nitrogen as either elemental nitrogen, ammoniacal nitrogen, or anionic nitrates. Chen et al. [49] portrayed bioslurry nitrogen to be 2- to 2.7-fold higher than that in mineral fertilizer. The use of the two additives caused more degradation of the proteins and, therefore, these samples had more nitrogen content than their corresponding control sample. The total Kjeldahl nitrogen and free nitrates content on the 28th day of retention time values belonged to the same population (95% confidence level, n = 14). However, the total ammoniacal nitrogen level of the control sample (10.803 ± 0.652 g/L) did not fit into the population of the samples with additives, i.e., T. brownii (14.599 ± 0.425 g/L) and Acanthaceae spp. (15.767 ± 1.243 g/L) (α = 0.05, n = 14). Biogas slurry ammonium levels are known to increase linearly over time [50]. It is worth noting that the Acanthaceae spp. sample had the highest nitrogen content values during most periods of the retention time, and thus the best bioslurry as far as nitrogen is concerned. Figure 3 illustrates the UV-VIS spectra of nitrate levels for the three samples on retention day 28.

6.7. Bioslurry Sulfur Content. Like nitrogen, sulfur is an essential nutrient and thus a major plant requirement [51]. Kitchen waste is prone to a combination of several proteins and elucidation of sulfur content as a bioslurry component is necessary. The bioslurry from the three samples was analyzed for total sulfur (TS) and free sulfate content at various retention periods as summarized in Table 7.
6.8. Bioslurry Phosphorus Content. Together with nitrogen and potassium, phosphorus is one of the three basic fertilizer requirements [41, 51]. Phosphorus is abundant in kitchen waste biomass due to the presence of the various proteins present [52]. The levels of various forms of phosphorus which are total phosphorus (TP), total phosphoric acid (TPA), and phosphorus as free phosphates in the three bioslurry samples were analyzed over the retention period as summarized in Table 8.

The concentrations of all three phosphorus analytes in all samples increased linearly over the retention period. The total phosphorus content in the control sample (1.960 ± 0.088 g/L) was proven to be an outlier at a 95% confidence level when compared to that of *T. brownii* (2.520 ± 0.250 g/L) and *Acanthaceae* spp. (2.420 ± 0.648 g/L) sample (n = 14). The levels of total phosphoric acid in the *T. brownii* sample (3.477 ± 0.023 g/L) were much higher compared to the rest and also significantly different.
Absorbance (a.u)

Absorbance (a.u)

0.2

0.3

0.4

0.5

0.6

0.7

0.8

1.2

1.4

0820 830 840 850 860 870 880 890 900 910

370 380 390 400 410 420 430 440 450 460

Wavelength (nm)

Figure 4: Bioslurry sulfate levels after retention day 28.

| Samples     | Parameter     | Day 1        | Day 7        | Day 14       | Day 21       | Day 28       |
|-------------|---------------|--------------|--------------|--------------|--------------|--------------|
| Control     | TP (g/L)      | 0.780 ± 0.020| 1.335 ± 0.025| 1.560 ± 0.019| 2.305 ± 0.062| 1.960 ± 0.088|
|             | TPA (g/L)     | 2.399 ± 0.112| 2.745 ± 0.002| 2.835 ± 0.563| 2.925 ± 0.156| 2.963 ± 0.333|
|             | Free PO₄³⁻ (ppm)| 465.350 ± 0.000| 466.900 ± 0.000| 468.520 ± 0.000| 475.940 ± 0.000| 477.430 ± 0.000|
| T. brownii  | TP (g/L)      | 0.587 ± 0.023| 1.232 ± 0.055| 2.420 ± 0.648| 2.490 ± 0.171| 2.520 ± 0.250|
|             | TPA (g/L)     | 2.822 ± 0.346| 3.040 ± 0.422| 3.040 ± 0.000| 3.389 ± 0.001| 3.477 ± 0.023|
|             | Free PO₄³⁻ (ppm)| 465.350 ± 0.000| 467.730 ± 0.000| 468.520 ± 0.000| 476.240 ± 0.000| 477.630 ± 0.000|
| Acanthaceae spp. | TP (g/L) | 0.660 ± 0.000| 1.345 ± 0.076| 1.525 ± 0.454| 1.675 ± 0.112| 2.420 ± 0.648|
|             | TPA (g/L)     | 2.567 ± 0.316| 2.655 ± 0.814| 2.694 ± 0.000| 2.771 ± 0.169| 2.771 ± 0.231|
|             | Free PO₄³⁻ (ppm)| 465.400 ± 0.000| 470.990 ± 0.000| 477.430 ± 0.000| 480.940 ± 0.000| 486.140 ± 0.000|

Table 8: Variation of total phosphorus, total phosphoric acid, and free phosphates in bioslurry over the retention period.

The levels of phosphate after 28 days of retention period belonged to the same population. These levels are illustrated by UV-VIS Spectra in Figure 5:

The phosphate levels after retention day 28 were similar to those of bioslurry attained using pure animal manure, i.e., 488 mg/kg [48].

6.9. Bioslurry Soluble Silicic Acid. Soluble silicic acid (orthosilicic acid) is a micronutrient which is very important in the growth and yields of most grasses (including maize, rice, and wheat) [53, 54]. Soluble silicic acid is usually sprayed as a foliar [55]. The levels of soluble silicic acid in the control and Acanthaceae spp. samples fluctuated throughout the retention period. Optimal soluble silicic acid levels were obtained after 21 days of the retention period for the control sample (31.667 ± 0.000 mg/L) and after 14 retention days for the sample with Acanthaceae spp. extract (32.778 ± 1.543 mg/L). Soluble silicic acid levels in the T. brownii sample increased linearly over the retention period. This is attributed to the continuous mineralization of silica in the biogas slurry over time. Gorrepati et al. [56] proved that the availability of silica in a solution is related to its pH value. Under acidic conditions, silica precipitates out by first polymerizing and then flocculating. The availability of silica for plant uptake is, therefore, reduced. Therefore, there was a drop in the soluble silicic acid values between day 21 and day 28 due to the decrease in pH of the biogas slurry. The levels after retention day 28 in the T. brownii sample (38.334 ± 2.492 mg/L) were proven to be significantly different from those of the control sample (25.001 ± 0.000 mg/L) and Acanthaceae spp. (20.556 ± 0.533 mg/L) (α = 0.05, n = 14). Table 9 illustrates the trends in soluble silicic acid content in the three samples over the retention period.
Soluble silicic acid is vital in reinforcing plant cell walls, increasing tolerance to drought and heavy metals, pests, and diseases [57].

7. Conclusions

Whereas T. brownii additives were found to be highly conjugated, Acanthaceae spp. additives had more biometal concentrations. These factors helped in the degradation of the biogas substrate for the formation of more available nutrients. The samples with these two additives were found to have more available nutrients compared to the control sample in all parameters tested. The levels of the plant nutrients analyzed were found to increase over time with retention day 28 having the highest values. The samples with additives had more plant available nutrients compared to the control. T. brownii sample had more total Kjeldahl nitrogen, total ammoniacal nitrogen, total sulfur, total phosphorus, total phosphoric acid, and appreciably higher soluble silicic acid values compared to the rest. On the other hand, the Acanthaceae spp. sample had the highest potassium, calcium, nitrates, sulfates, and free phosphates levels compared to the other samples.

The use of T. brownii and Acanthaceae spp. additives was thus proven to increase the available plant nutrients in bioslurry.

Abbreviations

FT-IR: Fourier transform infrared
UV-VIS: Ultraviolet visible
VOS/TAC: Volatile organic salts/total anorganic carbon
EC: Electrical conductivity
LOI: Loss on ignition
OC: Organic carbon
TAN: Total ammoniacal nitrogen
TKN: Total Kjeldahl nitrogen
TP: Total phosphorus
TPA: Total phosphoric acid
TS: Total sulfur.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Table 9: Variation of soluble silicic acid with time in the bioslurry samples.

| Samples           | Day 1          | Day 7          | Day 14         | Day 21         | Day 28         |
|-------------------|----------------|----------------|----------------|----------------|----------------|
| Control           | 27.778 ± 0.125 | 26.667 ± 0.145 | 30.001 ± 0.000 | 31.667 ± 0.000 | 25.001 ± 0.000 |
| T. brownii        | 23.889 ± 0.463 | 26.667 ± 0.145 | 31.112 ± 2.357 | 41.112 ± 1.253 | 38.334 ± 2.492 |
| Acanthaceae spp.  | 25.475 ± 0.357 | 28.889 ± 3.142 | 32.778 ± 1.543 | 30.001 ± 0.000 | 20.556 ± 0.533 |

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