THE EFFECTS OF INDIGENOUS CATALYTIC ADDITIVES ON QUANTITY AND QUALITY OF BIOGAS AND BIO-SLURRY PRODUCED FROM UNCOOKED KITCHEN WASTES

BY

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SM01/JP/MN/3645/2017

RESEARCH THESIS SUBMITTED TO THE SCHOOL OF SCIENCE AND INFORMATION SCIENCES, DEPARTMENT OF MATHEMATICS AND PHYSICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN CHEMISTRY.

MAASAI MARA UNIVERSITY

DECLARATION

Declaration by student

I declare that the work presented here is my original work and has never been submitted to any institution for the award of a certificate, diploma or degree.

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DEDICATION

I dedicate this work to my loving parents, Mr. and Mrs. Abdallah.

ACKNOWLEDGMENTS

I am grateful to all those who assisted me in fulfilling this thesis. My supervisors Dr. Aloys Osano, Dr. Justin Maghanga, and Dr. Martin Magu all worked tirelessly alongside me to ensure all was put into place. My supervisors walked with me right from concept development, proposal build-up, presentations, sampling, lab analysis, characterization, fabrication, monitoring of biogas reactors, data collection, writeup, and analysis. All these steps required critical and analytical review which they didn't fail to do. Even after all these steps were undertaken, my supervisors did not get tired to read and re-read to correct all possible errors. It is worth mentioning that most of these reviews were done during holidays, weekends and odd hours. All these calls for sheer determination and sacrifice. I don't take this sacrifice for granted. Thank you.

I wish to thank all lab technologists who aided me in analysis and characterization. Mr. Patrick Lumumba was instrumental in assisting me work at odd hours and on weekends to see that I met my objectives. Mr. Koti Musumba was particularly very helpful and worked alongside me not only teaching me how to operate the equipment but also on lab skills. Mr. Koti volunteered to work in odd hours and weekends to see that I met my goals. I am also forever grateful to Ms. Linda Mesoppirr and Mr. Kenneth Rutto who assisted me in my lab analysis

I sincerely appreciate my friends Mwendwa Geoffrey and Suter Evans who were of great significance in data analysis and use of statistical software as well as encouraging me.

Lastly, I am thankful to all friends and relatives who helped me finish my thesis on time.

To God be Glory and Honour!

ABSTRACT

Biogas production in third world countries is still un-optimized and produce very minimal outputs. Optimization of biogas quality by increasing the temperature of the bio-digester is quite technical. On the other hand, commercial enzymes used to increase biogas quantity are also expensive and substrate-specific. *T. brownii* and *Acanthaceae spp.* extracts were successfully used to hasten saccharification and fermentation of cellulose during the preparation of traditional alcohols. This study aimed at exploiting the potentials of these indigenous extracts in catalyzing biogas yields, methane levels, and bio-slurry plant nutrients. The additives and kitchen waste substrate were characterized for possible bio-catalytic and anaerobic digestion properties. A 28-day retention period was used. The *Acanthaceae spp.* extracts had more bio-metal concentration while the *T. brownii* extracts exhibited more organic compounds. The order of biogas output was *T. brownii* (15861.4ml/gVS); *Acanthaceae spp*. (13219.6ml/gVS) and the control (7444.8ml/gVS) at an average temperature of 19.5$\pm$0.5°C over the 28-day retention period. Methane levels were in the order of *T. brownii* (43.375$\pm$0.922%), control (41.750$\pm$1.401%) and *Acanthaceae spp.* (39.275$\pm$0.263%) on retention day-28. The Inferior Calorific Power (ICP) of the biogas systems increased over the retention time. The ICP values on retention-day 28 were 3538.86 Kcal/Kg_{biogas} (*T. brownii*), 3196.98 Kcal/Kg_{biogas} (*Acanthaceae spp.*) and 3398.45 Kcal/Kg_{biogas} (control). *T. brownii* bio-slurry had more lime content, total Kjehdahls nitrogen, total sulfur, total phosphorus, total phosphoric acid and soluble silicic acid. The *Acanthaceae spp.* bio-slurry had more calcium, potassium, nitrates, total ammoniacal nitrogen, sulfates and phosphates. The additives were proven to increase biogas yields, methane levels and available plant nutrients showing their viability to optimize biogas systems.

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LIST OF ACRONYMS AND ABBREVIATIONS

AAS	-	Atomic Absorption Spectrophotometer
AD	-	Anaerobic Digester
ALK	–	Alkalinity
CHP	-	Combined Heat and Power
EC	–	Electrical Conductivity
FT-IR	-	Fourier Transform Infra-Red
HRT	-	Hydraulic Retention Time
ICP	-	Inferior Calorific Power
LOI	–	Loss On Ignition
TIC	–	Total Inorganic Carbon
TAN	–	Total Ammoniacal Nitrogen
TDS	-	Total Dissolved Solids
TKN	–	Total Kjehdahls Nitrogen
TP	–	Total Phosphorus
TPA	–	Total Phosphoric Acid
TS	-	Total Solids
TSS	-	Total Suspended Solids
UV-VIS	-	Ultra Violet -Visible

VFA – Volatile Fatty Acids

VOS – Volatile Organic Salts

VS – Volatile Solids

CHAPTER ONE

INTRODUCTION

1.1: Background of Study

There is a justifiable need for portable, efficient and affordable renewable energy systems in Kenya. In 2018, the government banned charcoal burning hence by extension, the ban on wood logging minimized firewood sources. Petroleum mining is still at its infant stages while coal remains a scarce and eco-unfriendly resource in the country. As a developing nation, nuclear and wind energy at industrially quantifiable units still remain a dream come true for the country's energy needs. This floored the need for other cheaper but reliable energy sources. Regular international conventions have stressed on the need for clean energy. Biofuels are potential sources of renewable energy that are cheap to acquire yet clean (Franco et al., 2015).

Biofuels are energy sources that originate from biological compounds (Abbasi, 2018, Rokem & Greenblatt, 2015). These fuels span from bio-hydrogen, bio-ethanol, bio-gasoline, bio-diesel, bio-kerosene to bio-methane (biogas). Biofuel production from wastes is a green way of generating energy while minimizing environmental wastes. Most biofuels produce clean energies. Biomass waste is abundant and an indefinite source of energy that can be harnessed to generate energy while still providing bio-slurry fertilizer as a byproduct. However, the production efficiency of most of these biofuels is still low. A lot of resources and time are used in the production and purification of these biofuels. Unlike other biofuels, biogas production from most of the biomass sources is naturally spontaneous and quite efficient at diverse conditions (Bušić et al., 2018). Biogas production

also requires less technical knowledge and relatively cheap. Its production is thus quite widespread and applicable in many regions worldwide.

Various biogas substrate has been tested and proven to produce biogas. Poultry and pig dung are far much more effective due to presence of bacteria in situ (Quintanar-Orozco et al., 2018). Human dung is capable of producing biogas. However, the gas is likely to contain more free ammonia which should be water-scrubbed and thus making the process more costly (Fernández-Gutiérrez*et* et al., 2017). Food and kitchen wastes, better referred to as Food Waste Leachate, FWL is also a cheap and viable source of biogas substrate. Appropriate buffering systems have to be implemented for efficiency in production (Idi et al., 2015). Several studies have focused on biogas production from kitchen wastes. This scheme serves two problems simultaneously; solving land pollution while generating clean fuel. Kitchen wastes are quite abundant in both rural and urban areas. However, in rural areas, these wastes can alternatively be used in farms as organic manure or to feed cattle. There is little competition for use of kitchen wastes to produce biogas in urban areas. Both cooked and uncooked kitchen refuse are viable biogas production sources (Paritosh et al., 2017). Since kitchen wastes originate from diverse sources, the resultant biomass contains several non-cellulosic impurities. These impurities include lignin, proteins, fats and oils. Degradation of these biomasses to biogas is a longer pathway compared to that followed when cellulosic biomass is used. It is therefore pertinent to optimize biogas production from kitchen waste sources.

Several types of catalysts have successfully been tested and proven to increase biogas quantity and quality. These catalysts range from chemical ones (such as trace metals and methanoic acids) to biological ones (such as enzymes and some fungi strains). Chemical

catalysts are used to aid in hydrolysis of lignocellulosic biomass to shorter chain polymers (Kucharska et al., 2018). Acid, alkali and lime pretreatment can also be used in the process. Metals of transitional elements origin can equally be used to catalyze the methanogenetic redox reaction, leading to more biogas formation. Saccharification of biomass to yield soluble monomers is an organic reaction and fundamental stage of biogas production. Formic acid, one of the biogas catalysts have successfully been used to hasten this process (saccharification) (Den et al., 2018). Enzymes and fungi catalysts help to speed up glycolysis of fermentable sugars leading to pyruvate and eventually acetic acid, a precursor of methane production from biomass. While these catalysts offer a wide array of significances, they also possess some drawbacks that deter local biogas investors. For example, formic acid is quite expensive and it is not economically feasible to apply it in small-scale biogas units. Trace metal catalysts are quite unavailable. On the other hand, enzymes and fungi are substrate and conditions-specific (Nigam, 2013). Most of the enzymes have a particular pH and temperature range that they work on. Fungi are also susceptible to random changes in the environment. This complicates the use of these catalysts, especially to biogas users with less technical knowledge. It is thus feasible to venture into indigenous bio-catalysts.

Indigenous bio-catalysts are plant extracts that were traditionally used to catalyze domestic processes such as fermentation and cooking. Ancient Aandia community of the western slopes of Mt. Kenya used *Terminalia brownii* (*Mutundu)* leaves extract to hasten local brew (*Muratina*) production. The crude extracts quickened saccharification and fermentation of ground wheat brans (cellulose) for a quicker and more concentrated brew to be processed. On the other hand, '*Miti ni dawa'* (*Acanthaceae spp.)* alcohol has for long enjoyed populace in the Maasai region. The water extracts of several indigenous shrub barks are

used to accelerate the fermentation of sugarcane during the preparation of this alcoholic concoction. These bio-catalysts are presumed to contain catalytic properties such as trace metals, formic acid, fermentation enzymes and a good ecological niche to support the growth of fermentative fungi. This research aimed at exploiting the potentials of these native extracts in catalyzing the hydrolysis of various biogas substrates to attain more biogas yields and methane levels at ambient operating conditions. The levels of essential nutrients in the bio-slurry fertilizer formed were also analyzed.

1.2 Statement of the problem

Accumulation of kitchen wastes in most domestic households leads to land pollution. The problem is more severe in urban areas where there are no direct means to re-use these wastes. Kitchen wastes thus accumulate in municipal landfills in the process of harboring pathogens. These pathogens transmit harmful diseases such as cholera, typhoid, and malaria

Biogas from non-cellulosic biomasses such as kitchen wastes takes a longer duration to produce. The gas produced is also quite little. This is because some of the impurities hinder anaerobic digestion process to produce biogas. Biogas from such systems is little and of poor quality. Commercial additives used to curb this problem are expensive and out of reach to many small-scale biogas users. Some of the additives are also substrate-specific limiting their use in other biogas substrates.

Commercial fertilizers are quite expensive. The use of these fertilizers has also been associated with land and water pollution. Unoptimized bio-slurry production from kitchen waste is quite inefficient owing to the numerous impurities in the biomass that inhibit bio-

mineralization of nutrients. A lot of time is thus taken to decompose kitchen wastes to yield available plant nutrients.

1.3 Objectives

1.3.1 Main Objective

To investigate the effects of indigenous catalytic additives on the quantity and quality of biogas and bio-slurry produced from uncooked kitchen waste.

1.3.2 Specific Objectives

1. To determine the physicochemical parameters and chemical constituents of *T. brownii* and *Acanthaceae spp.* aqueous extracts and biogas substrate prepared from uncooked kitchen waste mixed with the extract as the bio-catalyst.
2. To determine the effects of the two indigenous catalytic additives on the quantity and quality of biogas produced from uncooked kitchen waste.
3. To evaluate the effects of the indigenous catalytic additives on the quantity and quality of bio-slurry produced from uncooked kitchen waste.

1.4 Research Justification

There is a need for conversion of kitchen wastes into alternative sources such as biofuels. The conversion of kitchen wastes into biofuels is one of the viable schemes that have been exploited. This scheme not only reduces environmental pollution resulting from the kitchen wastes but also lead to more clean energy production. Biogas production from kitchen waste is quite easy and cheap.

Several biocatalysts (*T. brownii* and *Acanthaceae spp*.) have successfully been used to optimize the fermentation of traditional alcohol, porridge, and milk by several communities

in Kenya. These extracts are not only easy to extract but are also widely available in several tropical regions. Crude extracts of these plants are used to hasten saccharification and fermentation processes. This minimizes the use of organic solvents for extraction further lowering production costs. These bio-catalysts are compatible with a wide range of biomass substrate thus applicable for the production of both bio-ethanol and bio-methane. Bio-catalytic extracts perform well at ambient temperature and pressure conditions. These catalysts have the potential to increase biogas quantity and quality from kitchen wastes.

The catalytic properties of *T. brownii* and *Acanthaceae spp.* extracts are presumed to hasten bio-mineralization of kitchen waste substrate. This will lead to the faster and more quantitative formation of available plant nutrients from kitchen waste.

1.5 Significance of the Study

The research explored kitchen wastes as suitable biomass with the potential to produce biogas. This implies that environmental pollution as a result of the accumulation of these wastes will be reduced. By extension, diseases that are transmitted by pathogens that thrive in kitchen waste pollutants will also decrease.

There will be more biogas generation from kitchen waste biomass. Increment in biogas production will supplement carbon-based fuels. Carbon-based fuels production has recently fluctuated due to political influence. These fuels are also associated with the emission of toxic pollutants into the atmosphere. Increased energy from biogas will not only avert these challenges but also reduce the cost and accessibility of clean fuel.

The use of kitchen wastes for biogas production will increase their demand. With this 'new-found value' of kitchen wastes, accumulation of the biomass in municipal dumpsites and landfills will reduce. Therefore, there will be a reduction in land pollution. By extension,

water pollution as a result of leach out of nutrients from the landfills into water bodies will also reduce.

Reduction in land reduces the breeding sites of pathogens. Pathogens that thrive in waste dumpsites such as plasmodial mosquitoes and cholera- and typhoid-causing bacteria reduce. By extension, resources allocated to treat these diseases can be put into alternative use for more growth of the economy. Contaminated water is prone to eutrophication. This phenomenon is attributable to increased waterborne diseases, such as bilharzia. The 'blue-baby disease' is common in water rich in nitrates which results from eutrophication.

Optimization of bio-slurry from kitchen wastes using biocatalytic additives will motivate more farmers to indulge into its production. This will attract several benefits such as reduced agricultural costs (since bio-slurry is cheaper than commercial fertilizer), green farming and overall increased food security. Bio-slurry production can also be commercialized to provide more revenue for biogas investors.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biogas Production

In nature, organic material is decomposed by metabolically active microorganisms in a humid atmosphere to a simpler matter. If the breakdown is occurring without the presence of air, so-called anaerobic digestion, methane is formed. The gas is called biogas and contains carbon dioxide, ammonia, hydrogen sulphide, water vapor, free hydrogen, oxygen, nitrogen and siloxane (Salazar et al., 2016). The methane achieved after purification is known as biomethane (≥ 96 volume % of CH_4). The biogas production process is illustrated in figure 2.1 below.

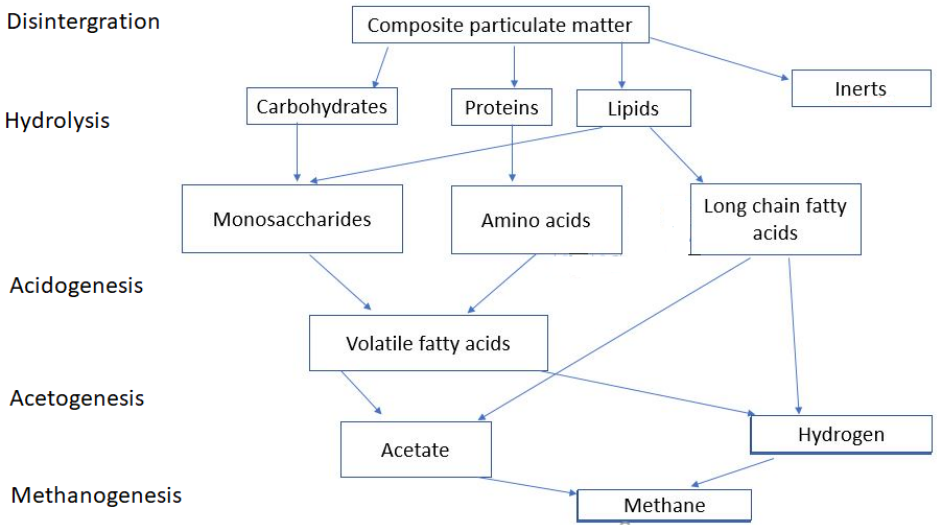

Figure 2.1: Biogas production process (Batstone et al., 2002)

The figure above can be summarized by the equation below;

$$\text{Complex Organics} \xrightarrow[\text{Fermentation}]{\text{Hydrolisis +}} \text{Organic acids + hydrogen} \xrightarrow[\text{Formation}]{\text{Methane}} \text{Methane + Carbon dioxide} \quad (1)$$

Organic matter is degraded gradually in several steps. Bacteria and enzymes are involved in their decomposition. This is attributable to a sequential breakdown of biogas feedstock

to biogas aided by an anaerobic microorganism. Some of these microbes include acidogenic bacteria, acetogenic bacteria and methanogens which harmoniously grow and produce reduced end-products. These organisms are crucial in establishing a good environment for methane formation. (Hillesland, 2018).

2.2 Portable Biogas Units

Over time, researchers have invested in portable biogas systems (figure 2.2). These systems aim at mobilizing heat, especially for cooking during parties, hikers, and campers as well as other functions. Portable biogas systems are small in size for ease of their movement. The flexibility of portable biogas units to adapt to different substrates and temperature regimes is critical for their success.

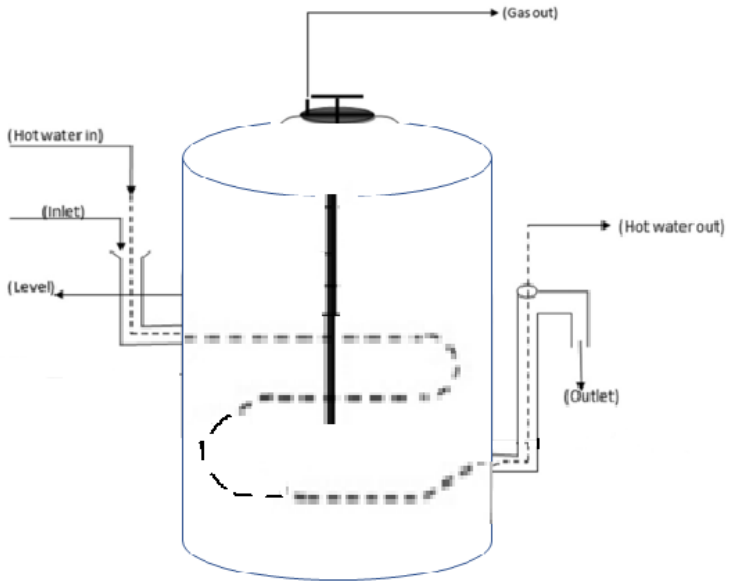

Figure 2.2: How a portable biogas digester looks (Tasnim et al., 2017).

Portable bio-digesters can have a fixed collecting gas or a floating one. Regular cleaning in spans of 8-10 months is required for optimal biogas performance (Gatune, 2018). Such biogas systems are suitable for use with kitchen wastes due to the limited supply of these biomass substrates. This is because they can be tailored for small-scale users such as most urban-dwellers whose only reachable biomass would be kitchen wastes.

2.3 Anaerobic Digester Parameters

The effectiveness of biogas systems is determined by monitoring the yields, methane levels and retention time taken. The retention time of a biogas digester is the time required for the decomposition of organic material to undergo completion. It depends on several parameters such as feedstock composition, temperature, particle size, inoculum, pH, organic loading rate and biogas additives.

2.3.1 Physical Parameters

Feedstocks with high Total Solids (TS) feed have prolonged retention time while high temperature decreases the retention time. Biogas digester Total Solids content should not be less than 10% (Farraji et al., 2016) and not exceed 22- 40% (Weber et al., 2018). COD and VS of biogas feedstock are also related with retention time (Han et al., 2016). Particle size reduction should be encouraged. This is because it enables the suspension of the particles that lowers the settling time and subsequently the flow of particles with the fluid. The temperature of production is very pertinent to high and quality biogas needs. It can be broadly categorized as;

i. Cryophilic (< 20˚C). Very little production take place.
ii. Mesophillic, 20-45˚C. The bacteria have low metabolic rates. Digestion requires longer retention times. This temperature is conducive for producing quality effluents.
iii. Thermophillic, 50-65˚C. Fermentation rate is proportional to temperature increase. It is at this temperatures where bacteria activity is at its optimum leading to efficient digestion of substrate (Yu et al., 2016).

Methanogenic bacteria thrive at an optimum pH of 6.6 -7.0 (Kasina et al., 2017). Methanogenic and acidogenic bacteria are in competition for substrate. A lower pH will

increase acidogenic bacteria. This favor acidogenesis which reduces the pH of the medium and inhibits the methanogenesis process (Vasconcelos, 2016). $CaCO_3$ is a suitable buffer for biogas systems and can also be used to test the alkalinity of AD medium.

2.3.2 Inoculum

Micro-organisms produce biogas by digesting the organic compounds. Sufficient quantities of microorganisms are needed for successful biogas production (Manyi-Loh et al., 2013). The number of micro-organisms in fresh material is below the required quantity of microbes. Sufficient inoculum must therefore be added at the starting of the process. The sources of micro-organisms affect the biogas production. Different types of microorganisms have different capabilities in digesting particular type of material. By applying suitable cultures efficient biogas production can be achieved. Bacterial cultures can be obtained from cattle manure, sewage waste, and other biogas producing facilities or artificial cultures (Goswami et al., 2016).

Inoculum from cow and goat dung is by far the most available source of inoculum in most regions of the country. This inoculum has previously been used to successfully harbor microorganisms used in anaerobic digestion. Cow and goat dung also contain significantly high amounts of methanogens necessary for methane generation.

2.3.3 Activators and Inhibitors

Activators enhance the biogas production while inhibitors reduce biogas production. Activators or inhibitors are added in small amounts in order to enhance or reduce biogas production in digesters, respectively. These substances can be enzymes from organic or inorganic compounds. Leaves of plants, legumes and microbial cultures are examples of additives which are used for enhancing biogas production. *Leucacena leucocephala*,

Acacia auriculiformis, *Dalbergia Sisoo* were reported as enhancing agents of biogas production (Kumar et al., 2013). *Leucacena leucocephala* and *Acacia auriculiformis* plants are common in Kenya and other tropical regions (Maghembe & Chirwa, 1996). However, *Dalbergia Sisoo* plant is only common in Asia. Microbial cultures like actinomycetes and mixed consortia were reported as enhancing biogas production with cattle dung. These cultures stimulate enzymatic activities which produces biogas. Inorganic compounds that enhance biogas production are iron salts like $FeCl_3$ and $FeSO_4$, and heavy metals (Kumar et al., 2013). Adapted microorganisms can endure the effects of inhibitors and can avoid the effects on biogas production. Some of the inhibitors which affect the biogas production are light, disinfectants, hydrogen sulphide and ammonia (Liao and Chen, 2018). These compounds affect the biogas production negatively when presents in higher concentrations. *T. brownii* is a semi-deciduous tropical plant common in several parts of Kenya (Worldagroforestry, 2019). The leaves of this plant are oval-shaped and range between 7 to 10cm with a wide pointed tip. Their edges are quite wavy (Worldagroforestry, 2019). The crude extracts of this plant were frequently used by some communities in Central Kenya to hasten saccharification of biomass. This reduced the hydrolysis time taken to convert the cellulosic biomass into fermentation products such as ethanol and sour porridge. On the other hand, *Acanthaceae spp.* are also tropical herbs found in semi-arid regions. These species are common in The Lower Rift valley part of Kenya. The plants are angiospermic in nature and popular for their beautiful and diverse flowers (Angiosperm Phylogeny Group, 2009). The Maa community of Kenya successfully used the crude extracts of their barks in preparation of traditional liquor. The extracts were reported to have some natural catalytic effect on the hydrolysis of cellulosic biomass.

2.4 Biogas Feedstocks

2.4.1 Types of Biogas Feedstocks Used

Several substrates can be used in anaerobic digestion for fermentation and thus biogas production. Most rural and urban organic wastes are biogas substrates. The suitability of a feedstock in biogas production is dependent on several factors including its carbon: nitrogen ratio, degradability and pH. Animal dung is seen to be the best substrate owing to its nutrient balance and conducive environment for methanogenic archaea (Low et al., 2016). It is therefore added onto another substrate as inoculum to provide these crucial anaerobes. Some of the commonly used biogas substrates include;

a) Agricultural residues which offer an excellent biogas substrate due to their nutrient matrix. Most of these feedstocks have adequate carbon and water necessary for biogas digestion. The feedstocks are also porous enough for easy degradability. Some of the commonly used agricultural residues include wheat and rice straw, maize stalk, potato vines etc. (Martínez-Gutiérrez, 2018). Agricultural residues are suitable candidates for organic manure. The residues are also feedstock for cattle and other farm animals. They thus have conflicting interests on whether to be used as biogas substrate, organic manure or animal feed. This overrides the use of agricultural residues as a novel biogas substrate.

b) Industrial effluents and sewerage deposits. These are slurry collections from factories and industries. They contain organic compounds up to certain limits depending on the type of effluent. Extreme pH values and the presence of toxic inhibitors may, however, deter anaerobes from thriving (Birgel et al., 2015).

c) Municipal landfill. Include domestic food leftovers especially those industrially processed. Municipal landfills have enough substrate to provide biogas

spontaneously without external influence but the absence of anaerobic media leads to landfill gas production. Landfill gas can, however, be upgraded to biomethane using relevant technologies (Hoo et al., 2018). However, municipal landfills have a lot of inorganic impurities that require sorting before considering them as suitable biogas substrate. This increases the production cost of biogas generation.

d) Kitchen refuse. Cooked food and uncooked vegetable peels have adequate carbon and water to produce biogas. The use of fats and oils during cooking as well as very low pH values may, however, inhibit anaerobes.

Table 2.1: Common biogas feedstocks and their corresponding biogas yields.

Biogas Feedstock	**Biogas Yield (M^3/T)**
Cattle slurry	15-20 (10% Dry Matter)
Pig slurry	15-25 (8% Dry Matter)
Poultry	30-100 (20% Dry Matter)
Grass silage	160-200 (28% Dry Matter)
Whole wheat crop	185 (33% Dry Matter)
Maize silage	200-220 (33% Dry Matter)
Maize grain	560 (80% Dry Matter)
Crude glycerine	580-1000 (80% Dry Matter)
Wheat grain	610 (85% Dry Matter)
Grass	298-467

Source: The official information portal on anaerobic digestion, 2019

This biomass is generated on a daily basis as people cook; either in their homes or in hotels, restaurants or institutions. Since there is no direct alternative use of kitchen wastes in most urban centers, it is quite feasible to use it in biogas generation. Additionally, a large fraction of kitchen waste biomass is composed of organic matter (Paritosh et al., 2017). Therefore,

less sorting is required before feeding the biomass into bio-digesters. Table 2.1 above illustrates some of the common biogas substrates used. Kitchen waste is a novel biogas substrate due to its availability, especially in urban centers.

2.4.2 Biogas Feedstock Pretreatment Methods

For optimum biogas production, the substrate should be treated. Not only are the yields increased but also the retention period is drastically reduced. Pretreatment primarily involves fermenting feedstock in a separate digester from the main one, to enable hydrolysis process to be initiated (Amin et al., 2017). Anaerobic media, warm temperature, and dark environment are ideal conditions for pretreatment. Some salts can also be added to assist in saccharification of tough biomass. There are five common pretreatment methods; Dilute acid, Concentrated acid, Lime, Sodium hydroxide and Water hydrolysis. Acid pretreatment affects pH of the substrate thus not common for biogas feedstock preparation.

a) Lime pretreatment: dilute calcium oxide and hydroxide are perfect acid buffers for biomass. These solutions are also strong enough to react with lignocellulosic biomass for easier enzyme digestion. Lime is cheap enough for mass biomass pretreatment. Lime can be removed by washing with excess distilled water.
b) Sodium hydroxide pretreatment: caustic soda is slightly more alkaline than lime therefore a better hydrolysis solution. Tough lignocellulosic biomass can be effectively degraded using this solution to enhance better enzyme digestion. Sodium hydroxide is also cheap and widely available. Excess sodium hydroxide can be removed by washing with weak acids, like citric acid.
c) Water pretreatment: It's a green method to treat biomass. The azeotropic nature of water offers both acidic and alkaline media necessary to degrade biomass. The

method is however slow and can be quickened by increasing temperature of the water.

2.5 Optimization of Biogas Production

Natural biogas formation takes place at low rates. Attaining the solid and hydraulic retention periods using cow dung slurry and substrate takes about 1 to 2 weeks, for a digester operating at ambient temperatures. Even then, methane levels are still low. When hard substrate such as kitchen waste is used, the retention periods are further prolonged.

Quite a number of steps have been taken to accelerate this process including; proper biogas feedstock pre-treatment, optimization of reactor design and conditions, use of mixed substrate consortia and additives. Proper biogas feedstock pre-treatment is crucial in breaking down strong glycosidic and amine bonds found in the substrate (Achinas et al., 2017). Several pretreatment measures such as concentrated acid hydrolysis, dilute acid hydrolysis, lime treating, water treating, caustic treating, and others have all been successfully exploited.

The anaerobic digestion process is spontaneous but slow. The initial stage (hydrolysis) is slow due to the presence of strong glycosidic and amine bonds that require to be to be degraded. The last stage of biogas formation, methanogenesis is also slow and largely dependent on bacteria archea present. It is, therefore, crucial to beef up these two important stages for optimal and quality biogas production.

Several researches have been conducted exploring on possible biogas catalysts ranging from inorganic salts and acids to biological leaves. Publication of knowledge generated is a risky affair, owing to the commercial benefits of the knowledge. Present literature on biogas catalysts, therefore, remains coded in order not to reveal the exact compounds or ratios of elements present. Nevertheless, logic has it that biogas catalysts should be those

that can hasten saccharification and fermentation of cellulose since both are anaerobic digestion processes. The contrast would be the condition of the bacteria culture required for methanogenesis. Whereas fermentation is an acidic process, biogas formation works best between pH 7-8.0 (Surra et al., 2018).

Several additives have over a long time been used to hasten cellulose fermentation. To apply the same to biogas production, appropriate buffering is required. Hard alkalis should not be used since methanogenic bacteria are pH-sensitive (Jimenez et al., 2015). Above pH 9.0, very few of these strains can survive. Biogenetic engineering to synthesize, culture and multiply these strains is ongoing though the whole exercise is very expensive. Catalytic biogas additives can basically be divided into two; inorganic salts and acids and biological compounds.

2.5.1 Inorganic Salts and Acids

Most of these catalysts are used in hydrolysis to break down the strong glycosidic and amine bonds of substrate. Strong acids are perfect compounds for hydrolysis. However, they form strongly acidic products with little applications necessitating buffering. Formic (methanoic) acid is a weak acid that has been found to catalyze cellulose degradation (Deb et al., 2019). Its applicability and performance in biogas systems have also been evaluated and found to work quite positively. Several transition metal salts can catalyze biogas production. The chlorides and oxides of nickel, cobalt and tungsten increase biogas production (Paulo et al., 2015). Ferric oxides and chlorides have been successively used to accelerate and increase biogas yields to several folds (Liu et al., 2018).

2.5.2 Biological Compounds

They range from the use of insects (termites), fungi, bacteria to bark and leaves extracts. Termites can eat down strong wood and reduce it to weak soil matter within a short period of time. Termites contain formic acid which is an excellent hydrolysis agent. Unfortunately, these animals cannot survive at anaerobic conditions and can thus only be used during substrate pretreatment to quicken hydrolysis. Some anaerobic fungi such as antinomyecetes have been tested positively in biogas optimization (Dollhofer et al., 2015). Culturing enzymes and bacteria required for hydrolysis is an expensive process out of reach for small scale biogas operators.

Extracts from various trees contain a matrix of natural products whose reactions can be utilized to degrade biomass. Most extracts occur in a pH conducive for methanogenic bacteria and other enzymes. Their correlation is therefore synergetic. When the extracts are mixed with substrate, the parts of the extracts that do not catalyze biogas production can as well be used as substrate.

2.6 Viability of Biogas Slurry as a Fertilizer

The slurry leaving a biogas digester is termed as bio-slurry or digestate (Boldrin et al., 2016). The biological reactions that occur during biogas formation leave behind a slurry that can be used as a fertilizer or soil conditioner. The retention period of digestate in a biogas digester is dependent on very many factors (Ziganshin et al., 2016). The physical appearance of digestate is brown and watery. The density of bio-slurry is slightly higher compared to that of the substrate (Hastik et al., 2016). Its pH is slightly higher compared to that of the substrate. There is no significant difference between the temperature of the digestate to that of the substrate. Depending on the type of substrate used, digestate is slightly more odor compared to the substrate used.

Digestate is an excellent organic fertilizer at whichever retention period it has been tapped. Digestion of substrate, which is by itself also a fertilizer frees elements in the slurry (Fierro et al., 2016). Their solubility is thus increased making it easy for uptake by plants. Being watery, digestate solves the problem of water as well as that of soil nutrient. Digestate contains all essential nutrients of Nitrogen, Phosphorus and Potassium (NPK) just as in commercial fertilizers (Peng et al., 2018). Micronutrients such as calcium and magnesium are also present in the bio-slurry. Farmers can use it in liquid form or compost it using the sun.

Some of the crucial parameters in bio-slurry that support growth of crops include; pH, LOI, electrical conductivity, nitrogen, phosphorus, sulfur and soluble silicic acid. These parameters are discussed below;

2.6.1 Bio-slurry pH value

Different crops thrive at different pH values. The optimal pH value for most of the crops is 6.0-7.0. It is therefore crucial to elucidate the exact pH of bio-slurry and any variations taking place with increasing retention time. Soil properties necessitating addition of lime to lower the pH vary with regions (Zhang et al., 2019). Some compounds present in bio-slurry increase the pH while others will reduce it. The same applies for soil pH. Abundance of sodium, calcium, potassium and magnesium ions in the soil (or bio-slurry) lead to increased pH. The colloids present in these compounds become replaced with hydrogen ions. Abundance of aluminium ions in the soil (or bio-slurry) increase acidity (Meriño-Gergichevich et al., 2010)

2.6.2 Bio-slurry electrical conductivity value

Electrical conductivity of bio-slurry fertilizer is directly related to the polarity and concentrations of ions present. High conductivity values imply presence of more soluble ions (such as potassium or sodium) as well as a more aqueous or polar solution. Bio-slurry electrical conductivity value is related to the bio-slurry pH. With increasing pH, most ions can easily solubilize and therefore the electrical conductivity increases (Siyavula, 2019).

2.6.3 Bio-slurry Loss on Ignition (LOI) content

The LOI values of a fertilizer depict the stability of that fertilizer at increasing temperature conditions. High LOI values imply the fertilizer sample is quite stable and can therefore be applied at any time of the day or season. Low LOI values imply the fertilizer sample is quite volatile and should be applied in the morning or evening. Volatile fertilizers (such as most ammonia fertilizers) are not applied by top-dressing, broadcasting or spraying methods to minimize their chances of volatilizing away into the atmosphere.

2.6.4 Bio-slurry nitrogen levels

Nitrogen is the most vital element in fertilizer. Nitrogen controls several critical functions in crops including plant growth, immunity and reproduction (Mur et al., 2017). Nitrogen can be found in different formulations naturally with the most common ones being urea and fodder (Rose et al., 2016). Leguminous plants such as beans increase the soil nitrogen content by converting atmospheric nitrogen to soil nitrogen. The strength of a fertilizer sample is measured from its net nitrogen content. Several commercial fertilizers with nitrogen such as calcium ammonium nitrate, diammonium phosphate, nitrogen-phosphorus-potassium are commonly available. Bio-slurry is a good source of nitrogen due to presence of protein in biogas substrate.

2.6.5 Bio-slurry phosphorus levels

Alongside nitrogen and potassium, phosphorus is also a key fertilizer (and bio-slurry) element. Like nitrogen, phosphorus in bio-slurry is as a result of mineralization of biomass substrate containing proteins. Animal dung is a good source of phosphorus and therefore bio-slurry is quite rich in phosphorus. Phosphorus is critical in several enzymes which control important functions of plants (Plaxton & Tran, 2011). Phosphorus is also involved in plant respiration which provide energy for plants.

2.6.6 Bio-slurry sulfur content

Sulfur plays a great role in plant metabolism. Though considered a secondary nutrient, sulfur also plays a great role in plant growth. Sulfur in bio-slurry also originate from protein matter found in the biogas substrate (Manyi-Loh et al., 2013). Peu et al, observed high sulfur content in bio-slurry containing seaweeds and pig dung as the primary substrates. High sulfur concentrations in bio-slurry imply more hydrogen sulfide levels in the biogas produced from the same material (Peu et al., 2011). Bio-slurry sulfur levels increase over the retention period as more sulfur ions are being ionized from the solid biomass.

2.6.7 Bio-slurry soluble silicic acid

Silica aid plants cell wall to stiffen and become stronger. The stems can therefore accommodate more uptake of water and nutrients resulting to faster growth and maturity of plants. Silicon also helps to alleviate the toxicity of other salts and metals in grasses (Luyckz et al., 2017). Silicon is usually added to plants in liquid or solid state during the planting season (Laane, 2018). Silica in biogas slurry originate from silicon trapped in volatile matter.

CHAPTER THREE

METHODOLOGY

3.1 Materials

All reagents used were lab grade expect for analytical grade reagents which are specified. All reagents were purchased by Sigma-Aldrich Co. (South Africa) and sourced from Maasai mara university laboratories, Kenya.

Sulfuric acid (98% pure), sodium hydroxide flakes, potassium sulfate, anhydrous copper sulfate, anhydrous titanium dioxide, alundum boiling chips, methyl red indicator, methanol (95%), ethanol tributyl citrate, paraffin, antifoam A, lysine monohydrochloride, hydrochloric acid (36%), potassium dichromate, ferrous ammonium sulfate, magnesium sulphate heptahydrate, ammonia solution (25%), ethanol (95%), universal indicator solution, aluminium chloride, barium chloride, potassium hydroxide pellets, formaldehyde (52.58%), quinoline, sodium molybdate, perchloric acid, hydrogen peroxide, phenolphthalein indicator and quimosiac solution.

The listed reagents were all analytical grade (all from Sigma-Aldrich, South Africa); Potassium bromide, AAS standards for zinc, cobalt, chromium, copper and iron, potassium nitrate, salicyclic acid, ascorbic acid, nitric acid, sodium sulphate granules, sodium chloride, glycerol, potassium hydrogen phosphate, ammonium molybdate, ammonium metavanadate, hydrazine sulphate, ammonium acetate, acetic acid.

3.2 Experimental Design

Three anaerobic digester setups of capacity 40.0 liters operated at mesophilic temperature regime were used to monitor biogas production patterns, biogas composition and levels of available nutrients in bio-slurry. The digesters were all maintained in batch mode. A 28-

day retention period was used. Biogas substrate comprising of uncooked kitchen waste and inoculum was used. The composition of the kitchen waste was; 20% cabbage peels, 20% kale peels, 15% potato peels, 15% carrot peels and 30% cooked starch leftovers. The substrate was pretreated using distilled water (10% water in 90% solid kitchen waste substrate) for 24 hours prior to loading into the bio-digesters. Two of the digesters were dosed with at 5% $^{v}/_{v}$ additive (*T. brownii* and *Acanthaceae spp*. water extracts) while the third bio-digester was the control setup. This setup had substrate similar to the other bio-digesters but did not have any additive.

3.2.1 Sampling and Study Area

Substrate samples were collected from Total area in Narok Town (35.8771˚E, 1.0875˚S) and Maasai Mara University (35.8783˚E, 1.0877˚S) within Narok County, Kenya. The region is as outlined in the map shown in Fig 3.1 below;

Figure 3.1: Map showing Narok Town and Maasai Mara University, Kenya. (Downloaded from mapsoftheworld, 2019 and modified)

The additive *T. brownii* were sourced from Kutus, Kirinyaga county, Kenya (0.4993˚S, 37.2785˚E) while *Acanthaceae spp.* concoction shrubs were obtained from Narok Town. Substrate characterization, biogas digester monitoring and characterization was done in

Maasai Mara University, Kenya, laboratories. VOS/TIC, biogas composition and UV-VIS analysis was done in Taita Taveta University, Kenya laboratories. UV-VIS analysis of the bio-slurry for the 28th retention day were done in Vaal University, South Africa.

3.2.2 Sampling Method

Sampling was conducted for the inoculum, substrate and additives. For the inoculum, cow dung and goat droppings were used. Fresh kitchen waste substrate was preferred over aged substrate to avoid any chances of side reactions having taken place in old substrate. Fresh additives were also used due to the same reasons mentioned above.

3.3 Analysis of bio-catalytic additives, kitchen waste substrate, biogas and bio-slurry production

3.3.1 Extraction and determination of chemical constituents in the bio-catalysts

3.3.1.1 Extraction

a) *T. brownii*

Freshly plucked leaves were washed in distilled water. The leaves were then shredded into small pieces. 5g of these leaves were dissolved in 100 ml distilled water and left at room temperature overnight to obtain the extracts.

b) *Acanthaceae spp.*

The barks of *Acanthaceae spp.* were washed in distilled water then ground to fine powder. 10 g of this powder were dissolved in 100 ml distilled water and left at room temperature overnight to obtain the extracts.

3.3.1.2 Determination of chemical constituents in the bio-catalysts

Chemical constituents were elucidated to determine if their properties could potentially inhibit anaerobic digestion process.

3.3.1.2.1 pH

The extracts obtained above were characterized for pH using a pH meter. The pH meter was calibrated using appropriate buffers before testing distilled water and samples made using the distilled water.

3.3.1.2.2 Electrical Conductivity

The extracts were put into a 250ml plastic beaker and swirled for uniformity. The probe of a conductivity meter was the inserted and extracts conductivity measured.

3.3.1.2.3 Dissolved Oxygen

The extracts were put into a 250ml plastic beaker and swirled for uniformity. The probe of the oxygen meter was the inserted and extracts dissolved oxygen read against the temperature of the solution.

3.3.1.2.4 Total Suspended Solids (TSS)

The mass of 100.0 ml 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).

3.3.1.2.5 Total Dissolved Solids (TDS)

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

3.3.1.2.6 IR Functional Groups

The extracts were concentrated to ensure all the water dried. The samples were then cast into pellets using KBr pellet before analyzing for functional groups using IR Spectrometer (Shimadzu-119 FT-IR).

3.3.1.2.7 Absorption peaks Analysis

Extracts were diluted serially using distilled water until a clear spectrum could be seen on the UV VIS spectrometer (Jasco-6850 UV-VIS). A scan was then run between 190-900 nm wavelength. The positions and intensities of pronounced peaks were monitored.

3.3.1.2.8 Bio-metal analysis

Extracts obtained were triple filtered using Whatman # 42 filter papers before analyzing for Zn, Cu, Co, Fe and Cr using Flame Atomic Absorption Spectrometer (PG-990 AAS). The working standards were prepared from the respective AAS-standard salts. A 1000 parts per million (ppm) stock solution was prepared before serially diluting it to the range of the machine detection limit (MDL) of each of the salt. The conditions for the AAS are as summarized in table 3.1 below;

Table 3.1: AAS conditions used to analyze the bio-metals

Bio-metal	Wavelength	Bandwidth	Lamp current	Flame	Sensitivity
Cr	357.9nm	0.4nm	5.0ma	Air/Acetylene	0.05mg/L
Co	240.7nm	0.4nm	5.0ma	Air/Acetylene	0.05mg/L
Cu	324.7nm	0.4nm	5.0ma	Air/Acetylene	0.03mg/L
Fe	248.3nm	0.2nm	5.0ma	Air/Acetylene	0.05mg/L
Zn	213.9nm	0.4nm	4.0ma	Air/Acetylene	0.01mg/L

The specific type of ion present in the samples was analyzed by speciation analysis; which is a lab simulation study using the metal concentration and other parameters such as pH,

temperature and conductivity. The software (PH-Redox-Equilibrium-Concentration (PHREEQC) was used for this speciation.

3.3.2 Determination of chemical constituents in kitchen waste substrate

3.3.2.1 Total Solids

10.000 g of 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.

$$\%T = \frac{M2}{M1} \times 100\% \qquad \textbf{(1)}$$

3.3.2.2 Volatile Solids

10.000 g of sample was weighed, M_1 using an Analytical balance. The sample was then placed in an oven conditioned at 540˚C for 1 hour before removing, cooling and reweighing. The new mass was recorded as M_2.

$$\%VS = \frac{M2}{M1} \times 100\% \qquad \textbf{(2)}$$

3.3.2.3 Alkalinity

A raw sample was hydro-distilled (1:1) and the distillate titrated against standard 0.05N H_2SO_4 solution up to pH 4.0 (pH meter, Hanna G-114). The volume of the sample solution used was used to determine the concentration of Alkalinity in the sample.

3.3.2.4 Volatile Fatty Acids

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

3.3.2.5 Total Kjehdahl's Nitrogen (TKN)

The method is according to Kimie et al., (2013) and Masayoshi, (1988).

3.3.2.5.1 Digestion

For sample digestion, 1.00g of ground sample was put into a digestion flask (W). A reagent blank and concentrated HCl added. A second subsample for lab dry matter determination was also done. 15g K_2SO_4, 0.04g anhydrous $CuSO_4$, 1.0g alundum granules were added followed by 20mL H_2S0_4. (1.0mL H_2SO_4 was then added for each 0.1g fat or 0.2g organic compound with weight is more than 1g). A flask was placed on pre-heated burning (adjusted to take 250mL standard water at 25°C to boil in 5 min). It was warmed until white fumes clear of the flask, swirling gently. The sample was then cooled off and 250mL water gradually involved to cool it.

3.3.2.5.2 Distillation

Using a clean and dry beaker 85ml of 20% HCl was put into a titration flask. 2ml of tributyl citrate as antifoam was added to lessen foaming followed by 1.0g of alundum chips. Slowly downside of the flask, 80ml 0f 45% NaOH was added. (The solution was not mixed until after flask was linked to distillation equipment or ammonia will be lost). Instantly the flask was connected to distillation equipment and distilled until at least 150mL distillate collected in titrating flask.

3.3.2.5.3 Titration

The excess acid was titrated against 1N NaOH to orange endpoint (color consist of red to lemon to yellow). A reagent blank (B) was also titrated.

3.3.2.6 Organic Carbon determination by Walkley-Black Method

The method is according to Kimie et al., (2013) and Masayoshi, (1988).

A weighed amount (50 mg) of the dried, ground sample was treated with 5 ml of 0.4 N potassium dichromate solution ($K_2Cr_2O_7$) followed by the addition of 10 ml of concentrated sulfuric acid. The mixture was gently swirled and left at room temperature in a fume hood for 16-18 hours and then 100 ml of triple-distilled water added to the mixture. The excess of dichromate was back-titrated with the standard 0.2 N ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$) solution. Blank titration of the acidic dichromate with ferrous ammonium sulfate solution was performed at the beginning of the batch analysis using the same procedure with no sample added. One ml of 0.2 N ferrous ammonium sulfate is equivalent to the 0.009807g of $K_2Cr_2O_7$ or 0.0006g of carbon.

Organic carbon (%) = (B - S) x 0.0006)/ m) x 100 (**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.

3.3.2.7 Total Phosphorus Analysis

The method is according to Kimie et al., (2013) and Masayoshi, (1988).

A filter paper was weighed and stored in a desiccator. 3.000g of the sample will be dissolved in 40.0 ml of distilled water and a different filter paper used to filter the mixture. 45 ml of 10% $MgSO_4.7H_2O$ was added to the filtrate followed by 150 ml of 2M NH_3 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 two 5 ml portions of distilled water and two 10 ml portions of 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.

3.3.3 Determination of biogas yields, composition and Inferior Calorific Power (ICP)

Biogas from the bio-digesters was collected in 3000ml tubings with gate valves being used to regulate gas outflow. The gas collected was analyzed for composition using a biogas test-kit (Multitek-545). The ICP values of the biogas samples were calculated using Dulong's formula for superior calorific power (PCS) before deducting the heat from condensation of water (as shown in the equations below);

$$PCS = 8.140 \times C + 34{,}400 \times (H - O/8) + 2220 \times S \qquad (4)$$

Where C is the percentage by weight of carbon in the fuel, H is the percentage by weight of hydrogen in the fuel, O is calculated as part of hydrogen where hydrogen is 1/8 of the oxygen percent by weight and S is the percentage by weight of hydrogen in the fuel but maybe nullified by oxy-nitrogen compounds present.

The ICP value was calculated from the PCS value above as;

$$CVn = PCS - 25(f + w) \qquad (5)$$

Where CVn is the ICP value, f is the water content in fuel by percentage weight and w is the water content generated by the combustion of any hydrogen gas present in the fuel.

3.3.4 Bio-Slurry Analysis for Essential Plant Nutrients

3.3.4.1 Nitrates

Nitrate solution (1000 μgmL^{-1}) was prepared by dissolving 0.7220 g potassium nitrate in water and diluting to 100 ml. Working standard solutions were prepared by appropriate dilution. 10 mL of nitrate stock solution was pipetted to a beaker, 5 mL of Conc. HCl and 2 mL of Zn/NaCl granular mixture was added and allowed to stand for 30 minutes. With

occasional stirring to form nitrite, the solution was filtered to 100 mL standard flask using Whatman #41 filter paper and diluted up to the mark.

Nitrate standards were prepared by dissolving 8.0 g of salicyclic acid in 100ml of 1M H_2SO_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.

3.3.4.2 Sulphates

To prepare the standards, 1.479 g anhydrous sodium sulphate was dissolved in distilled water and thoroughly mixed then topped up to the 100 ml mark in a standard volumetric flask. 1.0 ml of this mixture was assumed to have 1.0 mg of sulphate ions.

For standard preparation, 10 g of NaCl and 10 ml of concentrated HCl acid were added to 40ml of glycerol solution. A yellowish color was formed. 5ml of this solution was added to 45ml of analyte solution and the absorbance read at 410-430 nm.

3.3.3.3 Phosphates

A standard stock solution was made by dissolving 0.11g KH_2PO_4 in 250ml distilled water. Serial dilution was then done to prepare different standard concentrations.

A conditioning reagent was added. It was made by dissolving 1.7081g of ammonium molybdate/ Ammonium metavanadate and ascorbic acid (5.82g/300 ml distilled water) in 150 ml warm water. The solution was cooled before diluting to 250ml. 0.125g of hydrazine sulphate in 100 ml distilled water was added.

Analyte samples were diluted by a factor of 10 and added the conditioning reagent before measuring the absorbances at 830-860 nm by UV-VIS spectrophotometer against those of the blank and standards.

3.3.4.4 Potassium

Using a clean and dry calibrated measuring cylinder, 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 using UV-VIS (Kabir et al., 2017).

3.3.4.5 pH, Dissolved Oxygen and Electrical Conductivity

The above parameters were analyzed using a pH meter, Oxygen meter, and Conductivity meter respectively.

3.3.4.6 Loss On Ignition (LOI)

Exactly, 1.0 g of sample was put into a pre-weighed crucible and subjected to high temperature (>540°C) in an oven for 1 hour, cooled in a desiccator then reweighed again.

3.3.4.7 Bio-Fertilizer Alkalinity (Lime Content)

A weighed 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 added dropwise to the mixture (with pH probe inserted) until pH 4.3 is attained. The number of drops used was then be quantified.

3.3.4.8 Total Kjehdahls Nitrogen, Organic Carbon and Total Phosphorus

For nitrogen and phosphorus determination, the methods previously used during substrate characterization were used.

3.3.4.9 Total Ammoniacal Nitrogen (TAN) by Formaldehyde Method

The method is according to Kimie et al., (2013) and Masayoshi, (1988).

Using an electric balance, 3.0 g of crude sample was weighed into a clean beaker and 100.0 ml of 3M HCl acid added. The mixture was swirled to completely mix and effervescence. 4.0 ml of 24% aluminum chloride solution followed by 3 drops of methyl red indicator were added turning the mixture color to pink. After filtration, 100 ml of saturated potassium hydroxide solution (17%) was added until the color changes to yellow. 100.0 ml of 1M HCl acid was then added followed by 10.0ml of 50% formaldehyde solution. The mixture was then standardized using 0.1N NaOH solution. For the blank determination, 100 ml of 1M HCl acid and 10.0 ml of formaldehyde acid was used.

$$\% NH_3 = (V_S - V_B) \times C \times f \times V_1/V_2 \times 14.007/W \times 100/1000 \quad \textbf{(6)}$$

Where; V_S- sample volume, V_B- blank volume, C- concentration of titrant in mol/liter, f- ammonia factor, V_1- volume of sample solution, V_2- volume of sample solution transferred after filtration, W is the weight of sample in grams.

3.3.4.10 Total Phosphoric Acid (TPA) by Quinoline Gravimetric Analysis

The method is according to Kimie et al., (2013) and Masayoshi, (1988).

With an electric weighing balance, 1.0 g of sample was weighed into a round-bottomed flask. Catalysts 10.0 g potassium sulphate and 1.0 g of hydrated copper sulfate was added followed by 20.0 ml of concentrated sulphuric acid together with alundum boiling chips. The mixture was allowed to digest until white fumes clear the flask. Thereafter, 100 ml of distilled water was added until the color of the mixture starts to change. The total volume was recorded as V_1. 10.0 ml of this solution (V_2) was mixed with 10.0 ml of conc sulphuric acid/nitric acid mixture (1:1) before adding into 50.0 ml of Quimosiac solution (prepared

using quinoline and sodium molybdate). The mixture was then filtered onto a pre-weighed filter paper. The filter paper was then dried at 220˚C for 30 minutes and reweighed again.

$(T\text{-}P_2O_5)\ \% = A \times 32.07 \times 100/1000 \times V_1/V_2 \times 1/W$ **(7)**

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

3.3.4.11 Soluble Silicic Acid by Perchloric Acid Method

The method is according to Kimie et al., (2013) and Masayoshi, (1988).

Exactly 1.0g of the sample was added onto 50.0ml of concentrated HCl acid to allow for effervescence before filtering. The solution obtained is V_1. 30.0ml of the above solution (V_2) was added concentrated 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 50ml 1M HCl acid and reheating (while still covered) at 70-80˚C. The mixture was then filtered using a pre-weighed 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 high temperature (>1200˚C) using a pre-weighed crucible and the mass of residue taken after cooling.

$(S\text{-}SiO_2)\ \% = A \times V_1/V_2 \times 1/W \times 100$ **(8)**

Where A is the mass of precipitate.

3.3.4.12 Total Sulfur Content by Barium Chloride Gravimetric Method

The method is according to Abe et al., (2014) and Yasushi, (2011).

1.0 g of sample (W) was added onto 50.0ml 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.

T. Sulfur % = (A x 0.343) x W x V_2/V_1 x 100 **(9)**

3.4 Data Analysis

Data from the physical-chemicals, bio-metal concentrations, biogas yields and bio-slurry tests conducted was reported as mean ± standard deviation. f-test analysis was conducted to determine whether different data from physical-chemical parameters and bio-slurry parameters belong to the same population. The analyses were checked at 95% confidence level and 2 degrees of freedom. Statistical packages used included Microsoft Excel and OriginLab (version6.5).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Chemical constituents of the bio-catalytic additives used

4.1.1 Properties of the aqueous plant extracts

The *T. brownii* extracts were green in color and quite viscous with a sharp smell, similar to that of fermented vegetables. These properties indicated presence of bacteria as well as other anaerobes in the extract. One of the favorable properties of a fermentation bio-catalyst is provision of conducive environment for anaerobes to exist. Sharp smell of extracts has been attributed to being by-products of microbial processes (Bowler et al., 2011). It is thus feasible to conclude that the extracts of *T. brownii* supported microbial activity. This is further supported by the formation of the viscous broth over time due to microbial reactions taking place. The extracts of the additives are shown in figure 4.1 below;

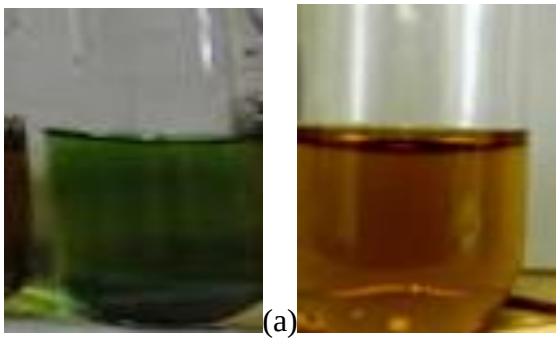
(a) (b)

Figure 4.1: Water extracts of *T. brownii* leaves (a) and *Acanthaceae spp.* barks (b)

According to Sunderg et al., (2013), there is a correlation between low pH values and survival of microbes emitting a high odor. Schaffner and Beuchat, (1986) demonstrated that the viscosity of fermentative leguminous extracts (from soybeans) was directly correlated with its pH. Decrease in pH was associated with more viscosity. Acidic pH favors coagulation of solutions leading to more viscosity (Rasnani et al., 2011). The

viscosity indicates the use of whole broths as biocatalysts which is quite recommended in most biocatalyst industries (Tufvesson et al., 2011). These broths solve many hurdles associated with the isolation of biocatalysts to specific enzymes. Some of these challenges include lack of catalyst stability in a reaction mixture, need for cofactor addition, enzyme coupling in cascade reaction systems and encapsulation of target enzymes in a microbial cell (Fernandes et al., 2018). These extracts were frothy citing the possibility of high organic load present. *Acanthaceae spp.* extracts were brown in color and watery. The *Acanthaceae spp*. extracts were odorless. Residues in the extract settled at the bottom of the test tube indicating the possibility of high total solids (Magu et al., 2016a).

4.1.2 pH Values of Additives

Additive pH is very important in biogas production (Zhang, 2017). High deviation from neutral pH, especially towards the acidic end is known to kill methanogenic archaea (Mohammed et al., 2017). Table 4.1 below shows the average pH values of the extracts against the temperature of the solutions.

Table 4.1: Additive pH values

Sample	**pH**	**Temperature ($^{\circ}$C)**
Distilled water	6.872 ±0.223	21.3
T. brownii	6.713±0.045	21.3
Acanthaceae spp.	7.160 ±0.006	21.3

From the table above, *T. brownii* and *Acanthaceae spp.* crude extracts were quite neutral. These findings indicate lack of volatile fatty acids in the extracts which would lead to reduced pH. *T. brownii* extracts were, however, more acidic compared to the *Acanthaceae spp.* extracts attributable to more volatiles and acidic microbes. However, there was no significant difference in the pH values of both additives ($p \leq 0.05$, $n = 2$). The pH values of *D. sisoo* and *L. leucocephala* water extracts (also used in biogas optimization) were

found to range between pH 6-8 (Zayed et al., 2018). The pH of *A. auriculiformis* was slightly acidic ranging between pH 4.5-6.5 (Cabi, 2019). Both extracts were however in a pH range conducive for the survival of methanogenic bacteria (Mohammed et al., 2017).

4.1.3 Solid content in the bio-catalytic additives

The two extracts were quite watery with relatively little solid matter. It is thus feasible to conclude that the catalysts were homogenous in regard to the state of the biogas substrate. The solid contents of the extracts are summarized in table 4.2 below. The total solids content in *Acanthaceae spp.* was higher than that in *T. brownii* which could be attributed to more inorganic matter content as supported by the TDS values. On the other hand, *T. brownii* VS content was higher due to more organic matter which is in agreement with its TSS value. Zhiqiang and Jian, (2016). indicated that the optimum solid content for biogas production is 8%. More solid content implies more organic load which is too much for the anaerobic microorganisms to decompose. A lower solid content, on the other hand, starves the anaerobic microorganisms.

Table 4.2: The solid content in the bio-catalytic additives

Sample	***T. brownii***	***Acanthaceae spp.***
Total solids (%)	9.112±0.013	11.762±0.111
Volatile solids (%)	6.772±0.238	6.113±0.151
Total dissolved solids (%)	2.328±0.013	10.010±0.000
Total suspended solids (%)	6.784 ± 1.257	1.752±0.058

The TSS values of *T. brownii* were by far much higher compared to those *of Acanthaceae spp.* This can be attributed to the viscous nature of the *T. brownii* extracts. The extracts of *Acanthaceae spp.* were, however, less viscous and watery which explains why they had a

higher TDS value of 10.010±0.000% compared to 2.328±0.013% of *T. brownii.* High TDS compared to TSS in the extracts imply the presence of more soluble and inorganic ions present. Such ions do not yield precipitates or coagulate easily and are therefore less viscous. It is likely that the inorganic compounds present in *Acanthaceae spp.* are thus more than *T. brownii* which is likely to have more organic compounds.

4.1.4 Additives Electrical Conductivity

Electrical conductivity is a useful measure of the nature of ions present (Maghanga et al., 2017). The Electrical conductivity of the additives and distilled water used for extraction were also analyzed as shown in Table 4.3 below.

Table 4.3: Additives Electrical Conductivity

Sample	**Electrical Conductivity**	**Temperature (°C)**
D. Water	40.065±0.103 (µS)	22.5
T. brownii extracts	48.428±0.998 (mS)	22.5
Acanthaceae spp. Extracts	57.472±0.012 (mS)	22.5

Acanthaceae spp. electrical conductivity values were by far higher than those of *T. brownii* with a margin of about 9.044mS. These findings are supported by the high TDS and TS values of *Acanthaceae spp.* extracts. Increased electrical conductivity values are likely to have an effect on the bio-slurry quality as was seen under bio-slurry analysis. High electrical conductivity values suggest more ionic compounds present and vice versa (Maghanga et al., 2017). Li et al., (2019) found out that the conductivity of fermentation species is affected by pH, ionic concentrations, reducing sugars and ethanol concentrations in the biomass substrate. At high pH values, there is more ionization of the ions present in the biocatalyst. This leads to higher electrical conductivity of the *Acanthaceae spp.* extracts

(which had a higher pH value). There is however no significant difference in the conductivity values of these two biocatalysts ($p \leq 0.05$, $n = 2$).

4.1.5 Dissolved Oxygen of Additives

Total dissolved oxygen is a measure of aerobic activity present in a sample (Rowe et al., 2017). Biomass substrate is expected to have some dissolved oxygen due to the micro-organisms present (Moreno-Casasola et al., 2017). The additives dissolved oxygen are summarized in Table 4.4 below;

Table 4.4: Dissolved oxygen values for the additives

Sample	**Dissolved Oxygen (%)**	**Temperature (°C)**
D. Water	4.633±0.153	23.5
T. brownii	10.467±0.306	23.5
Acanthaceae spp.	6.600±0.100	23.5

T. brownii had high dissolved oxygen values of up to 10.467±0.306% at the same temperature of 23.5°C. This is due to its organic matrix which encompasses a lot of molecular oxygen. However, these values of oxygen in the additives decrease drastically when put in a fermentation setup (such as in a bio-digester). The anoxic environment present combined with other conditions facilitate glycolysis of biomass in the bio-digesters. Oxygen present aids in the respiration of the biomass to glucose molecules as a parallel and synergistic reaction to the ongoing fermentation of biomass. High levels of dissolved oxygen is, however, a barrier to booming methanogenic archaea. A biogas digester should, however, have less oxygen to allow for anaerobic activity to occur (Laramee et al., 2018).

4.1.6 Bio-Metal Analysis

Various extracts are known to inhibit different concentrations of bio-metals (Swaleh et al., 2016; Osano et al., 2004). Five metals were analyzed for presence in the two additives and their values are as summarized in Table 4.5 below;

Table 4.5: Bio-metal concentration in the two additives

Sample	Copper (mg/Kg)	Zinc(mg/Kg)	Iron(mg/Kg)	Chromium(mg/Kg)	Cobalt(mg/Kg)
T. brownii	1.440±0.037	1.385±0.006	4.852±1.308	0.246±0.103	4.887±0.303
Acanthaceae spp.	1.517±0.202	1.502±0.097	9.193±0.827	0.492±0.154	9.755±0.446

Transition metals are good catalysts since they can form intermediate complexes of lower energy enabling reactions to proceed quickly and using less energy (Ahmed et al., 2016). From the above table 4.5, iron and cobalt were the most concentrated metals in the additive samples averaging about 4.852±1.308mg/Kg and 4.887 ± 0.303mg/Kgin *T. brownii* extracts and 9.193±0.827mg/Kg and 9.755 ± 0.446mg/Kgin*Acanthaceae spp.* extracts for both respectively. The abundance of iron ions as a catalyst has been reported to improve biogas quality by reducing the amount of hydrogen sulfide gases (Wintsche et al., 2016). Zinc concentration was relatively equal in both additives at about 1.385 ± 0.006mg/Kg and 1.502±0.097mg/Kg in *T. brownii* and *Acanthaceae spp.* respectively. Chromium ions were the least abundant in the extracts. Generally, *Acanthaceae spp.* extracts had higher bio-metal concentration compared to *T. brownii* extracts. These results conform to the higher TS and TDS values of *Acanthaceae spp*. compared to those of *T. brownii* since most metals are soluble in water at normal environmental conditions (Abbasian et al., 2015). An increase in bio-metal concentration is suitable in enhancing biomass degradation (Wang & Ho, 2016) to form biogas. The last stage of the biomass-to-biogas process is

methanogenesis. This stage is redox in nature and involves methanogenetic bacteria acting as electron acceptors. Cobalt ions have been reported to enhance the methanogenesis process (Paulo et al., 2017).

4.1.6.1 Speciation of bio-metals

The specific ions present in the bio-metals can be determined by speciation of these elements using prior knowledge of their pH, temperature and electrical conductivity (Magu et al., 2016b). Only specific bio-metal ions are responsible for hastening the methanogenesis process (Yu et al., 2016a). Lab simulation analysis was conducted using the software (PHREEQC). This is a standard and validated method for speciation of ions in the absence of more comparative chemical techniques.

a) Copper

Timely dosage of copper ions in biogas digesters is known to recover the process (Guo et al., 2012). This prolongs the retention period of biogas processes to achieve more biogas. The specific concentrations of major copper ions are as indicated in Table 4.6 below;

Table 4.6: Speciation of copper ions in the extracts

Sample	**Cu^{+}**	**$Cu(OH)_2$**	**Cu^{+2}**	**$CuOH^{+}$**	**$Cu_2(OH)_2$**
T. brownii (%)	1.573	63.145	32.138	3.069	0.075
Acanthaceae spp. (%)	1.560	62.268	32.349	3.043	0.078

Copper (ii) ions predominated copper (i) ions with copper (ii) hydroxide being the most abundant ion at pH 6.5 and 22.5˚C. Other copper (ii) ions also formed a large portion of copper ions i.e 32.2%. The abundance of copper ions in this environment was similar in both additives. Copper (ii) ions have been reported to inhibit the cell growth and survival rate of *Saccharomyces cerevisiae,* BH8 enzymes (Sun et al., 2018). This occurs in the late

fermentation stage just before the generation of pyruvate (Sun et al., 2018). This has an overall negative effect on biogas production which is a direct analogue of the fermentation process. Fermentative fungi present in the bio-catalysts are likely to be affected by the copper (ii) ions. No effects have been reported on the major ion, $Cu(OH)_2$ (63.145% and 62.268% in *T. brownii* and *Acanthaceae spp.* extracts respectively).

b) Iron

Iron is an important bio-metal known to catalyze anaerobic digestion processes. Iron (ii) ions play a crucial role in metalloenzymes (Shakeri-Yekta et al., 2014). Ariunbaatar et al., (2016) found out that supplementing iron (ii) in food waste biomass increased the rate of volatile fatty acids consumption and stimulated the microbial activities present. Iron (ii) ions potentially inhibit hydrogen sulfide by forming iron sulfide. This increases the purity of the biogas output. Ferrous salts were more abundant than ferric salts in the additive extracts at the given condition. Various iron (ii) ions formed 77.8% of the iron salts while $Fe(OH)_3$ and $Fe(OH)^{2+}$ also formed a large portion of the remaining ions. The abundance distribution of major iron ions at pH 6.5 and 22.5˚C is summarized in table 4.7 below.

Table 4.7: Speciation of iron ions in the extracts

Sample	**Fe^{+2}**	**$FeOH^{+}$**	**$Fe(OH)_3$**	**$Fe(OH)^{2+}$**	**$Fe(OH)^{-}_4$**	**$FeOH^{+2}$**
T. brownii (%)	77.636	0.194	11.739	10.329	0.098	0.004
Acanthaceae spp. (%)	77.946	0.193	11.549	10.213	0.097	0.004

Ferric salts in biogas digesters have been proven to passivate bio-mineralization of CuO and ZnO beyond 120mg/L (Liu et al., 2019). The distribution of ions in both additives was however similar. According to Liu et al., (2019), these ions have a toxic effect on a naerobic microorganisms at concentrations higher than 120mg/L.

c) Zinc

Zinc (ii) ions are required for the synthesis of enzymes involved in carbon dioxide reduction with hydrogen to form methane (Goswami et al., 2016). This is the hydrogenotrophic methanogenesis pathway. These ions are translocated by enzyme transporters in in *Methanothermobacter marburgensis* and *M. thermautotrophicus* bacteria responsible for methane production (Wang et al., 2009). The distribution and abundance of various zinc ions in *T. brownii* and *Acanthaceae spp.* extracts are summarized in table 4.8 below.

Table 4.8: Speciation of zinc ions in the extracts

Sample	**Zn^{2+}**	**$ZnOH^{+}$**	**$Zn(OH)_2$**
T. brownii (%)	99.026	0.8564	0.1172
Acanthaceae spp. (%)	99.04	0.84	0.115

Almost all (99%) ions in zinc were from divalent zinc in both additives. However, unlike in other bio-metals, the abundance of the hydroxide of zinc was not predominant. Other zinc (ii) ions formed more composition although the distribution was similar for both types of extracts.

d) Chromium

Chromium (iii) ions have been reported to inhibit growth of microorganisms in anaerobic digestion processes (Mudhoo & Kumar, 2013). Other chromium ions enhance methanogenesis process. The distribution of abundancy of chromium ions in the extracts at 25°C and pH 6.5 are summarized in table 4.9 below. The hydroxides of chromium were prevalent in oxidation states i, ii and iii in both *T. brownii* and *Acanthaceae spp*. extracts at the given conditions. The distribution of ionic abundance was also similar in both

additives. Chromium hydroxide ions have the ability to catalyze biomass degradation to smaller particles (Wang & Ho, 2016).

Table 4.9: Speciation of chromium ions in the extracts

Sample	$Cr(OH)^{2+}$	$Cr(OH)_3$	$Cr(OH)_2^{+}$	Cr^{+3}	CrO_2^{-}	$Cr(OH)_4^{-}$
T. brownii (%)	73.140	17.114	9.701	0.044	0.001	0.001
Acanthaceae spp. (%)	73.132	17.102	9.720	0.044	0.001	0.001

e) Cobalt

There was diversity in the occurrence of cobalt ions in the two extracts, as shown in table 4.10 above. From the table, most of the cobalt ions in *T. brownii* extracts occurred as cobalt (ii) while only 43.451% of the *Acanthaceae spp*. extracts were in this form.

Table 4.10: Speciation of cobalt ions in the extracts

Sample	$HCoO_2^{-}$	$Co(OH)_2$	Co^{2+}	Co_2OH^{+3}
T. brownii (%)	5.581	0.000	94.419	0.000
Acanthaceae spp. (%)	56.549	0.001	43.451	0.000

Cobalt (ii) ions are involved in synthesis of cobalamin in the enzymes involved in methanogenesis process (Goswami et al., 2016). Cobalt concentration in *Acanthaceae spp*. extracts was almost equally occurred as cobalt (ii) and as $HCoO_2^{-}$. Collins et al., 2010; reported that occurrence of cobalt ions in plant samples is greatly affected by its carboxylation by organic acids present. They found cobalt (ii) ions to be the most prevalent in ordinary neutral pH for several plant samples used.

4.1.7 Functional Group Analysis

The FT-IR profiles of both extracts were similar towards the blue end but differed in functional group peaks and intensity towards the right of their FT-IR spectra. The collated FT-IR Spectra of the two additives is as shown in Figure 4.2.

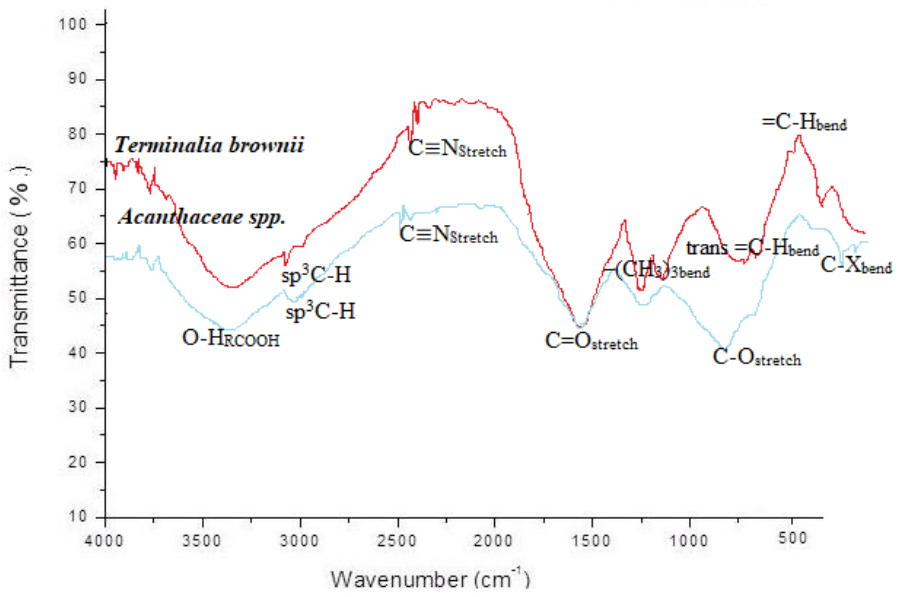

Figure 4.2: FT-IR Spectra of *T. brownii* (Red) and *Acanthaceae spp.* extracts (blue)

Carboxylic-$OH_{stretch}$, sp^3 C-H and $Amide_{stretch}$ peaks were present in both additives at an almost equal intensity. These peaks indicated presence of weak carboxylic acids present in the extracts (Bartyzel, 2017). The carbonyl peak at 1680cm^{-1} in *T. brownii* extracts was more pronounced compared to that in *Acanthaceaespp*. In contrast, the C-$O_{stretch}$ peak at 1035cm^{-1} in *Acanthaceae spp*. was more pronounced while *T. brownii* extract had more fingerprint peaks, key amongst them being trans =C-H_{bend} and =C-H_{bend} peaks. Of more interest was the C-X_{bend} peak at 560cm^{-1} in the *T. brownii* indicating presence of diverse compounds in the extracts. Increased organic compounds diversity enhance more reactivity (Breydo & Uversky, 2015). From the above profiles, it is clear that both species have organic acids; which is justified by their pH values being slightly acidic. *T. brownii* extracts

had more major peaks compared to *Acanthacea spp.* possible implying the extracts were rich in various organic compounds.

4.2 Determination of chemical constituents in Kitchen Waste Substrate

4.2.1 Substrate pH

All samples had slightly acidic pH possibly due to use of slightly acidic water for preparation as well as presence of weak carboxylic acids as evidenced by their FT-IR peaks in 4.2 above. Table 4.11 below summarizes the pH values of the substrate and distilled water used to prepare the extracts.

Table 4.11: Substrate pH

Sample	**D. Water**	**Control**	***T. brownii***	***Acanthaceae spp.***
Average pH	6.842±0.026	6.300 ± 0.001	6.070 ±0.001	6.750 ±0.017

The sample pH was however in the range of optimal biogas production and did not require any buffering. The pH of the samples did not deviate from each other significantly. Therefore, all samples had no bias in biogas production as far as pH levels were concerned. Jayaraj et al., (2014) confirmed that biogas production and biomass generation from kitchen wastes were high at neutral pH. According to the study, biogas yields and degradation efficiencies decreased with decreasing pH.

4.2.2 Substrate Total Solids and Volatile Solids

The solid content in biogas digesters is very crucial for more biogas yields and methane levels (Kamdem et al., 2018). The optimal TS range should be between 10-20% (Jimenez et al., 2015). Table 4.12 below indicates the solid content of the kitchen waste substrate used. The findings obtained were distinct from each other. The samples with additives had

slightly more solid content than the control sample. However, all samples statistically belonged to the same population at 95% confidence level (n = 2). The percentage of volatile solids in the samples were high expect in the *Acanthaceae spp*. sample whose volatile solids content did not belong to the same population with the rest ($\alpha = 0.05$, n = 2). The substrate was found to have average total solids for biogas production.

Table 4.12: The total solids and volatile solids in in biogas substrate used

Sample	**Control**	***T. brownii***	***Acanthaceae spp.***
Average TS (g/L)	11.157±0.669	12.320±0.317	12.361 ±3.056
Average VS (g/L)	11.283±0.008	12.293±0.294	8.737±0.304

Increased water content is known to increase the methane levels (Zhang et al., 2018). On contrary, very little solid content implies that the bacteria community will have less substrate to feed on and produce biogas and bio-slurry. Very high TS ratios increase the organic load to overwhelm the anaerobic archaea present which can choke these micro-organisms (Safferman & Wallace, 2015).

4.2.3 Substrate Total Dissolved Solids (TDS) and Total Suspended Solids (TSS)

An equilibrium between TDS and TSS should however be attained for optimal biogas and bio-slurry production. The TDS values were higher than the TSS values in all samples. All samples were however in the same population for both TSS and TDS values ($\alpha = 0.05$, n = 2). Table 4.13 below summarizes the TDS and TSS of the biogas samples.

Table 4.13: Substrate total dissolved solids and total suspended solids

Sample	Control	*T. brownii*	*Acanthaceae spp.*
Average TDS (g/L)	7.252±0.672	7.689±0.284	8.314±3.058
Average TSS (g/L)	3.904±0.003	4.976±0.002	4.047±0.003

While total dissolved solids are indicators of inorganic compounds present, total suspended solids are directly correlated to the organic load present in a biogas digester (Torsten, 2018). *T. brownii* had the highest TSS value of $4.976 \pm 0.002 g/L$ indicating high prospects of more biogas. Increased organic content provides more substrate for production of more biogas (Bansal et al., 2017).

4.2.4 Substrate Volatile Organic Salts (VOS)-Total Inorganic Carbon (TIC) ratio, Volatile Fatty Acids (VFAs) and Alkalinity (ALK) values

Kitchen waste samples are expected to have high volatile acids values due to present of carbohydrates and proteins (Escamilla-Alvarado et al., 2017). High volatile acids cause formation of more carbon dioxide in biogas (Hung et al., 2015). The average VOS/TIC values for all the samples were higher than 0.7 implying more organic load in the samples. VOS/TIC values indicate the correlation of organic load present per population of anaerobic archea present in the sample (Moeller & Zhensdorf, 2016). High VOS/TIC values above 0.7 indicate that the organic loading rate is very high and feeding of the biogas digester should stop (Moosbrugger 1993). Very low VOS/TIC values of below 0.3 indicate that there are too many bacteria for the organic load to be converted into biogas. The optimum VOS/TIC values are thus in the range of 0.3-0.7. Table 4.14 below summarizes the VOS/TIC (taken before biogas production began), VFAs and ALK values in the substrate used.

Table 4.14: Substrate VOS/TIC, VFAs and ALK values

Sample	Control	*T. brownii*	*Acanthaceae spp.*
VOS/TIC	1.240 ±0.020	1.507±0.035	1.360 ±0.072
VFAs (mg/L)	14.580 ±0.181	12.667±0.257	11.440 ±0.081
ALK (mg/L)	0.900 ±0.132	0.517±0.029	0.467±0.058

From the above FOS/TIC results, it is evident that the kitchen waste substrate had excessive organic matter to last for a long retention period without replenishing. The levels of VFAs superseded those of ALK indicating that the samples had a lot of proteins and carbohydrates (Escamilla-Alvarado et al., 2017). The volatile acids level of the control sample (14.580 ±0.181mg/L) were found to be significantly different from those of *T. brownii* (12.667±0.257mg/L) and *Acanthaceae spp*. (11.440 ±0.081mg/L) at 95% confidence level (n = 2). The high levels of volatile acids in the *T. brownii* sample indicated abundance of cellulosic and proteins (amino acids) (Deng et al., 2018). These compounds (especially cellulose) are prime requirements for optimized biogas production. The ALK levels of the *Acanthaceae spp*. sample were higher than those of *T. brownii* indicating more buffering capacity of excess acids in the latter sample.

4.2.5 Substrate Electrical Conductivity

Acanthaceae spp. sample had the highest conductivity due to high total dissolved solids present. Choo, (2019) predicts a linear relationship between TDS and electrical conductivity of solutions. The dissolved solids can easily conduct electrons and therefore are associated with more conductivity. *T. brownii* sample had low conductivity values due to reduced TDS and more volatile solids present. Volatile organic compounds have the ability to mask ions preventing or lowering their ionization in solution. High electrical

conductivity is related to salinity prospecting the *Acanthaceae spp.* sample to be more saline and thus have better bio-slurry. All samples conductivity however belonged to the same population ($\alpha = 0.05$, $n = 2$). Increase in electrical conductivity have no direct effect on the biogas output but affect the quality of the bio-slurry formed. The conductivity of the samples is summarized in table 4.15 below;

Table 4.15: Substrate electrical conductivity

Sample	**D. Water**	**Control**	***T. brownii***	***Acanthaceae spp.***
E. Conductivity	6.950 ± 0.451μS	1.293±0.002mS	0.940 ± 0.004mS	1.315±0.015mS

Biomass with more conductivity is likely to have more inorganic solids and therefore the levels of some essential plant minerals (such as potassium) are higher in these biomasses.

4.2.6 Substrate Dissolved Oxygen

It is quite impossible to attain a totally anerobic environment in a bio-digestor without an oxygen pump (like the one used in this study). The control sample had the highest amount of dissolved oxygen (8.500±0.476%) compared to the samples with additives i.e 7.500 ± 0.183% for *T. brownii* and 7.650 ± 0.311% for *Acanthaceae spp*. respectively. The levels of dissolved oxygen in biogas substrate were analyzed and summarized in table 4.16 below;

Table 4.16: Substrate Dissolved oxygen

Sample	**D. Water**	**Control**	***T. brownii***	***Acanthaceae spp.***
D. Oxygen (%)	8.600 ± 0.516	8.500±0.476	7.500 ± 0.183	7.650 ± 0.311

All values however belonged to the same population ($\alpha = 0.05$, $n = 2$). Biogas substrates with high oxygen levels produce lower yields due to inhibition of the anaerobic pathways

(Ghosh & Ghangrekar, 2019). Botheju and Bakke (2011), found out that biogas digesters can withstand oxygenation up to 15% of oxygen in the bio-digesters without any negative implications. However, the more the oxygen content in the bio-digester, the more the toxicity on methanogenic bacteria which control methane production. Limitation of anoxic environment towards that with more oxygen also lower other biogas production steps.

4.2.7 Substrate Carbon, Nitrogen and Phosphorus content

The ratios of carbon, nitrogen and phosphorus are significant in predicting the quantities and quality of biogas from digester systems. All samples had a balanced carbon: nitrogen: phosphorus ratio. The control sample had higher values of these elements. However, only the nitrogen content of the control sample was significantly different from the rest at 95% confidence level (n = 2). The optimum carbon: nitrogen: phosphorus ratio is 15:1:0.4 (Wang et al., 2018). High carbon content in biogas slurry imply more glycosidic bonds to break (which are quite strong) and thus more solid retention time taken to produce biogas (Arroyo et al., 2018). Table 4.17 below summarizes the average values of these three critical parameters of biogas substrate;

Table 4.17: Carbon, nitrogen and phosphorus levels in biogas substrate

Sample	**Control**	***T. brownii***	***Acanthaceae spp.***
Carbon (g/L)	21.600 ± 2.400	22.330±0.215	21.980±0.413
Nitrogen (g/L)	3.067±0.540	1.213±0.428	1.307±0.323
Phosphorus (g/L)	0.780±0.020	0.587±0.023	0.660 ± 0.000

High nitrogen or phosphorus values lead to increased ammonia and phosphine gas in the biogas (Shahbaz et al., 2019). The ratios of samples analyzed had more nitrogen content citing possibility of more impurities in the biogas to be formed. This is because of the

nature of substrate used (kitchen waste.) All samples were thus liable for optimal biogas production.

4.3 Monitoring of Biogas Parameters Over 30-Day Retention Period

4.3.1 Biogas Yields

The average biogas volumes from the three digesters were recorded daily in the specified retention period. Biogas production was in the order *T. brownii* (15,861.4ml/gVS), *Acanthaceae spp*. (13,219.6ml/gVS) and control (7,444.8ml/gVS). The biogas yields were measured against the environmental temperature as summarized in table 4.18 below.

Table 4.18: Biogas volumes from the three digesters used in the 30-day retention period

Retention day	**Environmental Temperature (°C)**	**Biogas volumes (ml)**		
		T. brownii	**Control**	***Acanthaceae spp.***
1	23.0±0.5	3000.00	0.00	0.00
2	24.0±0.5	3000.00	0.00	0.00
3	24.0±0.5	6000.00	0.00	0.00
4	22.5±0.5	9000.00	3000.00	1500.00
5	23.0±0.5	12000.00	3000.00	0.00
6	22.5±0.5	9000.00	3000.00	0.00
7	23.0±0.5	9000.00	0.00	3000.00
8	23.5±0.5	6000.00	3000.00	3000.00
9	22.0±0.5	12000.00	3000.00	3000.00
10	21.5±0.5	9000.00	9000.00	6000.00
11	20.0±0.5	6000.00	9000.00	6000.00
12	20.5±0.5	9000.00	9000.00	6000.00
13	21.0±0.5	6000.00	6000.00	3000.00
14	21.0±0.5	6000.00	6000.00	6000.00

Retention day	Environmental Temperature (°C)	Biogas volumes (ml)		
		T. brownii	Control	*Acanthaceae spp.*
15	20.5±0.5	9000.00	0.00	6000.00
16	19.5±0.5	9000.00	3000.00	9000.00
17	18.5±0.5	9000.00	0.00	9000.00
18	19.0±0.5	6000.00	3000.00	6000.00
19	20.5±0.5	6000.00	0.00	3000.00
20	20.0±0.5	6000.00	3000.00	6000.00
21	20.5±0.5	9000.00	0.00	6000.00
22	18.5±0.5	6000.00	0.00	3000.00
23	15.5±0.5	3000.00	3000.00	6000.00
24	15.0±0.5	3000.00	3000.00	6000.00
25	16.0±0.5	3000.00	0.00	0.00
26	17.0±0.5	6000.00	3000.00	3000.00
27	16.5±0.5	3000.00	3000.00	6000.00
28	18.0±0.5	3000.00	3000.00	3000.00
29	18.5±0.5	3000.00	3000.00	3000.00
30	18.0±0.5	3000.00	3000.00	3000.00
TOTAL	**Avg Temp. = 19.5±0.5°C**	**195,000.00 ml**	**84,000.00ml**	**115,500.00 ml**

The sample with *T. brownii* 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 the control sample) at the same temperature. The use of the plant extracts was key in increasing biogas yields. Maurya et al., 2015; showed that use of plant extracts in a mixed-consortia biogas setup and biomass pre-treatment increased biofuel yields by appreciable margins.

The samples with the additives were able to produce more biogas volumes because of two key reasons; abundance of organic matter (especially *T. brownii*) and presence of transition metals in the form of bio-metals (especially *Acanthaceae spp*.). Organic compounds with alternating pi-bond systems affect the electron density of their neighboring organic compounds (biomass). This effect destabilizes the biomass making it susceptible to both nucleophilic and electrophilic attacks that lead to hastening of the biomass hydrolysis (Sanchez et al., 2018; Miranda-Quintana & Ayers, (2016). On the other hand, presence of transition metals is known to increase reactivity of both organic and inorganic reactions due to their formation of low energy intermediate complexes hastening reactions (Ahmed et al., 2016). Catalysts bind with the reactants interfering with the electron density distribution in the reactants and potentially reducing the strength of bonds previously formed (Lodish et al., 2000). The intermediate formed is called an activation complex. The complex has more sites of both nucleophilic and electrophilic attack and easily reacts with other reactants using less energy (Liljenberg et al., 2013). Thereafter, the catalyst dislodges itself from the complex and a product is formed. This pathway uses less activation energy compared to that without a catalyst (Siyavula, 2019b). There was very little biogas recorded for retention-days 15 and 25 in *Acanthaceae spp.* and control sample due to technical challenges in the bio-reactor. The trend of biogas production over the retention period is illustrated in figure 4.3 below.

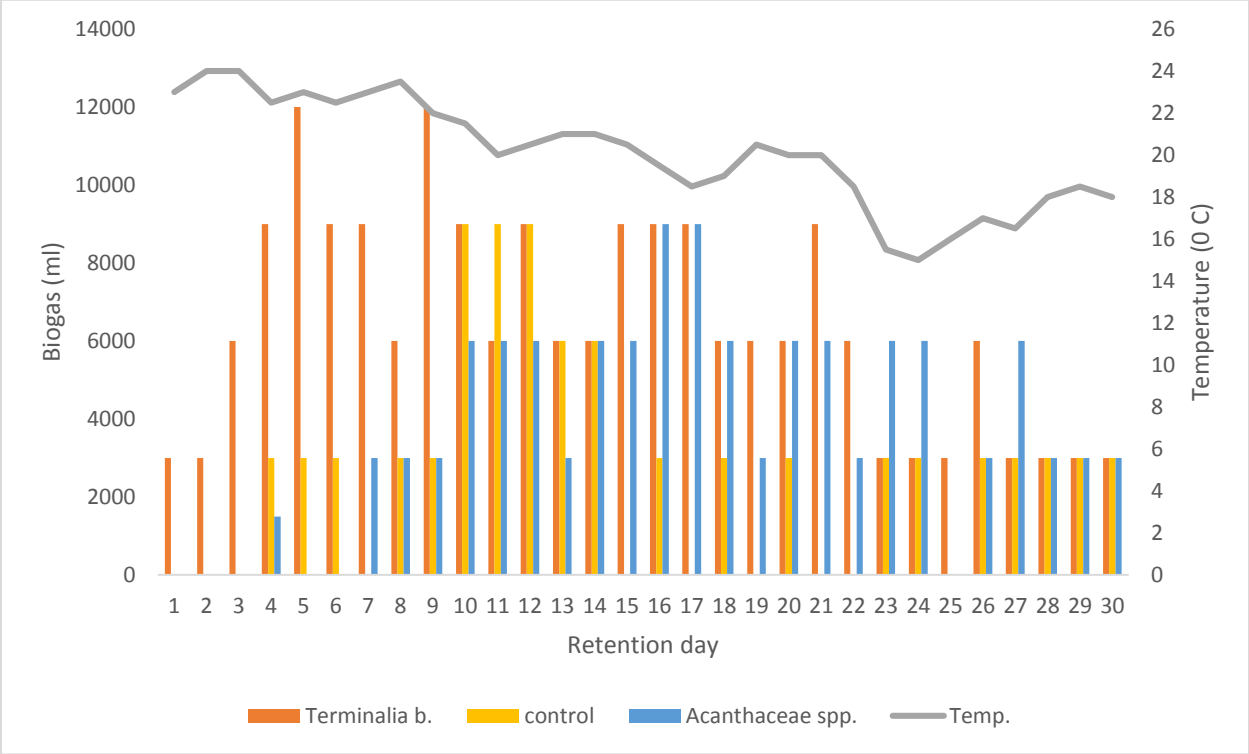

Figure 4.3: Variation of biogas volume with temperature over the retention period

Production of biogas was proportional to temperature variation. 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. At higher temperature, glycosidic bonds of the biomass are also weakened and can be easily degraded to biogas. Biogas production in *T. brownii* 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 (Yang et al., 2015). Upon commencement of biogas production, *Acanthaceae spp.* sample had consistent biogas yields averaging 3850ml/retention day. The control sample biogas yields averaged 2800ml/retention day. Biogas production from *T. brownii* sample was proven to be significantly different from the rest at 95% confidence level (n = 2).

4.3.2 Combustibility Points of Biogas Produced

Biogas samples were tested for combustibility daily. The exact days of full-glare blue flame were noted and summarized in table 4.19 below;

Table 4.19: Combustibility points of the biogas samples

Sample	Days taken to combust
Control	20
T. brownii	19
Acanthaceae spp.	17

Different biogas digesters attain biogas combustibility at different periods depending on the digester conditions, substrate or additives used. *Acanthaceae spp.* took the shortest retention time to produce combustible gas indicating ability of the additive to suppress CO_2, CO and other gases which do not support combustion or absorb water vapor.

Figure 4.4 below illustrates a gas sample from the digester burning in a blue flame.

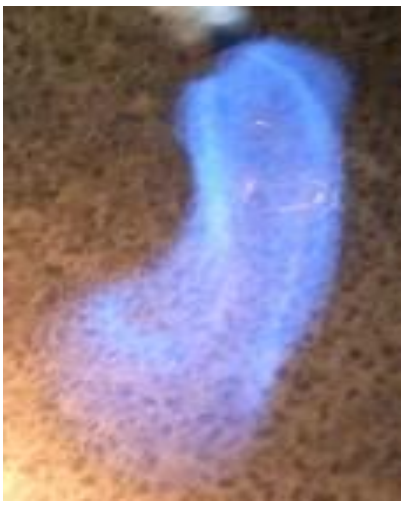

Figure 4.4: Flame of biogas sample from one of the digesters

The difference in retention periods taken to produce combustible gas between the control and *T. brownii* sample were not significantly apart, ($p \leq 0.05$, $n = 2$). The days taken to

combust is a direct reflection of methane level present. Previously, enzymatic bio-catalysts carbohydrases and proteases have been successfully used to increase methane production by 1.72 folds and 1.53 folds respectively (Mahdy et al., 2016).

4.3.3 Biogas Composition Analysis

Biogas composition was analyzed on a weekly basis for CH_4, CO_2, O_2, H_2S and CO gases. Only methane levels seemed to increase over retention time. *T. brownii* methane levels were highest ($43.475 \pm 0.922\%$) across the entire retention period with the *Acanthaceae spp.* sample having the least methane levels expect on the last retention week ($39.275 \pm 0.263\%$). *T. brownii* 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) (Mahdy et al., 2016). 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. 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. Wang *et al.,* (2019c) 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). All other impurities were minimal and decreased over retention time. Yu et al., (2016b); showed that using trace metals in biogas systems actually increase the methane levels as was proven in the *T. brownii* sample above. Table 4.20 below summarizes the trend in major gas levels and ICP values over the 30-day retention period.

Table 4.20: Raw biogas composition and ICP values over the retention period

Week	Bio-digesters	Biogas quality parameters					
		CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (ppm)	CO (ppm)	ICP (Kcal/Kg $_{biogas}$)
1	Control	14.450±0.968	27.250 ±1.708	25.150 ± 1.399	0.250 ± 0.500	47.750 ± 5.315	1176.230
	T. brownii	22.050±0.982	6. 000 ± 2.000	18.675±0.750	3.0000±2.449	116.250 ± 42.204	1798.940
	Acanthaceae spp.	20.825±2.353	23.500 ± 1.291	27.800 ± 1.111	0.750±1.500	5.000 ± 1.826	1693.120
2	Control	22.250 ±0.777	16.500 ± 3.109	15.150±1.100	0.000 ± 0.000	55.25±0.500	1831.500
	T. brownii	29.050 ±0.173	14.750 ± 0.500	15.7000 ± 0.216	1.000 ± 0.817	165.750 ± 4.573	2401.300
	Acanthaceae spp.	23.950 ±0.402	23. 000 ± 0.000	19.475±0.206	0.000 ± 0.000	71.500 ± 5.916	1949.530
3	Control	29.800 ±2.272	6.250 ± 0.655	18.675±0.655	0.250 ± 0.500	105.250 ± 5.498	2425.720
	T. brownii	35.650 ±4.638	21.500 ± 1.732	16.650 ± 0.889	1.750 ± 0.957	39.500 ± 12.396	2901.910
	Acanthaceae spp.	34.450 ±0.436	24. 000 ± 0.000	16.300 ± 0.100	0.000 ± 0.000	3.7500 ± 1.258	2804.230
4	Control	41.750 ± 1.401	17.000 ± 4.142	10.725±0.221	0.000 ± 0.000	13.000 ± 1.414	3398.450
	T. brownii	43.475±0.922	21. 00 ± 13.868	13.725±5.979	1.250 ± 1.500	48.250 ± 31.085	3538.865
	Acanthaceae spp.	39.275±0.263	23.500 ± 0.577	11.400±0.141	0.000 ± 0.000	14.250 ± 0.500	3196.985

T. brownii sample had higher hydrogen sulphide and carbon monoxide values compared to the rest of the samples but lower oxygen and carbon dioxide values. The carbon monoxide values of control sample were extremely high. *Acanthaceae spp.* sample had high carbon dioxide values but almost negligible hydrogen sulphide gas. This can be attributed to presence of abundant iron compounds in *Acanthaceae spp*. extracts which is known to scrub off hydrogen sulphide (Kucukhemek & Erdirencelebi, 2018). The ICP values of the biogas samples increased as the net methane levels increased with increase in the retention days. As the retention time progressed, more quality biogas was being generated (from ICP values). The ICP values of all the samples on the third week were significant enough to combust. These findings are supported by the combustion tests done in section 4.3.2 above.

The ICP values on the last retention day (day-28) corresponded to those of ethanol (Noyola et al., 2006).

4.4 Monitoring Biogas Substrate Over the Retention Period

Most parameters of biogas substrate in an anaerobic digester will gradually change as digestion proceeds (Wagner *et al*., 2014). Over time, elemental composition of the substrate such as dissolved oxygen, carbon, hydrogen and nitrogen and sulfur from protein substrate will also change (Ziels et al., 2018). These changes have an effect on the gas produced as well as the quality of bio-slurry formed (Ziels et al., 2018). It is thus essential to monitor substrate changes in parameter and composition over the given retention period.

4.4.1 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 *T. brownii* extracts had a steeper gradient in change of total solids implying higher degradation rates. As a rule of thumb, a good biocatalyst should be able to enhance fast biomass saccharification at ambient conditions. From the table 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 (Jimenez et al., 2015). Reduction in total solids content was highest in the *T. brownii* sample followed by the control and lastly the Acanthaceae spp. sample. In reference to the control setup, the *T. brownii* 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. Table 4.21 below summarizes the solid content change in the biogas substrate over time.

Table 4.21: 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
T. brownii	TS	12.320±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

Gumisiriza et al., (2017); used salts to hydrolyze kitchen waste biomass to biofuels at thermophilic temperatures. Most salt catalysts and enzymes used in biomass hydrolysis operate at thermophilic temperatures for enhanced degradability of strong cellulosic bonds (Mosier et al., 2005). The high conjugation effect of *T. brownii* extracts were 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.

4.4.2 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 4.22 below.

Table 4.22: 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	TSS	3.904±0.003	2.098±0.173	2.883±0.232	2.493±0.211	2.356±0.052
	TDS	3.252±0.672	6.394±0.210	2.780 ± 0.142	2.705±0.211	2.297±0.152
T. brownii	TSS	4.976±0.002	3.003±0.425	3.043±0.413	2.614±0.311	2.416±0.002
	TDS	7.689±0.284	2.126±0.113	1.662±0.115	1.371±0.315	0.125±0.415
Acanthaceae spp.	TSS	4.047±0.003	3.016±0.333	3.119±0.613	3.017±0.212	2.346±0.001
	TDS	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 (Jimenez et al., 2015). The TDS and TSS values of the additives decreased by larger margins compared to the control sample. The TSS value of *T. brownii* on retention day-28 was extremely low and significantly different from the rest ($p \leq 0.05$, n= 2). Increased TSS implies high organic load which is associated to more biogas yields (Wang et al., 2019b). More TDS values implies more inorganic matrix and therefore better fertilizer quality (Al-Wabel et al., 2018). *T. brownii* 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. 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 present (Liu and Tay, 2004). On the other hand, bio-digesters with these additives optimize on biomass substrate loaded for optimal energy production.

4.4.3 Substrate pH

Sample pH values fluctuated throughout the retention period. Table 4.23 below illustrates the change in substrate pH during the retention period.

Table 4.23: pH change during biogas retention period

Samples	**Sample pH**				
	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 ± 0.001	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

The highest pH achieved was 7.120 ± 0.022 fot the *T. brownii* extract on the 14th retention day. Anaerobic conditions in a biogas digester induce fermentation of organic matter (Klassen et al., 2015). The resultant products have varying pH values depending on the preceding steps (Lindner et al., 2015). After the 14th retention day sample pH decreased. 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 (Ma et al., 2015). 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 (Girotto et al., 2018). 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 (Refai et al., 2017). It is however worth noting that these cycles did not limit continuous biogas production. The use of batch digestors in the experiments limited the organic loading rate therefore limited entry of process buffers responsible for controlling pH drifts (Yuan & Zhu, 2016). Use of more inoculum is a sure means to boost the pH due to production of bicarbonate buffers by methanogenic bacteria (Liu & Tay, 2004).

4.4.4 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 (Gilbert et al., 2015). Enough inoculum should thus be added to increase the alkalinity levels and adjust pH to neutral. 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. 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. 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 (Vasconcelos et al., 2016). *T. brownii* 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 (Winde et al., 2018). The alkalinity and volatile acids levels of the substrate over the retention period are summarized in table 4.24 below;

Table 4.24: 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
T. brownii	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
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

4.4.6 Substrate Organic Carbon

Organic carbon represents the load that bacteria digest to produce biogas (Zehnsdorf et al., 2017). 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. The organic carbon content of the control sample on retention day-28 ($19.060 \pm 0.000 g/L$) was found to be significantly different from that of *T. brownii* ($17.800 \pm 0.698 g/L$) and *Acanthaceae spp.* ($18.000 \pm 2.400 g/L$) ($p \leq 0.05, n = 2$). 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 (Dykstra & Pavlostathis, 2017). These variations are summarized in table 4.25 below.

Table 4.25: Change in substrate organic carbon

Samples	**Sample Organic carbon (g/L)**				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	21.600±2.400	21.060 ± 0.245	19.990 ± 0.313	19.890 ± 0.451	19.060 ± 0.000
T. brownii	22.330±0.215	20.870±0.000	19.880±0.837	18.440±0.612	17.800 ± 0.698
Acanthaceae spp.	21.980±0.413	21.440±0.691	20.250±0.000	20.060 ± 0.521	18.000 ± 2.400

Decrease in 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 *T. brownii* 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.

4.5 Bio-Slurry Fertilizer Analysis

The elemental compositions of bio-slurry at different retention periods is crucial in defining its potential as an organic fertilizer (Zhang et al., 2017). Availability of these essential nutrients for reach by plant roots is also important (Osano et al., 2009). Nutrients ought to be unbound from their parent compounds and soluble for easy uptake and absorption by

plants (Caldelas & Weiss, 2017). Several bio-slurry parameters and elemental composition of elements and their ions were done at different retention periods and summarized below;

4.5.1 Bio-slurry pH

The pH value of a fertilizer is quite important since different plants produce optimally in different soil pH (Barga et al., 2017; Wamelink et al., 2019). Various soil microorganisms are also affected by pH changes. Table 4.26 below illustrates the changes in bio-slurry pH over the retention period used.

Table 4.26: Changes in pH value of bio-slurry

Samples	**Sample pH**				
	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 ± 0.001	5.770 ± 0.017	7.120 ± 0.022	6.230 ± 0.017	5.860 ± 0.003
Acanthaceae spp.	6.750 ± 0.020	5.830 ± 0.010	6.850 ± 0.017	6.210 ± 0.017	5.910 ± 0.010

Change in pH values in anaerobic systems are largely dictated by the type of biomass present and inoculum used (Kucharska et al., 2018). 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 (Vasconcelos et al., 2016). The pH of bio-slurry was thus quite acidic after day 7. Retention day 14 had a neutral pH due to methanogenesis which occur in neutral pH (Chaterjee et al., 2019). 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 bio-slurry. Thereafter, 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 gradual decrease in pH (Sabbadin et al., 2018). After 28-day retention time, *Acanthaceae spp.* sample had the highest pH of 5.910 ± 0.010 and thus the best sample for most plant growth (Türtscher et al., 2017). There was however no significant variation in pH as a result of use of the two additives at 95% confidence level (n = 2).

4.5.2 Bio-slurry Electrical Conductivity (EC)

Variation in EC values as a function of using the two additives is important in assessing the viability of the additives. The EC values fluctuated throughout the retention period. Table 4.27 below summarizes the changes in EC values over the retention period.

Table 4.27: Variation in bio-slurry EC over 28-day retention period

Samples	**Sample EC (mS)**				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	4.040±0.010	3.810 ± 0.003	5.350±0.003	4.990±0.021	4.520±0.003
T. brownii	4.420±0.010	3.750±0.017	5.570±0.003	5.490±0.003	4.770±0.021
Acanthaceae spp.	4.340±0.003	4.410±0.003	5.000±0.020	6.040±0.017	5.250±0.027

During methanogenesis stage (retention day-14) there was less organic matrix in the ions present were easily detected leading to higher EC values. An ultimate range of 4.520 ± 0.003 (Control sample) to 5.250 ± 0.027 mS (*Acanthaceae spp.)* was attained which is optimal for solubility of plant nutrients and their uptake by roots (Behera & Shukia, 2015). Electrical conductivity values in slurry depict presence of soluble ions for plant roots to uptake (Magu et al., 2017). High EC values imply that the bio-slurry is quite soluble and can thus require less water during application to plants.

4.5.3 Bio-slurry Total Solids (TS) and Loss On Ignition (LOI)

Total solids content in a fertilizer sample is crucial in determining its primary mode of application. Table 4.28 below summarizes the TS and LOI variation in the bio-slurry over the retention period.

Table 4.28: Variation of TS and LOI during biogas retention period

Samples	**Parameter**	**Sample days**				
		Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	TS (g/L)	11.157±0.669	8.492±0.313	5.663±0.451	5.198±0.333	4.653±0.616
	LOI (g/L)	11.283±0.787	7.956±0.881	5.106±0.661	4.508±0.791	4.312±0.669
T. brownii	TS (g/L)	12.320±0.317	5.129±0.022	4.751±0.394	3.985±0.612	3.664±0.217
	LOI (g/L)	12.294±0.991	4.597±1.013	4.313±0.787	3.845±0.799	3.552±0.813
Acanthaceae spp.	TS (g/L)	12.361±3.056	8.786±0.527	7.026±0.821	6.522±0.142	6.435±0.899
	LOI (g/L)	8.737±1.012	8.634±0.129	6.666±0.189	5.182±0.623	4.892±0.677

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 ($12.320 \pm 0.317 g/L$ to $5.129 \pm 0.022 g/L$). The LOI values in *T. brownii* sample were also quickly reduced. The bio-slurry 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 *Acanthaceae spp.* extract were quite high right from the onset indicating more particulate levels in this sample. This bio-slurry 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 (Zhang et al., 2016). On contrary, low TS content imply the

fertilizer can easily be applied by top-dressing. High LOI values imply that the bio-slurry fertilizer should not be on the soil surface to minimize evaporation. High LOI bio-slurry is volatile and most of it does not end in target location when applied as a top-dress. 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 95% confidence level (n = 2). This imply that the three types of additives should not be applied in a similar method for optimal absorption by plants.

4.5.4 Bio-slurry alkalinity (Lime content)

The alkalinity of bio-slurry implies its ability to buffer acidic soil. Many plants perform optimally in neutral and slightly basic soil (Maghanga et al., 2013). The control sample had the highest alkalinity level at the onset which drastically reduced after 7 days of retention period. This can be attributed to precursor reactions that lead to formation of volatile acids such as acetic, propanoic and butyric acid (Balderas-Hernández et al., 2018). The control sample had thus very little buffering capacity which have direct effects on the bio-slurry quality. While the alkalinity levels of *Acanthaceae spp.* samples had little variations, those of *T. brownii* sample largely fluctuated. *T. brownii* sample had the highest alkalinity level of 0.558 ± 0.075g/L after 28 days. This value was significantly different from the rest at 95% confidence level (n = 2). Table 4.29 below illustrates the variation in alkalinity values in the bio-slurry samples.

Table 4.29: Bio-slurry variation in lime content over the retention period

Samples	Bio-slurry alkalinity (lime content) (g/L)				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control (mg/L)	0.900±0.132	0.298±0.001	0.336±0.000	0.342±0.012	0.422±0.068
T. brownii (mg/L)	0.517±0.029	0.412±0.228	0.384±0.089	0.486±0.098	0.558±0.075
Acanthaceae spp. (mg/L)	0.467±0.058	0.384±0.089	0.412±0.128	0.422±0.068	0.480±0.000

Bio-slurry alkalinity is primarily contributed to its lime content and have previously been used as an indicator of lime levels (Vestergård et al., 2018). High alkali levels are desired for any bio-slurry as the fertilizer can work well in acidic soils.

4.5.5 Bio-slurry Oxygen, Calcium, Carbon and Potassium levels

Elemental composition of bio-slurry is quite useful as these elements are the required plant nutrients. Elucidation of whether an element is a micro or macro nutrient vary with different types of plants as well as their growth stage (Chaka & Osano, 2019). From the substrate analysis (in 4.5 above), volatile solids and total suspended solids declined over retention time. Similarly, oxygen and carbon content in the bio-slurry of all three samples decreased in a linear fashion. The dissolved oxygen at the onset of digestion is gradually converted into other by-products by anaerobic archea present in the slurry (Akhtar et al., 2016). Similarly, carbon is the main element in biomass for production of methane. Carbon present as carbohydrates, lipids or proteins is sequentially converted to low weight acids (acetic, propionic and butyric), then to acetic acid and hydrogen and ultimately to methane, carbon dioxide and other by-products (Ošlaj et al., 2019). 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 bio-slurry viability as far as oxygen and carbon are concerned. Wang et al., (2019a) showed that use of bio-slurry from poultry dung had almost similar carbon content with mineral fertilizer. Available carbon from the bio-slurries above were higher than that obtained when using slurry with pure animal manure origin (1.3%) (Kuligowski et al., 2010).

Table 4.30 below summarizes the abundance and variation of these elements in the bio-slurry over the retention period.

Table 4.30: Variation in oxygen, carbon, calcium and potassium in bio-slurry

Samples	Parameters	Sample days				
		Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	Oxygen (%)	9.200±0.017	8.300±0.013	8.100±0.013	8.100±0.100	7.800±0.013
	Carbon (g/L)	21.060±0.000	20.600±0.451	19.890±0.313	18.990±0.245	18.600±2.400
	Calcium(g/L)	3.700±0.007	4.000±0.000	5.300±0.013	5.800±0.002	6.000±0.000
	Potassium(ppm)	42.680±0.000	42.820±0.000	43.920±0.000	44.470±0.000	44.610±0.000
T. brownii	Oxygen (%)	9.400±0.100	8.600±0.023	8.000±0.013	7.500±0.013	7.400±0.100
	Carbon (g/L)	22.330±0.215	20.870±0.000	19.880±0.837	18.440±0.612	17.800 ± 0.698
	Calcium (g/L)	2.000±0.010	5.300±0.002	8.700±0.000	11.000±0.001	11.070±0.001
	Potassium(ppm)	44.060±0.000	44.880±0.000	45.290±0.000	46.260±0.000	46.540±0.000
Acanthaceae spp.	Oxygen (%)	9.300±0.013	9.200±0.017	8.700±0.042	7.800±0.013	7.520±0.100
	Carbon (g/L)	21.980±0.413	21.440±0.691	20.250±0.000	20.060 ± 0.521	18.000 ± 2.400
	Calcium (g/L)	6.700±0.001	8.700±0.002	10.000±0.010	11.000±0.010	11.700±0.013
	Potassium(ppm)	44.400±0.000	45.020±0.000	45.290±0.000	46.540±0.000	46.560±0.000

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 increment of the total dissolved solids (in 4.5 above). *Acanthaceae spp*. sample which had the highest total solids and total dissolved solids had the highest calcium and potassium levels.

4.5.6 Bio-slurry Nitrogen Content

The general trend in all three nitrogen forms analyzed was a linear increase in concentration over the retention period. Total Kjehdahls 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 4.31 below.

Table 4.31: Variation of Total Kjehdahls Nitrogen (TKN), Total Ammoniacal Nitrogen (TAN) and nitrogen as free nitrates in bio-slurry over the retention period.

Samples	Parameter	Sample days				
		Day 1	Day 7	Day 14	Day 21	Day 28
Control	TKN (g/L)	1.850±0.700	6.183±0.545	11.083±0.867	11.433±0.910	12.367±0.652
	TAN (g/L)	6.716±0.341	6.816±0.420	8.467±0.625	9.635±0.888	10.803±0.652
	Free NO^{-}_{3} (ppm)	420.81±0.00	421.30±0.000	423.01±0.000	423.07±0.000	424.01±0.000
T. brownii	TKN (g/L)	1.633±0.313	9.100±0.632	13.533±0.667	13.767±0.882	14.350±0.350
	TAN (g/L)	6.716±0.341	10.511±0.000	11.971±0.212	12.847±0.885	14.599±0.425
	Free NO^{-}_{3} (ppm)	421.36±0.000	422.46±0.000	424.01±0.000	425.61±0.000	425.77±0.000
Acanthaceae spp.	TKN (g/L)	1.633±0.313	7.000±0.585	11.433±1.022	12.367±0.652	13.183±0.435
	TAN (g/L)	7.884±0.611	10.803±0.552	11.971±0.756	13.431±0.857	15.767±1.243
	Free NO^{-}_{3} (ppm)	420.69±0.000	422.41±0.000	424.17±0.000	425.88±0.000	427.87±0.000

By far the most vital nutrient for plant growth and optimal production (Maghanga et al., 2013), elucidation of nitrogen levels in their various forms in bio-slurry is crucial. 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. Use of the two additives caused more degradation of the proteins and therefore these samples had more nitrogen content than their corresponding control sample. Since nitrogen senescence over time from older tissues to newer ones to facilitate growth of new cells, (Chaka & Osano, 2019), most fertilizers provide nitrogen as a supplement. 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 0.8% in cassava peels. The total Kjehdahls nitrogen and free nitrates content on the 28th day of retention time values belonged to the same population (95% confidence level, n = 2). However, the total ammoniacal nitrogen level of the control sample ($10.803 \pm 0.652 g/L$) did not fit into the population of the samples with additives, i.e *T. brownii* (14.599 ± 0.425g/L) and *Acanthaceae spp.* ($15.767 \pm 1.243 g/L$); ($\alpha = 0.05$, n = 2). Biogas slurry ammonium levels are known to increase linearly over time (García-Sánchez et al., 2015). It is worth noting that *Acanthaceae spp.* sample had the highest nitrogen content values during most periods of the retention time and thus the best bio-slurry as far as nitrogen is concerned.

4.5.7 Bio-slurry Sulfur Content

Like nitrogen, sulfur is an essential nutrient and thus a major plant requirement (Augusto et al., 2017). Kitchen waste is prone to combination of several proteins and elucidation of sulfur content as a bio-slurry component is necessary. The bio-slurry from the three

samples were analyzed for Both total sulfur and free sulphates concentrations increased linearly during the retention period. The concentrations of these compounds in the control sample was quite low compared to the samples with additives. While total sulfur concentration after day-28 retention period $(4.842 \pm 0.005) g/L$ belonged to the same population with the samples with additives, the free sulphates concentration of this sample was significantly different at 95% confidence level (n = 2). Total Sulfur (TS) and free sulphates content at various retention periods as summarized by table 4.32 below.

Table 4.32: Variation in total sulfur and free sulphates levels over the retention period

Samples	Parameter	Sample days				
		Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	TS (g/L)	2.178±0.315	2.435±0.331	2.950±0.000	3.293±0.000	4.842±0.005
	Free SO^{2-}_4 (ppm)	510.50±0.000	535.71±0.000	547.16±0.000	568.93±0.000	603.30±0.000
T. brownii	TS (g/L)	3.463±0.051	1.853±0.023	4.139±0.212	5.381±0.022	6.412±0.150
	Free SO^{2-}_4 (ppm)	533.42±0.000	542.58±0.000	634.23±0.000	707.55±0.000	829.55±0.000
Acanthaceae spp.	TS (g/L)	1.852±0.110	2.371±0.012	3.990±0.0120	4.921±0.121	5.612±0.151
	Free SO^{2-}_4 (ppm)	536.85±0.000	568.93±0.000	643.39±0.000	765.97±0.000	965.89±0.000

4.5.8 Bio-slurry Phosphorus Content

Together with nitrogen and potassium, phosphorus is one of the three basic fertilizer requirements (Augusto et al., 2017; Maghanga et al., 2013). Phosphorus is abundant in kitchen waste biomass due to presence of the various proteins present (Azevedo et al., 2015). The concentrations of all three phosphorus analytes in all samples increased linearly over the retention period. Like nitrogen content, phosphorus in the bio-slurry is as a result

of degradation of proteins releasing phosphorus in various gaseous, liquid and solid forms. 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 (Ezekoye et al., 2011). The levels of various forms of phosphorus; Total Phosphorus (TP), Total Phosphoric Acid (TPA) and phosphorus as free phosphates in the three bio-slurry samples was analyzed over the retention period as summarized in table 4.33.

Table 4.33: Variation of total phosphorus, total phosphoric acid and free phosphates in bio-slurry over the retention period

Samples	**Parameter**	**Sample days**				
		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 PO^{3-}_4 (ppm)	465.35±0.000	466.00±0.000	468.52±0.000	475.94±0.000	477.43±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±0.0013	3.477±0.023
	Free PO^{3-}_4 (ppm)	465.35±0.000	467.73±0.000	468.52±0.00	476.24±0.000	477.63±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 PO^{3-}_4 (ppm)	465.40±0.000	470.99±0.000	477.43±0.000	480.94±0.000	486.14±0.000

The total phosphorus content in the control sample ($1.960 \pm 0.088 g/L$) was lower than the sample with additives (*T. brownii* ($2.520 \pm 0.250 g/L$) and *Acanthaceae spp.* ($2.420 \pm 0.648 g/L$)). This is due to reduced biomineralization rate in the control sample.

The levels of total phosphoric acid in the *T. brownii* sample ($3.477 \pm 0.023 g/L$) were much higher compared to the rest and also significantly different ($\alpha = 0.05$, $n = 2$). The levels of phosphate after 28 days retention period belonged to the same population. The phosphate levels after retention-day 28 were similar to those of bio-slurry attained using pure animal manure i.e 488mg/Kg (Kuligowski et al., 2010).

4.5.9 Bio-slurry Soluble Silicic Acid

Soluble silicic acid (orthosilicic acid) is a micro nutrient which is very important in growth and yields of most grasses (including maize, rice and wheat) (Li et al., 2019; Hosseini et al, 2018). Soluble silicic acid is usually sprayed as a foliar (Nascimento et al., 2018). The levels of soluble silicic acid in the three bio-slurry samples was monitored over the retention period as summarized in table 4.34 below.

Table 4.34: Variation in soluble silicic acid in bio-slurry over the retention period

Samples	Soluble silicic acid (mg/L)				
	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

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 retention period ($31.667 \pm 0.000 mg/L$) for control sample and ($30.001 \pm 0.000\ mg/L$) for the sample with *Acanthaceae spp*. extract. Soluble silicic acid levels in *T. brownii* sample increased linearly over the retention period up to retention day-21. This is attributed

to continuous mineralization of silica in the biogas slurry over time. Gorrepati et al., (2010) proved that the availability of silica in a solution is related to its pH value. Under acidic conditions, silica precipitates out by first polymerizing 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 the decrease in pH of the biogas slurry. The levels after retention day 28 in *T. brownii* sample (38.334 ± 2.492 mg/L) were proven to be significantly different from those of the control sample ($25.001 \pm 0.000\ mg/L$) and *Acanthaceae spp*. ($20.556 \pm 0.533\ mg/L$) ($\alpha = 0.05$, $n = 2$). Soluble silicic acid is vital in reinforcing plant cell walls, increasing tolerance to drought and heavy metals, pests and diseases (Abstract of technical papers, 2005).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Both *T. brownii* and *Acanthaceae spp.* extracts were found to have a lot of similarities in physical-chemical parameters such as pH, electrical conductivity and solid content. The two extracts had closely related FT-IR spectra. However, *Acanthaceae spp.* extracts had more bio-metal content (especially iron and cobalt). The levels of chemical constituents and anaerobic digestion parameters in the kitchen waste substrate were found to be optimal.

T. brownii and *Acanthaceae spp.* additives increased biogas quantities by significant amounts. Biogas quality (inferior calorific value) was also boosted by the use of *T. brownii* bio-catalyst. It was however observed that *Acanthaceae spp*. had very trace levels of hydrogen sulphide. The two additives also stabilized parameters necessary for more biogas output (e.g pH and VFA/ALK) and had a faster biomass degradation rate.

The levels of the plant nutrients analyzed were found to increase with retention time. The samples with additives had more plant nutrients compared to the control. *T. brownii* sample had more Total Kjehdahls 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, *Acanthaceae spp.* sample had the highest potassium, calcium, nitrates, sulfates and free phosphates levels compared to the other samples.

In general, use of the two additives successfully increased biogas and bio-slurry quantities and qualities in the 28-day retention period.

5.2 Recommendations

The study recommends the following;

i. Research on more other traditional additives to be carried out in a bid to catalyze biogas and bio-slurry production

ii. Research on effects of the two additives; *T. brownii* and *Acanthaceae spp*. in different biogas substrate and consortia to improve biogas yields and ICP values.

iii. Determination of the effects of these additives with increasing temperature regimes should be carried out i.e. exploitation of biogas and bio-slurry quantities and qualities at thermophilic regimes.

iv. Research on the exact specie of *Acanthaceae spp.* with bio-catalytic behavior. In this study, the concoction of *Acanthaceae spp.* used contained several species of *Acanthaceae.*

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