CHAPTER ONE

1.0 INTRODUCTION

1.1. Background of study

Environmental pollution by solid wastes and lack of access to adequate energy resources are some of the major challenges facing the human populace in Sub Saharan Africa (Wei *et al*., 2014; Jain *et al.*, 2015; Chirambo, 2016; Ge *et al.*, 2016; Kamp and Forn, 2016; Mengistu *et al*., 2016; Mungwe *et al.*, 2016; Wang *et al*., 2016; Zou *et al*., 2016; Abadi *et al.*, 2017; Ohimain and Izah, 2017; Roopnarain and Adeleke, 2017; Russo *et al.*, 2017; Shane *et al.*, 2017). Out of 21 Sub-Saharan African countries, less than 10% have access to energy (Mshandete and Parawira, 2009). Therefore, there is serious need to search for alternative and renewable energy sources from locally available resources in the quest for human survival and national development in Africa (Valentine *et al*., 2012; Khoufi *et al.*, 2015; Giwa *et al.*, 2017). Besides, there is a need for the adoption of appropriate and economically feasible technologies for the effective management of solid and liquid wastes and energy recovery from them (Calabro *et al.*, 2015; Yasar *et al.*, 2017).

One of the major tools for national and international development is energy. Developing countries like Nigeria depend heavily on fuels from fossil origin. Adaramola and Oyewole (2011) reported the presence of enormous conventional energy resources (crude oil, tar sands, natural gas and coal) in Nigeria besides the huge amount of renewable/sustainable energy resources including hydro, solar, wind, biomass etc. The global quest for environmentally friendly and ecologically balanced and sustainable energy has been on the increase over the last few decades and this has forced the world to search for other alternate sources of energy (Lynd *et al*., 2015; Su *et al*., 2015).

However, the new alternative energy sources demand immense economic investment and technical power to operate, and this makes it little difficult for a developing country like Nigeria. Presently, energy from biogas is a reliable, abundant, accessible and economically feasible source of alternative and renewable energy which can be generated using agricultural, domestic and industrial materials employing simple technology (Kwietniewska and Tys, 2014). The prospect of this technology is bright because Nigeria

is rich in fossil fuels and other renewables (Mshandete and Parawira, 2009). The technology can be utilized to provide energy for households, rural communities, farms, and industries (Giwa *et al*., 2017).

1.2. Rationale for Biofuel Production in Africa

The quest for renewable and sustainable energy generation is fast becoming widespread across Africa due to the understanding that there is a need to seek an alternative to fuels of fossil origin which currently sustains the world's-energy need. Research into the generation of renewable fuels had been on-going in continents like Europe, South America, Asia and other developed countries bearing in mind the extinction nature of fossil fuels. Globally, attentions are been drawn to fuel generation from biomass and their derivatives such as lignin, triglycerides, cellulose, and hemicelluloses. The aim is to use such fuels for cooking, heating, as fuels in vehicles, jet engines, and other applications. Therefore, the integration of the African continent in the race for biofuel production is germane in the quest for survival and developments considering present and favourable factors like climate, soil, land mass among other environmental-friendly resources in different African countries (Ezeonu and Ezeonu, 2016). Africa is the second largest continent in the world after Asia making up 10% of the world's population which is equivalent to about 80% of the population in India sub-continent (Amigun *et al.*, 2008). As such, biofuels especially biogas, biodiesel, and bioethanol are being considered as the most potent alternatives to fossil fuels in the continental energy mix (Adeniyi *et al.*, 2007; Ayhan, 2008).

According to Soumonni and Cozzens (2008), there are two broad processes in biofuel development and these are first, the actual production from both edible and non-edible sources and secondly, the compatible technologies for the fuel usage. Nowadays, large scale biofuel projects are gaining considerable attentions and establishment of biofuel facilities is fast becoming widespread in the continent while issues of energy security and economic growth are also being discussed in several scientific gatherings (Soumonni and Cozzens, 2008).

1.3. Statement of the Research Problem

Several thousand tons of solid wastes are generated in Nigeria annually most of which end up as pollutants in the environment without being put to any meaningful usage. The biomass used in this research are found abundantly in all the six (6) geopolitical zones of Nigeria with very little documentations for use as biofuel feedstocks. They include shoots of *Tithonia diversifolia* (Mexican Sunflower), also known as "Awolowo" in South-Western Nigeria and *Chromolaena odorata* (Siam weed), also known as "Akintola" in South-Western Nigeria. Others are fruit peels of *Carica papaya* (Pawpaw), also known as "Ibepe" in South-Western Nigeria*, Telfairia ocidentalis* (Fluted pumpkin), also known as "Ugwu" in Nigeria and the hull or pod of *Arachis hypogaea* (Peanut or Groundnut), also called "Epa" in South-Western Nigeria. Despite the huge availability of these biomass in their various locations of production, they mostly end up as solid wastes in the environment as little or no usage has been sought for them over the years. Even when some of the biomass has been experimented on for biofuel production, the various arrays of microorganisms involved in their biodegradation are yet to be documented in biofuel literature. With the past and anticipated energy challenges earlier alluded to and the nation's overdependence on fossil fuels, these biomass need be examined and for their energy producing potentials.

1.4. Aim and Objectives of the Study

This study is aimed at generating biogas and biofertilizer from the mono and co-digestion of five locally available biomass listed in section 1.3 with poultry dropping as co-substrate while using a consortium of microorganisms from cattle's rumen content and optimisation for large scale production.

The specific objectives of this research are to:

i. perform a detailed investigation of the biogas production potentials of the five biomass in both mono and co-digestions
ii. characterize the substrates before and after digestion as well as the microbial consortium in both the rumen content and poultry droppings to genus level

iii. study the microbial succession in the mono and co-digestions regimes of the selected biomass

iv. carry out both microbial and statistical optimisation studies on biogas generated data using combinations of the microbial isolates from the research and also using Response Surface Methodology (RSM) and Artificial Neural Networks (ANN) in order to determine the most accurate and precise software for the optimisation study

v. evaluate the microbial and nutritive content of each digestate in all the digestion regimes before use as biofertilizers and subsequent field experiment with newly produced biofertilizers.

1.5. Justification of Study

Inadequate energy supply, environmental pollution and loss of soil fertility are some of the challenges being faced in Nigeria and other developing nations, especially in Africa. The energy consumption rate of the modern world is an indication that renewable and environmental-friendly energy need be generated from alternative sources. The mono digestion of substrates has been found to be limited in both quantity and quality of generated gas while co-digestion of substrates enhance the anaerobic digestion process as this leads to higher carbon/nitrogen balance and nutrient availability. Biofuel research in Nigeria is in its infancy as limited substrates have been utilized and significant effort has not been directed at evaluating the composition and/or succession of the microbes responsible for the bioconversions (Akinbami *et al.*, 1996). Most of the previous biogas researches utilized animal dung, poultry droppings, banana peels, human excreta, agricultural residues and kitchen wastes as feedstock substrates (Akinbami *et al.*, 1996, 2001; Okagbue, 1988; Ubalua, 2008; Alfa *et al.*, 2012; Adepoju *et al.*, 2016; Ibrahim and Imrana, 2016; Idire *et al.*, 2016). The use of succulent plants for biogas production has been limited to water lettuce, water hyacinth, cassava leaves, *Cymbopogon citrates* and *Eupatorium odoratum* (Odeyemi, 1981; Okagbue, 1988; Akinbami *et al.*, 1996; Akinbami*et al.*, 2001; Ilori *et al.*, 2007; Ubalua, 2008). Detail analysis of lignocellulosic component and optimization of biogas production processes and parameters are lacking in

the Nigerian energy literature and has therefore been addressed in this research.

1.6. Scope of Research

This research employed the use of shoots of *Tithonia diversifolia* (Mexican Sunflower), *Chromolaena odorata* (Siam weed), fruit peels of *Carica papaya* (Pawpaw), *Telfairia ocidentalis* (Fluted pumpkin), and the hull or pod of *Arachis hypogaea* (Peanut or Groundnut). Their digestions were carried out anaerobically using the chain digesters that were fabricated. The characterization of the microbial consortia in the feedstock and biofertilizers were carried out using basic morphological and biochemical parameters and presumptive isolates were confirmed using rapid API kits. After this, the experimental aspect of the research was carried out in the Teaching and Research Farms, Microbiology and Environmental Engineering Laboratories of Landmark University, Omu Aran, Kwara State while the microbiological procedures were carried out at the Laboratory of Microbiology, Covenant University, Ota, Ogun State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Biogas Development in Africa

Biogas generation via anaerobic digestion is very famous in the Americas, Asia, Europe and India Sub-Continent. However, the Sub-Saharan Africa region has over the last few decades witnessed a very slow acceptance and adoption of this technology despite significant individual, institutional, national and international efforts (Lynd *et al*., 2015). This slow pace of development has been linked to scarcity or unavailability of feedstock caused by poor agricultural practices (United States Department of Agriculture, 2008). Table 2.1 shows that as at 2005, only a few African countries have adopted the biogas technology with an insignificant number of biogas digesters/plants compared to what is obtainable in other continents (Mshandete and Parawira, 2009). In order to improve this situation, a new African initiative was launched in 2007 in order to install biogas digesters to not less than 2 million households by the year 2020 (van-Nes and Nhete, 2007; Ukpabi 2008). By the year 2010, the number of biogas plants in Africa has increased especially in Tanzania with about 4,000 digester units (Ocwieja, 2010). However, only about 60 % of these plants were functional while the remaining failed or performed below satisfaction due to reasons like planning and construction errors, poor community awareness, lack of adequate maintenance culture, misconception of the technology's benefits, and lack of technical know-how by end-users among others (Ocwieja, 2010).

Table 2.1: African Countries with Biogas Producing Digesters

Country	Number of small/medium digesters (100 m^3)	Number of large digesters (>100 m^3)	Region
Botswana	>100	1	South
Burkina Faso	>30	-	West
Burundi	>279	-	East
Egypt	>100	<100	North
Ethiopia	>100	>1	East
Ghana	>100	-	West
Cote D'Ivoire	>100	1	West
Kenya	>500	-	East
Lesotho	40	-	South
Malawi	-	1	South
Morocco	>100	-	North
Nigeria	Few	-	West
Rwanda	>100	>100	East
Senegal	>100	-	West
Sudan	>200	-	North
South Africa	>100	>100	South
Swaziland	>100	-	South
Tanzania	>1000	1	East
Tunisia	>40	-	North
Uganda	Few	-	East
Zambia	Few	-	East
Zimbabwe	>100	1	South

Source: Mshandete and Parawira (2009)

2.2. Biogas Development in Nigeria

Biogas technology's adoption and operation in Nigeria is still at the infancy stage. This slow pace which is similar to the situation in some other Sub-Saharan African countries is caused by unfavorable government policies, inadequate funding of technology and individual's unwillingness (Sokoto Energy Research Centre Information Brochure, 2004). To this end, several feedstocks which are economically suitable for biogas generation in Nigeria have been selectively identified. These include aquatic plants like water lettuce and water hyacinth; agricultural wastes like cow and piggery dung, poultry droppings, cassava leaves and processing waste; industrial wastes like municipal solid wastes and sewage (Okagbue, 1988; Akinbami *et al*., 1996, 2001). Also, the continuous assessment of other locally available materials for their use in biogas production has been made (Ubalua, 2008). The use of succulent plants has been limited to water lettuce, water hyacinth, cassava leaves, *Eupatorium odoratum* and *Cymbopogon citratus* (Odeyemi, 1983; Alfa *et al*., 2012). Similarly, the potential of poultry droppings, cow dung and kitchen/food wastes for biogas generation has been experimented upon (Lawal *et al*.,1995; Ojolo *et al*., 2007)

Acid formers previously isolated from biogas digesters include species of *Escherichia, Citrobacter, Bacillus, Pseudomonas, Klebsiella, Clostridium, Bacteroides, Salmonella, Aspergillus, Mucor, Rhizopus*, and *Penicillium* while methane formers previously implicated includes species of *Methanococcus, Methanosarcinae etc* (Alfa *et al*., 2014a). The author also reported that a correct balance must be reached between these groups of microorganisms in order to achieve success in the anaerobic operation.

2.3. Suitable Feedstock for Biogas Generation

One of the major steps in achieving anaerobic digestion success is the careful selection and identification of viable feedstocks. The world over, several feedstocks have been utilized including food wastes, animal dungs, agricultural and plant residues, wastewaters, Organic Fraction of Municipal Solid Wastes (OFMSW), energy crops etc. In Nigeria, substrates suitable for anaerobic digestion include aquatic plants such as water lettuce and water hyacinth; agricultural wastes/residues such as cow and piggery dung, *Cymbopogon citratus*, cassava leaves; municipal wastes such as human excreta, processing wastes,

urban refuse and industrial wastes (Akinbami *et al*., 2001; Okagbue, 1988; Ubalua, 2008; Alfa *et al*., 2012; Dahunsi and Oranusi, 2013). A whole lot of other locally available materials in Nigeria have been evaluated for their potentials for biogas generation (Odeyemi, 1983). Among these, the potentials of poultry manure, cow dung and kitchen wastes for biogas production have been demonstrated (Matthew, 1982; Akinluyi and Odeyemi, 1986; Abubakar, 1990; Lawal *et al*., 1995; Zuru *et al*., 1998; Ojolo *et al*., 2007).

Similarly, Ilori *et al*. (2007) demonstrated the biogas generation from the co-digestion of the peels of banana and plantain and obtained the highest gas volume with an equal mass of both substrates. In another study, the co-digestion of pig waste and cassava peels seeded with wood ash produced a significant increase in biogas yield when compared with the unseeded mixture of the substrates (Adeyanju, 2008). Fariku and Kidah (2008) have also reported the efficient generation of biogas from the anaerobic digestion of *Lophira lanceolata* fruit shells. The biogas producing potentials of Nigerian local algal biomass has been recognized by Weerasinghe and Naqvi (1983). Odeyemi (1981) in his comparative study of four substrates (*Eupatorium odoratum*, water lettuce, water hyacinth and cow dung) as potential substrates for biogas production concluded that *Eupatorium odoratum* was the best while cow dung was the poorest substrate in terms of gas yield. Ahmadu (2009) compared the biogas production from cow dung and chicken droppings while Igboro (2011) compared the biogas from cow dung from an abattoir and the National Animal Production Institute, Zaria, with the abattoir waste generating the highest volume of gas. Igboro *et al*. (2011) also designed a biogas stove burner which was effectively tested with the biogas produced from cow dung and other feed materials.

2.4. Anaerobic Digestion (AD)

Anaerobic digestion is a biological process that converts complex substrates into biogas and digestate by microbial action in the absence of oxygen through four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Roopnarain and Adeleke, 2017). Anaerobic digestion (AD) is one of the oldest and well-studied technologies for stabilizing organic wastes (Su *et al*., 2015; Cuetos *et al*., 2017; Shane *et al*., 2017). Among the treatment technologies available for treating organic solid wastes,

AD is very suitable because of its environmental friendliness (Hashisho *et al*., 2016; Yap *et al*., 2016) and high potential for energy recovery (Alfa *et al*., 2014a, b; Scano *et al*., 2014; Leite *et al*., 2016). Such positive aspects coupled with the recent concerns about rapid population growth, increasing energy demand, and global warming has promoted further research on the AD process development and improvement in order to enhance biogas production, achieve faster degradation rates and reduce the amount of final residue to be disposed (Liu *et al*., 2015; Zahedi *et al*., 2016; Zou *et al*., 2016).

Most researchers have reported the hydrolysis stage to be the rate-limiting step for complex organic substrates due to the formation of complex heterocyclic compounds or non-desirable volatile fatty acids (VFA) during this step (Ferrer *et al*., 2008; Vavilin *et al*., 2008; Fernandez *et al*., 2009; Romano *et al*., 2009; Appels *et al*., 2010; Rafique *et al*., 2010; Izumi *et al*., 2010; Bordeleau and Droste, 2011; Fdez-Guelfo *et al*., 2011; Khalid *et al*., 2011; Ma *et al*., 2011; Raposo *et al*., 2011; Elliot and Mahmood, 2012; Gonzalez-Fernandez *et al*., 2012). However, methanogenesis has been documented to be the rate-limiting step for easily biodegradable substrates (Skiadas *et al*., 2005; Lu *et al*., 2008).

2.5. Classification of Anaerobic Digestion (AD) System

Anaerobic digestion systems are generally categorized into liquid AD (L-AD) and solid state AD (SS-AD) based on the total solids (TS) content of the medium (Kwietniewska and Tys, 2014). Anaerobic digestion systems operating at a TS content of more than 15% are known as SS-AD; otherwise, it belongs to the L-AD category (Rapport *et al*., 2008). Both L-AD and SS-AD have been extensively studied for methane production (Zheng *et al*., 2009; Li *et al*., 2011; Liew *et al*., 2011). L-AD systems are known to have higher reaction rates and shorter retention times while SS-ADs are advantageous in the treatment of lignocelluloses since it is characterized by smaller digester volume, lower energy requirements for heating and reduced material handling (Guendouz *et al*., 2008). As a result of the low moisture content of SS-AD, the resulting digestate is usually applied as soil biofertilizer or processed into pellets for easy use as fuel and thus provide for better handling than the digestate of liquid AD (Zhang *et al*., 2014; Jain *et al*., 2015).

2.6. Accelerants/Additives for AD

The production of biogas can be increased or sped up by using different approaches which include the addition of biological and/or chemical additives generally known as AD accelerants. These accelerants provide suitable surfaces for adsorption of the substrate and this result into high substrate concentration.The effects of this are favorable conditions for microbial proliferation and subsequent higher yield of biogas (Mao *et al.,* 2015).

2.6.1. Green Biomass

These comprise of different extracts of plants, leaves, and shoots of succulent plants, weeds, residues of crops among others found in the environment.These are often added to the AD system in order to enhance digester performance/stability as well as increasing biogas yield. Some plant materials have been documented to contain stimulants and which is capable of acting as accelerants for microbial metabolism (Giuliano *et al*., 2013). Powdered leaves of some plants and legumes (such as *Gulmohar, Leucacena leucocephala, Acacia auriculiformis, Dalbergia sisoo*, *Cymbopogon citratus* and *Eucalyptus tereticonius*) have been found to stimulate biogas production (Pang*et al*., 2008; Alfa *et al*., 2012; 2013a). The contribution of other additives such as *Pennisetum purpureum* and *Azadirachta indica* has been extensively reported (Yen and Brune, 2007; Romano and Zhang, 2008; Pang *et al*., 2008; Zhu and Li 2009; Astals *et al*., 2012).

2.6.2. Biological Additives

a. **Fungi**: Lignin-attacking fungi have found lots of useful applications in the pretreatment of lignocellulosic biomass for improved biogas production. Several classes of fungi have been used in pretreatment with white-rot fungi proving to be most effective via its secretion of lignin-degrading enzymes e.g., peroxidases and laccase and this has greatly increase methane yield (Cuetos *et al*., 2010; Alfa *et al*., 2012; Chiu *et al*., 2013; Vivekanand *et al*., 2013).

b. **Microbial Consortia**: Studies have shown that microbial consortia (usually comprising of cellulolytic bacteria, fungi, yeast etc) can bring about the solubilization of the cellulose and hemicellulose components of lignocellulosic biomass. Previous studies have reported

an increase in yield of methane via the use of microbial consortia (Rai, 2011; Mao *et al.,* 2015). Similarly, the addition of specific microbes with enzymes and/or yeasts has been demonstrated to increase biogas yield than the use of either of them (Pang *et al*., 2008; Abu-Dahrieha *et al*., 2011). However, the major challenge with the use of microbial agents as AD accelerants are the extensive process of their purification and the creation of a suitable environment for their proliferation and these investments are not cost effective thereby militating against its popularity and application (Zou *et al*., 2016).

c. Enzymes: These are chemicals usually obtained from different biological sources and are known to speed up biochemical reactions via their catalytic activities and as such are fundamental in substrate degradation by bacteria as well as fungi. Some of the commonly available enzymes in the AD system are cellulase and hemicellulase (Lu *et al*., 2007). Also, common exoenzymes e.g proteases, lipases, and chitinases have shown slightly good activities but only slightly enhanced biogas production (Jain *et al*., 2015; Mao *et al.,* 2015). Also, enzymes are costly and have, therefore, found limited application in the pretreatment of substrates for biogas generation.

2.6.3. Chemical Additives

These are predominantly used for the pretreatment of lignocellulosic biomass due to their effectiveness and low cost. The mode of action of these chemical reagents that in turn make lignocellulosic biomass more amenable to biodegradation by anaerobic microbes includes an increase in the surface area, removal of lignin and hemicellulose among others (Jain *et al*., 2015).

a. Alkali Reagents: Several alkalis are used as additives for the AD process out of which sodium hydroxide (NaOH) and potassium hydroxide (KOH) are the most efficient for biogas production improvement (Jain *et al*., 2015; Li *et al*., 2015). Alkalis act on their substrates by breaking the links between lignin, cellulose, and hemicelluloses and this usually results in the solubilization of these structural materials. This subsequently increases the substrate's surface area making it malleable to microbial attack. Increase in cellulose and hemicellulose degradation has been observed with increased sodium

hydroxide concentration and this led to higher methane production (Gerber, 2010; Jain *et al*., 2015).

b. Acid Reagents: Acid reagents are highly applicable in the pretreatment of lignocellulosic materials. These treatments are known to cause the disruption of chemical bonds which then leads to the solubilization of cellulose and hemicellulose and their subsequent hydrolysis into monosaccharide (Jain *et al*., 2015). The treatment is also suitable for hydrolytic microbes that thrive better at acidic pH. However, factors such as the enormous loss of fermentable sugars during the degradation of complex substrates, high costs of acids and the need to neutralize the acidic conditions before the AD process has made acidic treatment of little usage (Zirkler *et al*., 2014; Mao *et al*., 2015).

c. Oxidative Reagents: Oxygen speed up the reaction rate and free radicals production in feedstock prior to pretreatment. Hydrogen peroxide has also been implicated as a strong oxidation agent (Lu *et al*., 2007; Carrere *et al*., 2010). Another important oxidative agent which has found useful application during biomass pretreatment (Ozonolysis) is ozone and this often results in lignin degradation (Mao *et al*., 2015).

d. Inorganic Salts: Different inorganic salts, especially iron salts, have been applied to the AD systems and the resultant increases in the yield of methane (Skiadas *et al*., 2005). Methanogenesis has been observed to have been enhanced by adding salts like $MFeSO_4$ $FeCl_2$, and $FeCl_3$ to the digestion system (Esposito *et al*., 2011; Elliot and Mahmood, 2012; Jain *et al*., 2015). However, the disadvantage of this method is the difficulty in recovering the used chemicals and this eventually contribute to the menace of environmental pollution (Carrere *et al*., 2010; Mao *et al.,* 2015).

e. Macronutrients and Trace Elements: The inclusion of macro nutrients and trace elements as accelerants help the AD process to progress adequately and enhance better biogas production because of their environmental friendliness. This accelerating effect has been demonstrated in the bioconversion of various animal wastes, some energy crops, farm residues and municipal solid waste which were devoid of these elements prior to digestion. These trace elements are equally needed by microorganisms because they serve as building blocks for growth, supports for enzyme activities, chemical reactions and as

co-precipitates during the AD process (Elliot and Mahmood, 2007). Iron is known to reacts with hydrogen sulphide to form Iron sulphide; therefore, inclusion of certain amount of iron is useful in the release of corrosion in compressors and the reduction in hydrogen sulphide toxicity in biogas beside the stabilization of food waste AD process as reported (Elliot and Mahmood, 2007). Nickel addition is also effective in biogas production. In a batch mono-digestion of cattle dung, nickel addition improved both biogas yield enhancement and the methane content while addition of calcium and magnesium salts as energy supplements enhances methane production (Bougrier *et al*., 2006). Se and Co addition is an integral process in food waste digestion which ensures digester stability and operation at high ammonia concentrations (Zhu *et al*., 2009; Subramani and Ponkumar, 2012). The significant interaction effects produced by the addition of trace elements have considerably increased the smooth running of anaerobic digestion as well as improvement of methane content of biogas (Zhang and Banks, 2013). Macronutrient's requirements are also determined based on bacterial composition, growth yields and biomass composition. The nutrient ratio for carbon, nitrogen, phosphorus and sulphur is usually 600:15:5:1 respectively (De-Baere, 2008) while the optimum C:N:P ratio for methane production enhancement 200:5:1 respectively (Hansen *et al*., 2007). Carbon needed for biological activities is usually obtained from the substrate and is in turn used for the fortification of the structure of a microbial cell. Nitrogen is required for protein synthesis while sulfur is needed as amino acids constituent and also equally as an essential growth nutrient for methanogens (Jain *et al*., 2015; Mao *et al.,* 2015).

2.7. Anaerobic Co-Digestion

Co-digestion of substrates such as dairy manure, fats, oils, and grease (Lansing *et al*., 2010), slaughterhouse waste (Alvarez and Liden, 2008), human excreta (Dahunsi and Oranusi 2013), lemon grass (Alfa *et al*., 2014a), or energy crops (Amon *et al*., 2007; El-Mashad and Zhang, 2007; Lansing *et al*., 2010; Adanikin *et al*., 2017) resulting in higher biogas yield have been established, thus increasing the feasibility of AD technology especially for small to mid-sized dairy farmers (Klavon *et al*., 2013). Energy crop digestion is increasingly utilized due to the higher methane (CH_4) yield as against the backdrop of animal manure (Al-Seadi *et al*., 2008; Lansing *et al*., 2010). The most

commonly used energy crops are maize (Braun *et al*., 2009; Bruni *et al*., 2010), switch grass (Masse *et al*., 2010), sugar beets (Umetsu *et al*., 2006), sunflower grass and Sudan grass (Amon *et al*., 2007).

2.8. Properties of Lignocellulosic Biomass

Lignocellulosic biomass such as agricultural residues, green grass and energy crops is an abundant organic resource and large quantities of lignocellulosic materials accrued from different sources including agricultural, forestry, municipal, and other activities (Jain *et al*., 2015). Three major polymers i.e. cellulose, hemicellulose, and lignin make up lignocellulosic biomass of which the first two (known as carbohydrate components) can be microbiologically fermented post hydrolysis.This makes these biomass suitable feedstocks for biofuel production. The militating issue against the usage of lignocellulosic biomass however is the nature of these structural materials especially cellulose which makes them highly recalcitrant to microbial and enzymatic degradations (Atalla and Vanderhart, 1984; Ha *et al*., 1998).

In contrast to cellulose, hemicelluloses are more amorphous, random, and branched heterogenic polysaccharides of various pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose, and/or rhamnose), and acids (glucuronic acid, methyl glucuronic acid, and galacturonic acid). Short and branched chains of hemicelluloses help build a network with cellulose microfibrils and interact with lignin, rendering the cellulose-hemicellulose-lignin matrix extremely rigid. The amorphous and branched properties make hemicelluloses highly susceptible to biological, thermal, and chemical hydrolysis of their monomer compounds (Morohoshi 1991; Ademark *et al*., 1998). Moisture content, pH, and temperature are critical parameters in the thermo-chemical hydrolysis of hemicellulose (Bobleter, 1994; Fengel and Wegener, 1984; Garotte *et al*., 1999).

After cellulose, lignin is the second most abundant organic compound in nature. It is a large and complex aromatic and hydrophobic amorphous heteropolymer and is composed of phenylpropane units such as coniferyl alcohol and sinapyl alcohol with hydroxyl, methoxyl, and carbonyl functional groups. Lignin plays the role of cement for the crosslinking between cellulose and hemicellulose to form a rigid three-dimensional structure of the cell wall (Palmqvist and Hahn-Hägerdal, 2000). It is also water insoluble

and optically inert. Lignin has been shown to dissolve in water at high temperature (e.g. 180^{o} C), neutral pH, or acid/alkaline conditions depending on the precursors of the lignin (Grabber, 2005). These properties of lignin make it the most recalcitrant component of the plant cell wall, and the higher the lignin content, the greater the resistance of the biomass to chemical and biological degradation. Lignin is a major barrier to utilization of lignocellulosic biomass in bioconversion processes. In general, softwood contains more lignin than hardwood and most agricultural residues, so that softwood is generally the most recalcitrant to pretreatment and bioconversion.

2.9. Pretreatment to Improve the Digestibility of Lignocellulosic Biomass

The complexity of lignocellulosic biomass chemical structures ultimately determines the appropriate pretreatment method to be applied (Kim and Holtzapple, 2005, 2006). The pretreatment of feedstock for anaerobic digestion involves:

i. Removing the non-biodegradable materials, which are not affected by digestion and take up unnecessary space;
ii. Providing a uniform small particle size feedstock for efficient digestion;
iii. Protecting the downstream plant from components that may cause physical damage and remove materials which may decrease the quality of the digestate (Monnet, 2003)
iv. Disrupting such properties in order to improve the biomass response to the microbial and enzymatic attack.

Extensive research has been conducted on pretreatment methods to accelerate the hydrolysis step and to obtain suitable by-products as well as to improve the quality of useful components like nitrogen and phosphorus to be recycled (Carlsson *et al.,* 2012). The European Union Regulation EC1772/2002 has stipulated that substrates such as solid wastes, food waste, and slaughterhouse wastes require pasteurization or sterilization before and/or after digestion as a way of reducing the pathogen load. Following this regulation, application of pretreatment methods is an ideal and economical alternative to pasteurization and/or sterilization thereby obtaining a higher energy yield (Eggeman and Elander, 2005; Hendriks and Zeeman, 2009). However, pretreatment methods can be

unsustainable and environmental unfriendly despite the enhancement of bioconversion process and digester performance (Carball*o et al*., 2011). Different pretreatment methods impart different effects and this is also a function of the characteristics of the substrates in use hence, the systematic assessment and comparison of the applicability and sustainability of such methods at a full scale are pretty difficult. The following pretreatment methods are common:

2.9.1. Mechanical Pretreatment

These methods are usually used for increasing the specific surface area of substrates by disintegrating and/or grinding the solid particles of the substrates and releasing cell compounds in the long run. The advantage of an increased surface area is the provision of improved interaction between the substrate and the anaerobic bacteria, leading to better bioconversion (Carrere *et al*., 2010; Elliot and Mahmood, 2012). It has been reported that the size of particle plays a vital role in the maximum rate of substrate utilization by anaerobic organisms as well as enhancement of higher chemical oxygen demand degradation (Esposito *et al*., 2011). Mechanical pretreatment methods such as sonication, lysis-centrifuge, liquid shear, collision, a high-pressure homogenizer, maceration, and liquefaction are therefore employed for the reduction of substrate particle size.

Beside size reduction, other effects are obtained through the use of some mechanical methods. For example, maceration electroporation and liquefaction have been reported to have a better effect due to shearing than cutting of fibers (Hartmann *et al*., 2000; Shepherd, 2006; Carlsson and Kaldnes, 2008). Similarly, pretreatment by sonication i.e. use of a vibrating probe was reported to achieve mechanical disruption of the cell structure and floc matrix (Chua *et al*., 2002; Bougrier *et al*., 2006; Elliot and Mahmood, 2007). Mata-Alvarez *et al*., 2000 and Barjenbruch and Kopplow, 2003 found that a high-pressure homogenizer (HPH) increased the pressure up to several hundred bars and then homogenizes substrates under strong depressurization and the formed cavitation induces internal energy, which disrupts the cell membranes.

Mechanical pretreatment methods are not popular with all substrates. Lignocellulosic materials, manure, and waste water treatment plant sludge are some of the substrates to which these methods have been applied. Some of the benefits of mechanical pretreatment

include odour removal, easy application/implementation, efficient dewaterability of the final digestate and reasonable energy consumption while the disadvantages include the inability to remove/reduce pathogen load and scaling or clogging of equipment (Perez-Elvira *et al.*, 2006; Toreci *et al*., 2009).

2.9.2. Thermal Pretreatment

Thermal treatment of substrates is well studied and its application has been successfully carried out on a large scale (Carrere *et al*., 2010; Carlsson *et al*., 2012; Cesaro and Belgiorno, 2014; Serrano *et al*., 2017). This pretreatment type is effective in the disintegration of cell membranes thus leading to organic compounds solubilization which is achieved at higher temperature or at lower temperature, but longer treatment times (Marin *et al*., 2010; Protot *et al*., 2011) Some of its advantages are pathogen removal/reduction, improvement of dewatering performance and digestate viscosity reduction leading to better handling of digestate (Carlsson *et al*., 2012; Liu *et al*., 2012). Different thermal pretreatment procedures have been compared and no significant difference was found between the use of steam and electric heating, whereas higher biopolymer solubilization was obtained via the use of microwave heating (Mottet *et al*., 2009; Toreci *et al*., 2009; Marin *et al*., 2010). Various temperatures range (50–250^{o} C) has been applied to enhance the digestion of different substrates mainly lignocellulosic substrates. Temperatures above 160^{o} C were reported to cause not only the solubilization of hemicellulose but also solubilization of lignin component of lignocellulosic biomass in which the released compounds are mostly phenolic compounds serving as inhibitors to anaerobic microbes (Hendriks and Zeeman, 2009). This submission has earlier been made by Bougrier *et al*. (2006) who reported the high possibility of a chemical bond formation with the application of temperatures above 170^{o} C. A pronounced scenario is the Maillard reaction, occurring between carbohydrate monomers and amino acids, resulting in the formation of complex substrates that eventually retard the digestion process.

Mallaird reaction usually occurs at extreme thermal treatment temperatures above 150^{o} C, or lower temperatures (<100^{o} C) for a longer treatment (Carrere *et al*., 2010; Elliot and Mahmood, 2012). Besides the formation of complex chemical reactions, thermal

pretreatment can equally lead to loss of volatile organic acids and/or potential biomethane production from easily biodegradable biomass. The success of thermal pretreatment, therefore, depends on the substrate used as well as the temperature range applied.

a. Thermal Pretreatment at Lower Temperatures (<110^{o} C)

Numerous studies with thermal pretreatment below 100^{o} C failed to achieve the breakdown of structural molecules (Protot *et al*., 2011), whereas Skiadas *et al*. (2005) reported enormous pathogen removal by pretreating sludge at a lower temperature (70^{o} C) had a decisive effect on pathogen removal. These results probably led to the EU Regulation EC1772/2002 requiring solid wastes to be pretreated at least an hour at 70^{o} C and the aftermath of this was increased studies on thermal pretreatment at 70^{o} C in different parts of the world (Chamchoi *et al*., 2011; Gonzalez-Fernandez *et al*., 2012). Some of such research yielded higher biogas production while others did not e.g. Appels *et al*. (2010) obtained a slight biogas production improvement from sludge pretreated at 70^{o}C for 60 min, whereas the yield was increased 20 times when a 60 min pretreatment at 90^{o}C was applied. Other researchers (Climent *et al*., 2007; Ferrer *et al*., 2008; Rafique *et al*., 2010) achieved a maximal enhancement of between 30% and 78% biogas production by applying pretreatment at 70^{o} C especially for mesophilic while some failed in thermophilic AD (Raposo *et al*., 2011).

b. Thermal Pretreatment at Higher Temperature (>110^{o} C)

Thermal pretreatment of food waste and combination of fruit and vegetable wastes have been extensively studied at 175^{o} C with a 7.9% and 11.7% decrease in the methane production, respectively, as a result of melanoidin formation (Liu *et al*., 2012). A similar result was obtained by Rafique *et al*. (2010) when they studied pretreatment of pig manure at temperatures higher than 110^{o} C characterized with low gas yield. In their case, hardening and the dark brownish coloration of the substrate were observed indicative of Mallaird reactions. In another study, Ma *et al*. (2011) obtained a 24% methane production increase from food waste pretreated at 120^{o} C.

2.9.3. Chemical Pretreatment

This method is used for destroying organic compounds formed as a result of chemical reactions. Alkali pretreatment has been tagged to be the chemical treatment of choice in most studies because the AD system usually requires a pH adjustment by increasing alkalinity (Li *et al*., 2012). The use of acidic pretreatments and oxidative methods such as ozonation are also popular in the enhancement of biogas production and improvement of hydrolysis. The substrate composition and the applied pretreatment method usually determine the success of chemical pretreatment and this explains why the method is not suitable for easily degradable biomass with high carbohydrate composition because of their higher degradation rate and VFA accumulation which cause poor performance of the methanogenesis step (Wang *et al.,* 2011). It is however ideal for lignin-containing biomass (Fernandez *et al*., 2009).

a. Alkali Pretreatment

Solvation and saponification are the first sets of reactions that occur during alkali pretreatment of substrates and result is the swelling of solids and the increase in specific surface making substrates easily accessible to anaerobic digestion (Carlsson *et al*., 2012). These are then followed by COD solubilization via various simultaneous reactions such as saponification of uronic acids and acetyl esters, as well as neutralization of various acids formed by the breakdown of the particles (Modenbach and Nokes, 2012). However, the fact that the biomass itself consumes some of the alkali during alkali treatment leads to the requirement of much alkali for desired AD performance (Hendriks and Zeeman, 2009).

b. Acid Pretreatment

The application of acid pretreatment for lignocellulosic substrates is highly efficient, because of its ability to condense and precipitate the lignin component, hydrolyze hemicellulose into monosaccharides and the provision of a desirable environment for hydrolytic microbes (Hendriks and Zeeman, 2009; Mussoline *et al*., 2012). Disadvantages of acid pretreatment include the production of inhibitors, loss of fermentable sugars due to pronounced degradation of complex substrates, high cost of acids and alkali used for neutralization after acidic treatment (Taherzadeh and Karimi, 2008; Kumar and Murthy,

2011; Modenbach and Nokes, 2012). These factors have led to the avoidance of strong acidic pretreatment and the embracement of the combination of dilute acids with thermal pretreatment methods (Modenbach and Nokes, 2012).

2.9.4. Biological Pretreatment

This comprises the use of anaerobic and aerobic organisms and enzymes such as peptidase, carbohydrolase, and lipase. These methods are only applicable to few substrates such as organic solid wastes, waste water treatment sludge and pulp and paper wastes. Usually, some researchers regard the hydrolytic-acidogenic stage of a two-phase AD process as a biological pretreatment (Carrere *et al*., 2010; Ge *et al*., 2010), while others do not see it as a pretreatment method (Carlsson *et al.,* 2012). Also, more specific enzymes can be produced by acidogenic microbes through the optimization of the hydrolysis stage of AD.Such enzymes usually function in substrate breakdown (Parawira *et al.,* 2005). Separation of the acidogens from the methanogens also resulted in a higher methane yield and efficient COD removal over a shorter hydraulic retention time (Hartmann and Ahring, 2006).

Composting and other aerobic pretreatments of complex substrates prior to digestion have been considered an effective way of facilitating hydrolysis of complex substrates due to higher specific microbial growth and enzyme production during composting (Fdez-Guelfo *et al*., 2011; Lim and Wang, 2013). Various other researchers have reported the enhancement of anaerobic digestion, higher methane yield (80 to 90% increase) and COD removal efficiency (99.5%) via the use of aerobic pretreatment using microorganisms like *Geobacillus thermodenitrificans, Trametes pubescens,* and *Trichoderma reseei* (Melamane *et al*., 2007; Muthangya *et al*., 2009). However, other studies reported loss of volatile solids with no significant biogas enhancement by pretreating solid wastes aerobically prior to anaerobic digestion (Miah *et al*., 2005).

2.9.5. Combination of Various Pretreatments

a. Thermo-Chemical Pretreatment

Combination of pretreatment methods has been extensively studied as a way of obtaining biogas production enhancement and this because the various pretreatment methods rely on

different mechanisms to solubilize particulate organic matter before digestion (Valo *et al*., 2004). The combination of thermal (>110^{o} C) and chemical (hydrogen peroxide and lime) treatment failed for organic solid wastes with lower biogas generation due to the reactions between the amino acids and sugars forming melanoidins (Rafique *et al*., 2010; Shahriari *et al*., 2012). The use of alkaline alongside thermal treatment (70^{o} C) however resulted in a higher biogas and methane content (78% and 60% respectively) due to the reduction of the hemicellulosic component of the substrate (Rafique *et al*., 2010).

b. Thermo-Mechanical Pretreatment

The combination of mechanical and thermal pretreatments has also been studied for the enhancement the AD process. Report from researchers (Zhan *et al*., 2005; Wett *et al*., 2010; Elliot and Mahmood, 2012) established the enhancement of biogas production via a combination of grinding and ultrasonic pretreatment. Dewatering characteristics of the formed digestates were also improved reducing the cost of disposal.

2.10. Classification of Anaerobic Digesters

The biogas digestion system usually consists digestion tank inside of which microorganisms converts substrates fed into the tank into biogas under anaerobic condition.The tanks are usually furnished with two openings i.e. an inlet through which the materials to be digested is introduced and an outlet via which the remaining digestate is then removed (Ocwieja, 2010). Efficiency and stability of anaerobic digestion vary based on the digester type as well as the operating parameters being considered (Ostream, 2004).While there are very simple and easily operated but less efficient digesters, there is the complex and fully automated ones which are mostly industrial in nature and are designed to automatically detect slight environmental changes (Ocwieja, 2010).

In the design of digesters, therefore, considerations such as capacity, the orientation which can be vertical or horizontal, batch or continuous flow total solids content, number of stages, substrate mixing, type of pretreatment etc. Also, Jain *et al*. (2015) reported that most digesters are designed for process optimisation in order to fit for factors like geographical location of use and types of waste to be digested.

2.11. Capacity of Anaerobic Digesters

The capacity of a digester is usually determined by the availability and accessibility of raw materials. In this respect, farms, cities and commercial centers are the most feasible sites of construction.A good example is The Friesland plant in the Netherlands with a capacity of 230,000 metric tons per annum (Ostream, 2004). For the effective management of municipal solid wastes (MSW), in the developed nations, the smallest economic digester is about 50,000 tons per year (Igboro, 2011).

2.12. Basic Considerations for Digester Construction

The following criteria among many others are usually considered for the construction of an ideal anaerobic digester/plant: simplicity of construction and operation, cost effectiveness, and durability, efficiency of gas production per quantity of feedstock, construction with locally available materials and minimal requirement for repair and maintenance

2.13. Siting of Biogas Digester

In order to ensure the sustainability of a biogas installation, efforts must be made to select the best site for the plant. The factors that should be considered (Rai, 2011) are:

i. Distance between the proposed site and the location of gas consumption,

ii. Distance between the site and the raw material source,

iii. Distance between the site and effluent/digestate storage facility,

iv. Distance between the site and water sources in order to prevent water contamination,

v. Distance between the site and trees/bamboos in order to prevent damage to the facility caused by the roots of the plants,

vi. Ground water depth should be investigated. Construction will be relatively easy at locations where the ground water table is low.

vii. The ultimate bearing pressure of the foundation should be adequate to support the load of the biogas plant and the slurry inside.

viii. The direction of the prevailing wind should be considered so that the smell from the biogas plant will not be a nuisance to residential areas.

2.14. Design Theories of Anaerobic Digesters

Various models of anaerobic digester plants have been developed. There are three popular practical models of a biogas plant in developing countries (Karki *et al*., 2005). These are Floating Drum Plants, Fixed Dome Plant and Deenbandhu. These and other models developed and tested over the years are briefly discussed below.

2.15. Continuous and Batch Digesters

2.15.1. Continuous Digester: Consist of a single digester into which raw materials are fed on a regular basis without any form of interruption except when repairs and cleaning are required. This digestion type is usually completed in either a single or two stages as discussed below (Abu-Darieha *et al*., 2011; Rai, 2011).

i. **The Single Stage Process**: In this system, the bioconversion of substrate into biogas is completed in a single digestion tank which regularly receives feedstock while the digestate moves continuously via the outlet (Figure 2.1).

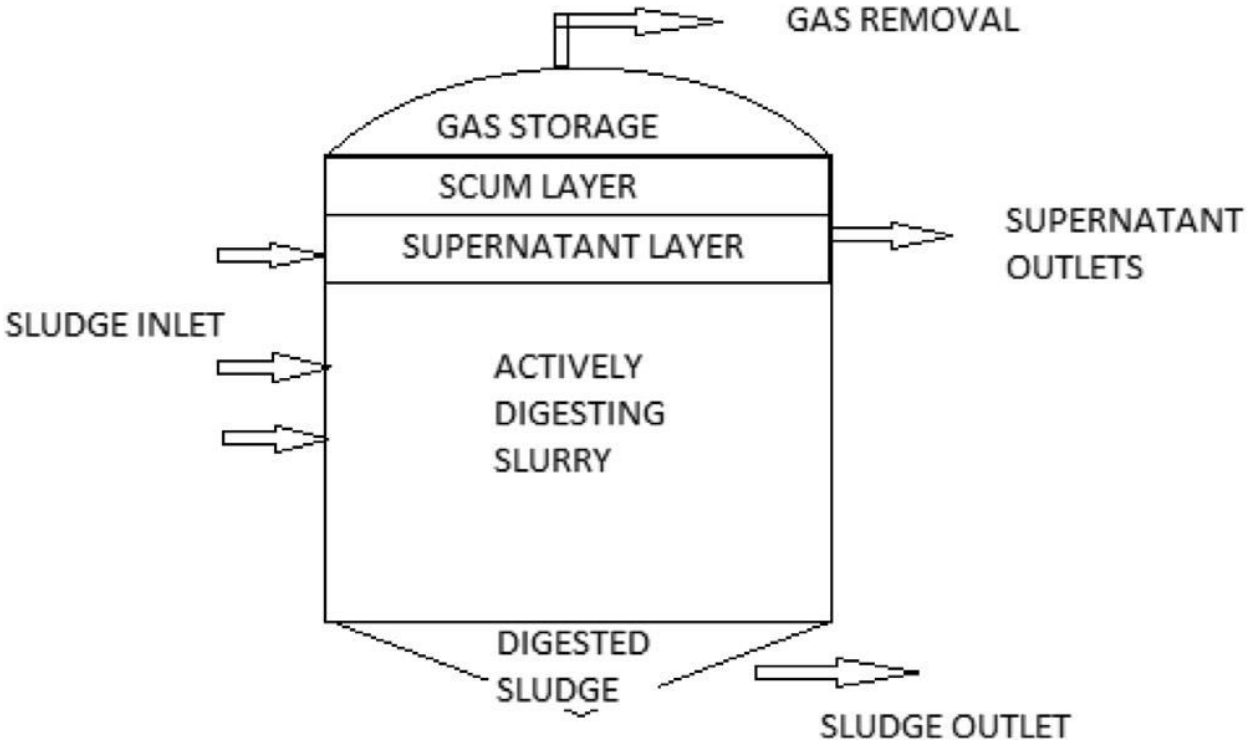


Figure 2.1: A Single Stage Process Conventional Digester (Rai, 2011)

ii. **The Double Stage Process**: In this digester type, there is physical separation between the acidogenesis and methanogenesis stages. The first stage which involves the production of acid is separately done in a tank and the products are charged into the other chamber where methanogenesis occurs and the generated biogas is collected (Figure 2.2). The multi-stage systems are characterized with a higher rate of loading, flexibility and improved process stability. However, the cost of building and operating has limited their applications (Abu-Darieha *et al*., 2011; Rai, 2011).

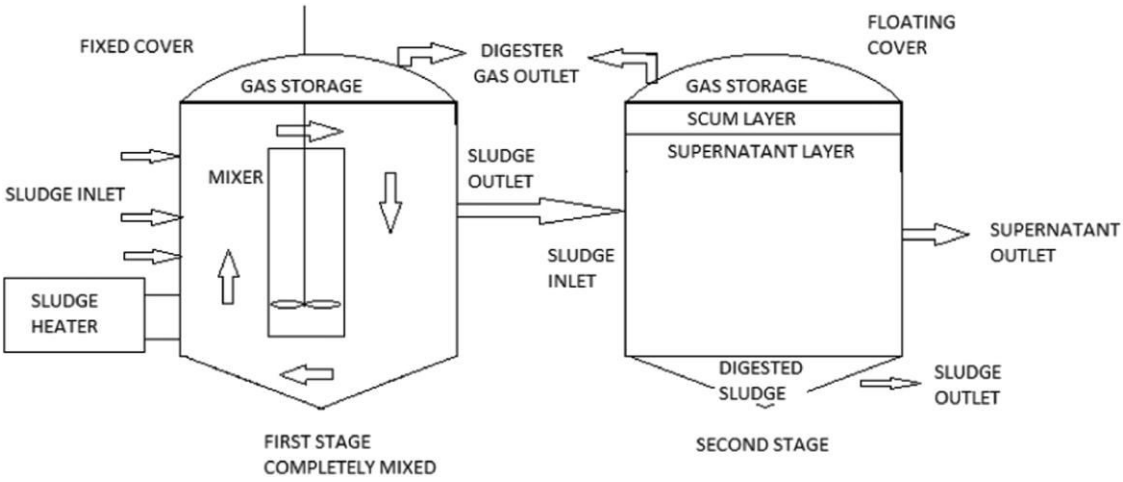


Figure 2.2: A Double Stage Process Conventional Digester (Rai, 2011)

2.15.2. The Batch Digester: In this type of plant, feeding of substrates is done at intervals and the digester is off-loaded once the anaerobic digestion process is complete. The digesters are fed and later emptied one after another after digestion in a synchronous manner which maintains constant gas generation through a common gas holder. The major challenge with this digester type is haphazard gas production and poor microbial population which affects the stability of the process and this gradually being surmounted by sequential and phased batch digesters (Rai, 2011). The different types of batch reactor are discussed below:

i. **Biocel Reactor**: One of the components of early research into the use of high solids digestions was the introduction of the Biocel reactor in the late 1980s and early 1990s. It has an initial goal of reducing cost through the simplification of material handling and elimination of mixing while simultaneously achieving relatively high rates of loading and bioconversion (Rapport *et al*., 2008; Abu-Darieha *et al*., 2011).

ii. **Sequential Batch Anaerobic Composting (SEBAC)**: Like the Biocel, the SEBAC reactor was equally developed in the early 1990s which aimed at eliminating substrate mixing and also minimizes handling while ensuring a high rate of bioconversion and system stability (Rapport *et al*., 2008; Abu-Darieha *et al*., 2011). It consists of two-or three-batch, leach-bed reactors with leachate

recirculation by a sprayer. Its advantage over the Biocel reactor is the sequence loading which makes it possible for leachate to be transferred between the reactors.

iii. **Anaerobic Phased Solids (APS) Digester**: The APS digester in the same way with the SEBAC system uses batch loading to stimulate rapid organic acid production in a two-stage digester system. However, the APS digester system combines high-solids reactors for the first stage with a low-solid mixed biofilm reactor in the second stage thereby surmounting the challenges imposed via the usage of leach bed reactors (Rapport *et al*., 2008; Abu-Darieha *et al*., 2011).

iv. **BioConverter**: The BioConverter digester is a single-stage, sequentially batched system. In its full-scale application, an equalization tank is used for pulping and metering feed into the batch reactors leading to a drop in the pH which is an indication that the tank may serve as a first-stage hydrolysis reactor (Rapport *et al*., 2008; Abu-Darieha *et al*., 2011).

2.15.3. The Drum and Dome Types

Out of the different models of these digesters, only two are important common (Rapport *et al*., 2008; Abu-Darieha *et al*., 2011).

2.15.4. The Floating Gas Holder Digester: This is commonly used in India and the fixed dome digester used in China both have different shapes ranging from cylindrical, rectangular, spherical etc. They are generally sited above or under the ground (Rai, 2011). The major challenges about this design cost of gas holder construction and corrosion (Figure 2.3).

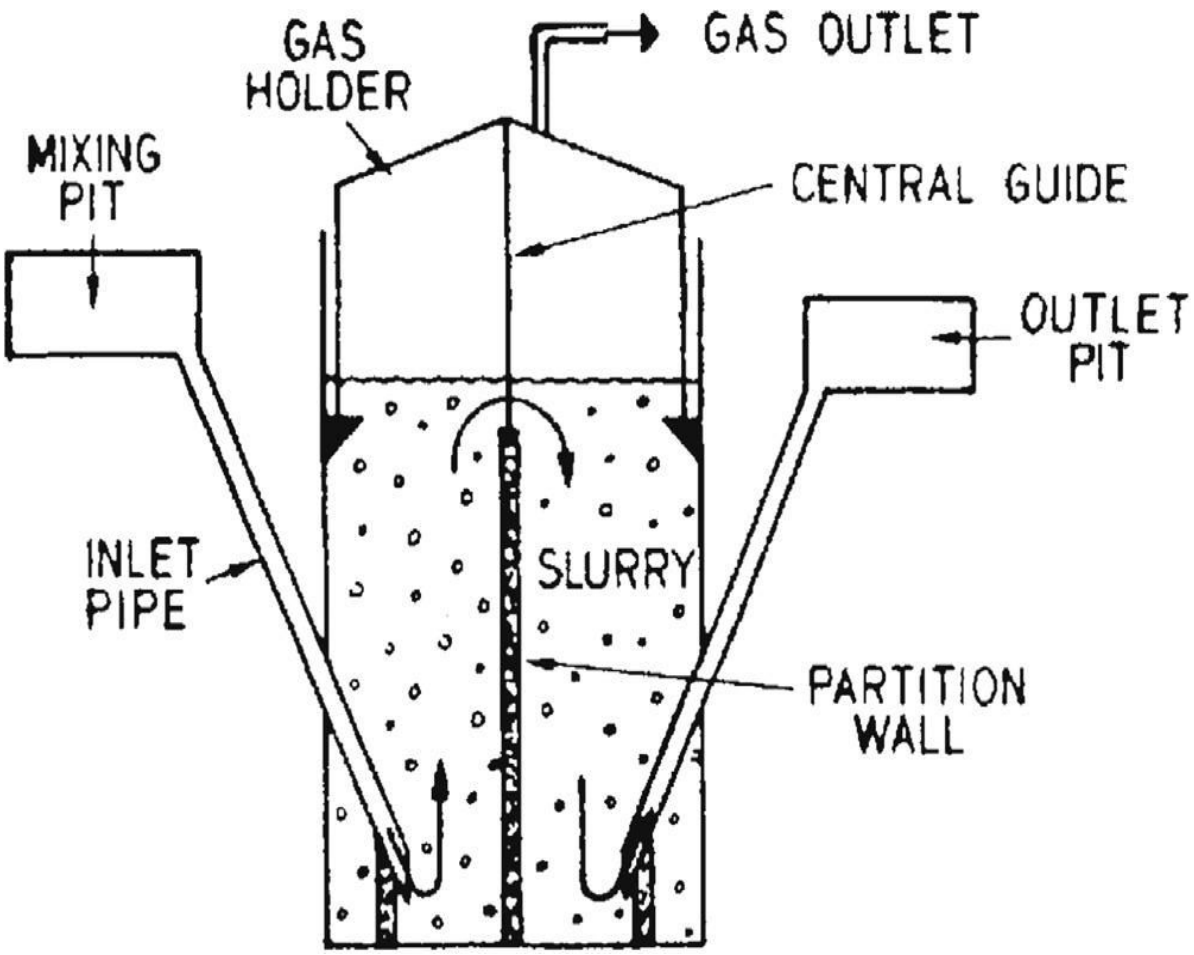


Figure 2.3: A Floating Gas Holder Digester (Rai, 2011)

The floating drum plants, however, have become obsolete with the advent of fixed dome plant due to comparatively high investment and maintenance cost of the former (Rai, 2011). The latter has the advantage of constant gas pressure excluding the need for adjustments in lamps, stoves and other appliances when used. Another advantage is the rising of the gas holder above the digester indicating availability of gas (Figure 2.4).

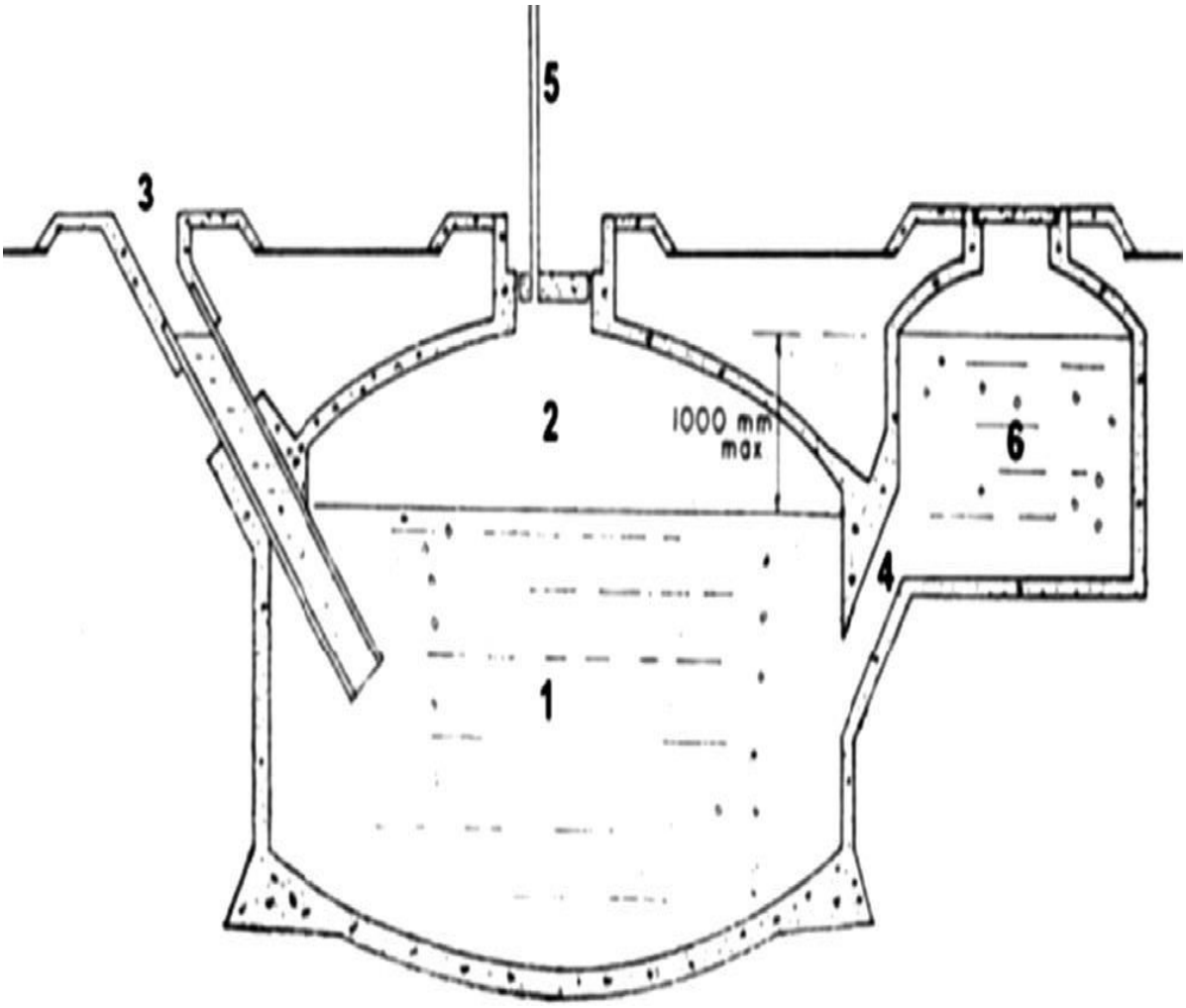


Figure 2.4: The Fixed Dome Digester (Rai, 2011)

1 = Slurry, 2 = Gas collection/staorage fixed dome, 3 = Inlet for slurry, 4 = Outlet for digstate, 5 = Outlet for gas, 6 = Outlet tank for digestate slurry

2.15.5. Fixed Dome Digester: This is also popularly called "The Chinese model biogas plant". It is a single unit comprising a fermentation chamber made of brick and constructed under the ground and a gas storage dome on top. The design has successfully eliminated the use of expensive mild steel for gas holder construction as it is prone to corrosion.This makes the shelf-life of this digester to be above 20 years unlike the floating drum design (Rai, 2011).

2.16. Biofertilizer

Biofertilizers are preparations containing latent cells of efficient microorganisms which help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil (Di Maria *et al*., 2017; Yasar *et al*., 2017). Digestate biofertilizers comprise microbial biomass, semi-degraded organic matter, and inorganic compounds, and therefore can be used as soil conditioners on farmlands (Alburquerque *et al*., 2012). Over reliance on inorganic chemical fertilizers has resulted in soil quality reduction, eutrophication and heavy metals pollution (Zhu *et al*., 2012). Therefore, biofertilizers are important in the provision of environmental benefits including the improvement of soil and food quality and safety as well as human and animal well-being health (Grigatti *et al*., 2011; Johansen *et al*., 2013). There are different types of digestate biofertilizers and their major differences are usually in the raw materials used for their production, forms of utilization and the source of microorganisms used in the preparation (Garfi *et al*., 2011).

Anaerobic digestate usually contains microorganisms like *Pseudomonas, Klebsiella, Salmonella, Penicillum, Shigella, Bacteriodes, Aspergillus, Bacillus* etc. all of which can be exploited in the production of biofertilizers because they quicken the microbial processes in the soil and increase the availability of nutrients that can be assimilated by plants (Tamil Nadu Agricultural University, 2008). *Klebsiella* and *Clostridium* species are free living nitrogen fixers while *Bacillus* and *Pseudomonas* species are phosphate solubilizers (Alfa *et al*., 2014b). It contains more readily available nutrients than the undigested products which make it better for crops fertilization (Goberna *et al*., 2010; Lansing *et al*., 2010; Garfi *et al*., 2011). Biofertilizers application stimulates plant growth by different mechanisms such as atmospheric nitrogen fixation, phosphorus solubilization, and mobilization, sequestration of iron by siderophores, phytohormones production etc (Babalola, 2010).

Different raw materials such as agricultural, municipal and domestic wastes are suitable for the cheap production of digestate biofertilizers, unlike chemical fertilizers that require high costs (Curry and Pillay, 2012; Dai *et al*., 2013). To this extent, the use of fiber and liquor from anaerobic digestion has led to improved fertilizer utilization and therefore less chemical consumption in many cropping systems around the world (Sun *et al*., 2015).

Fertilizer application/addition is a common soil management practice as it enhances the fertility of the soil and improves agricultural productivity (Shen *et al*., 2010). Inorganic fertilizers are usually high in nutrient and this explains why they are rapidly used up by crop plants. In order to meet the ever increasing demands from intensive agriculture in different parts of the world, the quantity inorganic fertilizers applied to soils in on the increase by the day (Savci, 2012). However, the increasing threats posed to biodiversity preservation, soil fertility maintenance, and resource conservation are at an alarming rate with increasing chemical fertilization (Dittmar *et al*., 2000). To this end, therefore, organic fertilizers from sources like animal droppings and dungs, human excreta or plant/vegetable residues provide benefits like higher and balanced nutrient supply and sustainable fertility of soils unlike the chemical inorganic ones (Chen, 2006).

Besides, fertilizers from organic sources have the ability to modify soil physical conditions via the improvement of soil aggregation, soil hydraulic conductivity reduction of mechanical resistance and bulk density (Hati *et al*., 2006; Bhattacharyya *et al*., 2007). Although organic fertilizers are comparatively lower in nutrient content and are characterized by low rate of nutrient release. These make biofertilizers to be slow in meeting the requirements of crops in a short time. However, they are highly efficient at enhancing soil nutrient and stability in the long run. Another approach is the combination of inorganic and organic fertilizers which has proved to be a better approach to increasing and sustaining soil fertility and yield of the crop (Bhattacharyya *et al*., 2008; Bandyopadhyay *et al*., 2010; Aguilera *et al*., 2012).

2.16.1 The Importance of Nitrogen in Crop Yield and Soil Improvement

Nitrogen cycling is a major component in agricultural systems because it is largely a major factor militating against crop plants growth and yield. Nitrogen loss is also a global problem which decreases agricultural values besides causing numerous environmental problems like eutrophication, water contamination, climate change and global warming (Choudhury and Kennedy, 2005; Stark and Richards, 2008). Therefore, there is an urgent need to prevent the loss of this vital nutrient and the understanding of the efficacy of different agricultural practices goes a long way in nitrogen conservation (Stark and Richards, 2008).

Microorganisms generally are highly sensitive to disruption of habitat and several bacteria and the archaea are known to play major roles in nitrogen compound's transformation resulting in the nitrogen cycle (Jangid *et al*., 2008; Simon and Klotz, 2013). Fertilizer application is known to affect soil physical, chemical, as well as the biological conditions and chemical fertilization, has been reported to differentially affect the abundance of microbes involved in soil nitrogen cycling (Bandyopadhyay *et al*., 2010). Earlier researches reported increase in the abundance of ammonia-oxidizing bacteria and denitrifiers due to NPK fertilization (Wakelin *et al*., 2007; Fan *et al*., 2011; Chen *et al*., 2012a; Chen *et al*., 2012b) while others established minimal effect of NPK fertilization on the microbial abundance (Shen *et al*., 2008; Mårtensson *et al*., 2009).

While chemical fertilization had been reported to negatively interfere with the microbial abundance in soil nitrogen cycling, organic fertilization, on the other hand, exerts positive effects on the functional microbes (Wakelin *et al*., 2007; Miller *et al*., 2008; Wang *et al*., 2013). Organic matter addition to the soil and the associated carbon content increase is a major issue of consideration in the study of the positive effects on microbial population in the soil. Organic substances are known to release abundant nutrients which are beneficial for the growth of microorganisms especially those involved in the cycling of nitrogen (Philippot *et al*., 2007; Miller *et al.,* 2008; Chen *et al*., 2012a). Despite the available data on the effects of inorganic and organic fertilizer addition on the nitrogen cycling microbial community, most of the studies are focused on a single component of the nitrogen cycle, while wide gap exists in knowledge on the entire process of nitrogen cycling community response to fertilization (Hai *et al*., 2009; Bru *et al*., 2011).

2.17. Experimental Design and Optimisation in Biogas Production

Most bioprocessing experiment requires adequate experimental design which most accommodates the standardization of important process parameters (Betiku *et al.,* 2014). In biogas production, the important process parameters include temperature, pH, retention time, total solids, volatile solids, inoculum ratio, loading rate etc. Most researches have reported the temperature for mesophilic AD between 30 and 40^{o} C using different substrates (Jain *et al.,* 2015). For pH, values usually range between 6.5 and 8.0 for the

efficient functioning of members of archaea (microbes responsible for methane production) (Zonta *et al.,* 2013). The ambient temperature of production affects the AD to a large extent and this is why most reports have suggested between 20 and 30 days as the retention time for most mesophilic AD (Nges and Liu, 2010; Mao *et al.,* 2015). Most liquid AD has been operated with a total solids content < 15% and ≥ 4% (Nagao *et al.,* 2012; Kougias *et al.,* 2013; Gou *et al.,* 2014; Jain *et al.,* 2015; Kim *et al.,* 2015).

2.18. Artificial Neural Networks (ANNs)

These are biologically stimulated mathematical models mimicking the neurons found in animals and employ a connectionist system to process information (Wasserman *et al.,* 1989). They are often used in the modeling of complex interactions between inputs and outputs data (Weiss and Kulikowski, 1991; Adepoju and Olawale, 2014).

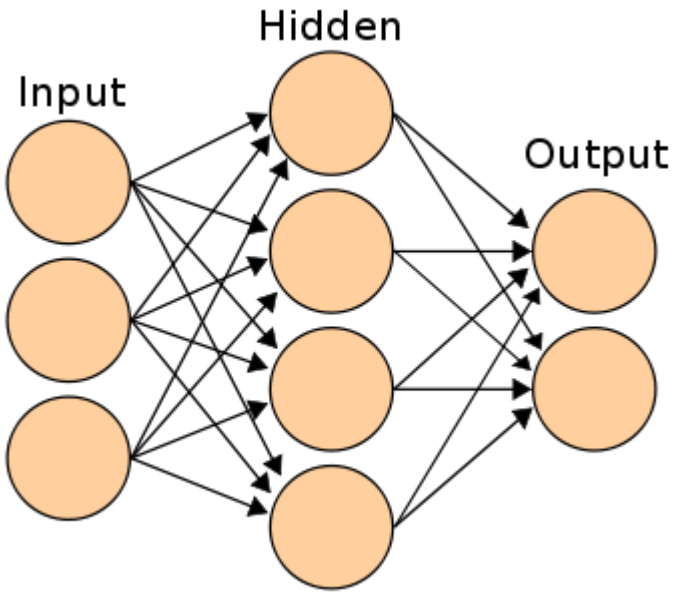


Figure 2.5: Structure of a Typical Neural Network

The simple network of processing elements contained in ANNs usually exhibit complex behavior predetermined by the connections between the processing elements and their different parameters (Abdi *et al.,* 1999; Betiku *et al*., 2015). The various functions in

ANNs are performed collectively and in parallel similar to the mode of function of biological neurons (Haykins, 1994; Betiku *et al*., 2015). Neural network are highly applicable in artificaial intelligence, statistics, cognitive psychology etc where they are often used as components in larger systems combining both adaptive and non-adaptive elements (Betiku and Ajala, 2014; Emeko *et al*., 2015).

2.18.1. Models

ANNs models are basically simple mathematical models which defines a function: $f:X\rightarrow Y$ or a distribution over X or both X and Y. However, models are sometimes cordially connected with a particular learning algorithm.

2.18.2. Network Function

The word *network* in ANNs refers to the interconnectivity existing between the neurons in the different layers of artificial systems (Masters, 1993) which are typically three layered in nature. The first layer transmit data through it input synapses to the second layer which in turn send more synapses to the third layer of output neurons. The numbers of layers of input and output neurons in a system is however determined by the complexity of the system.

2.18.3. Employing ANNs in Data Modeling

Schalkoff (1997) describes ANNs' greatest advantage to be their capability to "learn" from observed data when employed as arbitrary function approximation mechanisms from observed data. However, it was suggested that the following should be considered when employing them:

i. The model choice usually depends on the pattern of data's representation
ii. The selecting and tuning of any learning algorithm for the purpose of training on unseen data is usually preceeded by qualitative experimentation
iii. For robustness, appropriate models, cost function and learning algorithms must be selected

2.18.4. Real-life Applications Applications of ANNs

The versatility often seen in the application of ANNs is a function of their ability to infer a function from observed data which is mostly applicable in situations that is beyond the manual statistics. According to Schalkoff (1997), the application of ANNs tasks can fall within the following basic categories:

i. Function approximation and/or regression analysis
ii. Data classification
iii. Data processing
iv. Robotics

2.18.5. Current Research on ANNs Application

Most of the earlier researches on ANNs focused on the electrical characteristics of neurons (Abdi *et al.,* 1999) whereas modern investigations are concerned with the roles played by different neuromodulating substances on behavior and learning (Betiku *et al*., 2015).

2.19. Response Surface Methodology (RSM)

2.19.1. Factorial Design Selection

These designs are primarily employed in screening significant factors and in process modeling (Box and Wilson, 1951; Montingelli *et al*., 2016). Factorial design offered a number of factorial design types.

i. **2-Level Factorial Designs** – These designs are employed for the exploration of many factors while eventually setting each factor to only two levels.
ii. **General Factorial Designs** – These can be used in experimental design with each factor having a different number of levels ranging from 2 to 999.
iii. **Plackett- Burman Designs** – These highly confounded designs are used for non-significant but useful data.
iv. **Taguchi OA Designs** – These are a set of classic designs from the teachings of Taguchi which are often used for building a particular design. Note that all analyses will be completed using standardized ANOVA reports and interaction graphs
v. **Latin Square** designs may also be used in certain situations. Although these designs are not explicitly offered in the program, they can be built relatively easily.

2.19.2. Response Surface Design Selection

These are designs which help in quantifying the interactions between one or more measured responses and the vital input factors (Atkinson *et al.,* 2007). Most of the designs handle up to fifty numeric factors, with ten additional categorical factors (Cornell, 2002; Adepoju and Olawale, 2014).

2.19.3. Central Composite Design

This is the most popular RSM design consisting of three groups of design points: two-level factorial design points, axial points and center points which are designed to estimate the coefficients of a quadratic model. All point descriptions are usually represented by coded values of the factors (Gaffke and Heiligers, 1996; Montingelli *et al.,* 2016).

Factorial Points: There are two-level factorial parts of this design consisting of all possible combinations of the +1 and -1 levels of the factors. The four available design points are (-1, -1), (+1, -1), (-1, +1) and (+1, +1).

Star or Axial Points: These points have all of the factors except one set to the midpoint (0) with the exceptional one having the value +/- Alpha. The star points for a two-factor problem are: (-Alpha, 0), (+Alpha, 0), (0, -Alpha) and (0, +Alpha). Usually, the Alpha value is calculated in each design for both rotatability and orthogonality of blocks.

Center Points: These are points with all levels set to coded level 0 which is the midpoint of each factor range i.e. (0, 0). In order to arrive at a good estimate of experimental error, the center points are usually repeated 4-6 times.

2.19.4. Box-Behnken Design

These are response surface designs having only three coded levels i.e. -1, 0, and +1 and are available for between 3 and 21 factors formed from the combination of two-level incomplete block factorial designs. The quadratic model has been postulated as the most appropriate simply because there are only three available levels (Kiefer, 1985; Adepoju and Olawale, 2014).

2.19.5. 3-Level Factorial Design

These designs are located under the Response Surface, Miscellaneous design node in the program and are available for up to 4 factors. Because there are only 3 levels for each factor, the appropriate model is the quadratic model. These designs are usually run in one or 3 split three blocks of equal size (Gergone, 1974; Betiku *et al*., 2015).

2.19.6. One Factor RSM Design

This design allows development up to a cubic model for one numeric factor. The order of polynomial for approximation usually determines the number of levels required. Three levels of a single factor (-1, 0, 1) plus replicates allow a lack of fit and pure error determination for a linear model. Five levels of a single factor (-1, -0.5, 0, 0.5, 1) plus replicates allow a lack of fit and pure error determination for a quadratic model. Seven levels of a single factor (-1, -0.666, -0.333, 0, 0.333, 0.666, 1) plus replicates allow a lack of fit and pure error determination for a cubic model (Ghosh, 1996; Betiku *et al*., 2015).

2.20. Biomass Used in this Study

Below is the scientific summary of the five biomass used in this research:

2.20.1. *Tithonia diversifolia* (Mexican Sunflower) Shoot:

Plate 1: *Tithonia diversifolia* Shoot (Landmark University Orchad, Omu-Aran, Nigeria)

Tithonia diiversifolia (Mexican sunflower) has its origin from Mexico and Central America but is now widely distributed throughout tropics in Central and South America, Asia and Africa. It was introduced to Nigeria as an ornamental plant when its spores

which were attached to grains were imported through Ogbomoso in Oyo State, Nigeria (Akobundu and Agyakwa, 1987; Ayeni *et al*., 1997). It belongs to the Asteraceae family all of which are known to exhibit allelopathy. *T. diversifolia* is an aggressive weed with potentials to grow up to about 2.5 m and thrives comfortably on diverse soils (Chukwuka *et al*., 2007). Its high invasive capacity has made it a common weed in Nigeria especially on abandoned sites, waste lands, along major roads and waterways and on cultivated farmlands as a serious weed of crops over the decades (Taiwo and Makinde, 2005). It also has stimulatory and phytotoxic plant inhibitory attributes (Taiwo and Makinde, 2005). It is presently found abundant in the southern, eastern, western and partly in northern Nigerian States where it is put to no significant usage despite its high availability. Prior to this research, only one study in Nigeria (Adepoju *et al*., 2016) reported the investigation of biogas optimization from *Tithonia diversifolia.*

2.20.2. *Chromolaena odorata* (Siam Weed) Shoot

Plate 2: *Chromolaena odorata* Shoot (Landmark University Orchad, Omu-Aran, Nigeria)

Chromolaena odorata (Siam weed) (L.) King and Robinson (Asteraceae), formerly known as *Eupatorium odoratum* L., is a highly invasive alien plant usually impacting adversely on agriculture and conservation of biodiversity in its areas of dominance as a stubborn

weed thereby causing significant economic losses (Tefera *et al*., 2008; Perrings *et al*., 2010). As is notable for invasive alien plant, *C. odorata* is a huge threat to natural and derived ecosystems of its localities (Zacharides *et al*., 2009) thereby compromising ecosystems stability. It has the ability to smother existing native plant communities and has therefore attracted significant attention in several cropping systems (Adebayo and Uyi, 2010).

C. odorata is renowned to have originated from tropical Central and South America especially Mexico, the Caribbean and Brazil, from where it has spread to other localities due to its effective well developed dispersal mechanisms. It forms pure stands when fully established often in disturbed areas, grasslands, fallow areas and forestry plantations, and is highly competitive (Gauttier, 1992). It was introduced to Southern Nigeria from Sri Lanka in 1937 and has currently reached alarming population in the country (Uyi *et al*., 2013; Uyi and Igbinosa, 2013) and other African countries like Cameroon, Ghana where it is regarded as one of the worst weeds.

In a bid to control this weed's invasiveness, chemical, mechanical and biological control methods have been employed none of which proved to be cost effective and sustainable (Uyi *et al*., 2014). There are presently no control or proven management strategies to curtail the spread of the weed in Nigeria and other countries. Biogas generation from this biomass was first reported in the 80s and very early 90s by Akinluyi and Odeyemi, (1989) and Ejike and Okereke, (1991). However, these researches were only preliminary and no further works has been done on the biogas generation from this biomass since then.

2.20.3. *Arachis hypogaea* (Groundnut) Hulls

(a) (b)

Plate 3: *Arachis hypogaea* (a) The Hulls from Landmark University Farms (b) Groundnut Pyramids (Taphee and Jongur, 2014).

Arachis hypogaea (Groundnut) is a native of South America but its cultivation is now widespread globally. It was introduced to the African continent during the colonial era (Duke, 1981). It entered Africa during the Portuguese exploration. World total production as at 2007 was 34.9 million metric tons (Food and Agricultural Organization, 2007). Groundnut is produced in Africa majorly by Nigeria, Sudan, Senegal, Chad, Ghana, Congo and Niger. Groundnut pyramids were a success story of the Northern Nigeria (Kano State especially) prior to independence while its farming remains a popular practice in Northern Nigerian with the fruit pods being put to no usage (Taphee and Jongur, 2014). Prior to this research, the potentials of groundnut hulls in biogas generation in Nigeria have been reported in few recent studies (Yavini *et al*., 2014; Ibrahim and Imrana, 2016)

2.20.4. *Carica papaya* (Pawpaw) Fruit Peels

Plate 4: *Carica papaya* (FAO, 2012)

Carica papayas (Pawpaw) has also been reported to have its origin in Southern Mexico, Central and South America from where it spread to other locations where it is currently found (Anon, 2010). Pawpaw is a flowering plant belonging to the family *Caricaceae*, comprising up to 25 species usually growing as high as 10 m. Its cultivation is currently popular in most tropical countries Nigeria inclusive (Anon, 2010).

Production of *papayas* occurs in more than 60 countries worldwide, with the vast majority being grown in developing countries. According to the FAO, global production in year 2010 was estimated to be 11.22 million metric tons (FAOSTAT, 2012). The major papaya-producing countries are India, Brazil, Indonesia, Nigeria, and Mexico (Evans *et al*., 2012). In the year 2011, Nigerian pawpaw production was estimated to be about 750, 000 tons due to the popularity of the crop across the country especially in the South western zone (FAOSTAT, 2011). Despite the huge applications of pawpaw parts, the skin/peel has not been efficiently used and is often regarded as wastes in most pawpaw-producing localities. The peel is often removed and thrown away. Prior to this research, there is no documented report of biogas generation from fruit peels of Pawpaw in Nigeria and other countries where biogas research is pronounced.

2.20.5. *Telfairia ocidentalis* (Fluted Pumpkin) Fruit Peel

Plate 5: *Telfairia occidentalis* (a) A standing fruit (b) A cross section of fruit (Akoroda *et al.*, 1990)

Telfairia occidentalis (Fluted pumpkin) originated in South East Nigeria from where it is distributed to other parts of the country and other West African nations (Akoroda *et al.,* 1990; Schippers, 2002). It is a member of the family *Cucurbitaceae* and is a large perennial dioecious plant which climbs by means of bifid and tendrils which are usually coiled and growing to a height of more than 20 m (Eseyin *et al*., 2014). It is an important leaf and seed vegetable indigenous to Southern Nigeria and grown in the forest zone of west and central Africa (Okoli and Mgbeogu, 1983). Its countries of major dominance include Nigeria, Ghana and Sierra Leone.

The wide cultivation of *Telfairia occidentalis* is majorly for its palatable and nutritious leaves which have higher nutritive values than other tropical vegetables especially in terms of protein content (21 %) and in vitamins and minerals such as Calcium, Phosphorus and Iron (Eseyin *et al*., 2014). Despite the huge applications of fluted pumpkin in several parts of the world, the peels/skin of the fruits remain grossly unutilized and often are left in piles thereby constituting solid waste pollution despite its large size which ensures huge biomass production. The green colouration of the fruits indicates presence of chlorophyll which makes the fruit a source of enormous energy via photosynthesis. Therefore, a

permanent solution need be sought for the disposal/utilization of these waste. Prior to this research, only one study has reported the biogas generation from the waste of *Telfairia occidentalis* vegetable (Idire *et al*., 2016) while no report on the use of the fruit peel has not been documented.

Overall, though few researches have reported the use of these five biomass or their parts for biogas generation, details of pretreatment prior to digestion and use of appropriate tools for process parameters optimization have not been studied hence this study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Materials

The following materials were used in this study (All chemicals and reagents were analytical grades):

i. Poultry droppings (Obtained from the Landmark University Teaching and Research Farm)
ii. 20300 API 20 A (25 Strips) for anaerobes and 20160 API 20 E (100 Strips) for Enterobacteria (BioMerieux, Lyon, France)
iii. Twenty five (25) anaerobic digesters (Twenty five liters each)
iv. Thioglycolate broth (Rapid Labs., Essex, United Kingdom)
v. Waterproof sacks obtained the Landmark University Commercial Farms
vi. Two hundred and forty plastic planting experimental pots obtained from the Omu-Aran market (Used for biofertilizer phyto-assessment)
vii. NPK 15-15-15 Inorganic fertilizer (Shandong Lvfeng Fertilizer Co., Ltd Shandong Province, China)
viii. Low Nitrogen soil (< 0.5% Nitrogen via analysis) obtained from the Landmark University Teaching and Research Farm)
ix. Local Short Variety (LSV) maize seeds obtained at the Landmark University Teaching and Research Farm
x. An acre of land was acquired within the Teaching and Research farms of Landmark University on which the set up was installed. The direction of the prevailing wind was taken into consideration in the choice of the site.

3.2. Instrumentation

The list of instruments used in this study is shown in Appendix 1

3.3. Design of Pilot Scale Anaerobic Digesters

The design theory used for this study combines the Ajoy Karki's kitchen waste biogas model (Karki, 2002) and a gas holder system which was separate from the digester tank. The digester's shape was cylindrical in order to ensure adequate substrate mixing. The separate gas holder system was incorporated into this design to allow for ease of measurement of gas volume at atmospheric pressure. The succeeding sections give details of the principles and design consideration for the digester type adopted. The digester is a separate component, with the gas holder (inverted drum) floating in a separate water jacket. The theory behind the design is simply "downward delivery and upward displacement". The slurry on fermenting in the digester produces gas which is then delivered to the bottom of the water jacket via a pipe; the pipe extends above the surface of the water level (water seal) in the water jacket. The gas displaces the gas holder (upward) and gets trapped between the gas holder and the water seal. The displacement of the gas holder is dependent on the pressure and volume of the gas produced. Figure 3.1 shows a schematic view of the plant set up.

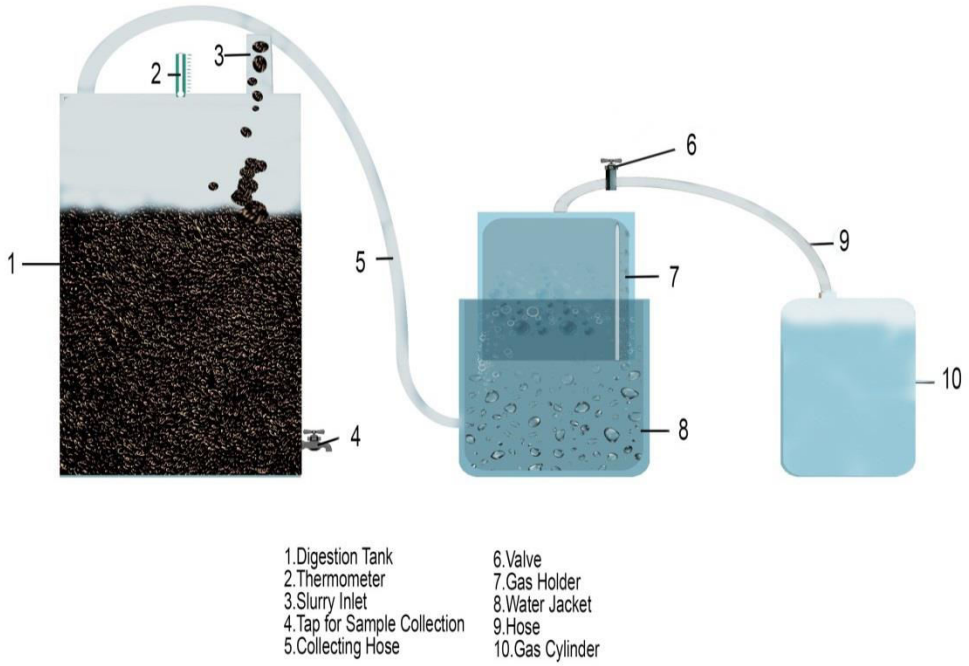


Figure 3.1: Set-up of the anaerobic digester used in the study

The choice of this set up was necessitated by the following objectives:

i. It is a simple design and construction with high tolerance to construction flaws and defects.

ii. It is the most suitable for small scale study of anaerobic digestion.

iii. It makes the best use possible of the restricted compound space.

iv. Low maintenance and adapted to the habits and perceptions of the intended users.

v. Collecting the gas outside the digester reduces pressure in the digester

vi. Gas is produced at steady/constant pressure, as weight of gas holder balances the pressure in the gas holder; volume of gas produced is immediately detected due to the positioning of the calibrated drum.

vii. There's superior sealing of the substrate, no risk of spillage of slurry into the gas holder, thus very hygienic.

viii. Gas holder can easily be protected from rust by painting regularly, thus facilitating gas tightness.

3.4. Anaerobic Digester Design Considerations

3.4.1. Digester Design:

i. Operating Volume:

The operating volume used in this work is 25 liters according to earlier design (Ahmadu, 2009). The operating volume of the digester (V_o) was determined based on the chosen retention time (RT) and the daily substrate loading (S_d) (m^3/d), and is given as:

$$V_o = S_d \times RT \ [m^3 = m^3/day \times number\ of\ days] \quad (3.1)$$

The RT is the time interval during which the fed substrate was allowed to be degraded by microbes in the digester and this was determined by the chosen digester temperature.

Kossmann et al., (2001) opined that a RT of minimum 30 days is appropriate for a simple biogas plant.

$$\text{Substrate input } (S_d) = \text{Biomass (B)} + \text{Water (W)} \ (m^3/\text{day}) \quad (3.2)$$

ii. Total Volume:

The total volume of the digester (V_T) which is 25 liters was greater than the operating volume so as to allow for biogas production and the rise of the slurry during fermentation (Ahmadu, 2009). The total volume is thus given as:

$$V_T = {V_o}/{0.8} \quad (3.3)$$

iii. Digester dimensions:

Having determined the total volume of the digester, a ratio for the dimensions can be adopted, depending on the chosen geometric shape of the digester. For a cylindrical digester, the chosen geometry for this work,

$$V_T = \pi r^2_d \, h_d \quad (3.4)$$

Where V_T = Total volume of digester

r_d = radius of digester

h_d = height of digester

iv. Digester Temperature:

The digester was designed to operate within the mesophilic temperature range (20-40^o C). This was achieved by natural heating from the sun. An absorptive surface is required for the digester; this is to absorb solar radiation during the day time. An insulating material is required for the digester at night in order to retain the heat within and keep temperature fluctuations within manageable limits.

3.4.2. Gas Holder System Design:

i. Gas holder volume (V_g):

According to Kossmann *et al*. (2001), the gas holder's volume (V_g) is a function of the relative gas generation and consumption rate (Ahmadu, 2009). The gas holder should be designed to:

- Cover the peak consumption rate (gc_{max}) for the period of maximum consumption (tc_{max}), $V_g = V_{g1}$ (3.5)

- Hold the produced gas during the longest Zero consumption period

(tz), $V_g = V_{g2}$ (3.6)

From equation (3.5)

$$Vg_1 = gc_{max} \text{ X } tc_{max} \quad = g\ c_{max}\ tc_{max} \tag{3.7}$$

From equation (3.6)

$$V_{g2} = G_h \text{ x } t_{Zmax} = Gh\ t_{Zmax} \tag{3.8}$$

Where,

gc_{max} = maximum hourly gas consumption (m^3/hr)

tc_{max} = time of maximum consumption (hr)

G_h = hourly gas production (m^3/hr) = G ÷ 24hrs/day

G = daily gas production (m^3/day)

t_Z = maximum zero consumption time (hr)

The larger value, (V_{g1} or V_{g2}) determines the size of the gas holder. A safety margin of 10-20% was then added (Ahmadu, 2009).

ii. Gas holder dimensions:

Having determined the volume of the gas holder, a desired ratio for the dimensions was then adopted, depending on the geometric shape of the design. For a cylindrical gas holder,

$$V_g = \pi r^2_g h_g \quad (3.9)$$

Where,

V_g = volume of gas holder `

r_g = radius of gas holder

h_g = height of gas holder (Ahmadu, 2009)

iii. Water jacket design:

The water jacket holds the water in which the gas holder floats and should be of the same geometrical shape as the gas holder. The radius was made to be a little larger than that of the gas holder to give clearance for sliding of the gas holder (Ahmadu, 2009).

$$R_j = r_g + c \quad (3.10)$$

Where, r_j = radius of water jacket

r_g = radius of gas holder

c = clearance/allowance

The height of the water jacket was equal to the height of the gas holder:

$$h_j = h_g \quad (3.11)$$

Where,

h_j = height of water jacket

h_g = height of gas holder

Volume of water jacket (V_j) is given by (Ahmadu, 2009):

$$V_j = \pi r_j^2 h_j \quad (3.12)$$

r_j = radius of water jacket

h_j = height of water jacket

3.4.3. Guide Frame Design:

The guide frame is to guide the gas holder in its upward displacement and prevent it from tilting. It's also to provide a maximum displacement position for the gas holder. It consists of two rods mounted on opposite sides of the gas holder, sliding through corresponding slides ways on the water jacket. Length of the rods were little less than height of gas holder to give allowance for welding onto the gas holder. Any convenient length can be taken for this allowance, this is be denoted c, thus

Length of guide frame (L_f)

$$L_f = h_g - c \tag{3.13}$$

Where h_g = height of gas holder.

c = allowance

On the guide frame is a maximum displacement point at a distance

$$L_f - d_{max} \tag{3.14}$$

Where d_{max} is a distance taken from the bottom tip of the frame, with a hole drilled at this point and a pin inserted. With this, at maximum displacement of the gas holder, a portion of it is still submerged in the water seal, thereby providing rigidity and safety. The guide frame merely guides the gas holder in its upward displacement, thus it's not under the action of any load or force. Therefore, any convenient safe diameter can be adopted (Ahmadu, 2009).

3.4.4. Force on Gas Holder (Fg):

The force on the gas holder is given as:

$$F_g = P_g \times A_g \tag{3.15}$$

P_g = Pressure in gas holder

A_g = Cross-sectional area of gas holder (Ahmadu, 2009)

3.4.5. Gas Pipe Diameter:

The gas pipe diameter was selected based on the flow rate of biogas through the pipe and the distance between the digester and gas holder (i.e. length of pipe required). The values can be checked from standard tables to determine the required pipe diameter (Ahmadu, 2009).

3.5. Material Selection for Digester Construction

As a general rule, the selection of all the materials was based on: Cost-effectiveness, availability and durability.

3.5.1. Materials for Digester Construction: The material used for the digester was a mild-steel. It was selected based on the following parameters:

i. Water/gas tightness in order to avoid leakage/seepage and the potential threat to soil and ground water quality and also prevent entering of air into the digester.

ii. Good tensile strength and ease of rolling by machine to required design geometry.

3.5.2. Materials for Digester's Gas Holder and Water Jacket: The material used for the gas holder was a thin sheet metal while that for water jacket was a mild-steel sheet metal painted to prevent corrosion and provide reflective surface. It was selected to meet the following requirements:

i. Relatively cheap.

ii. Provides reflective surface thereby minimizing heat build-up inside the gas holder and within the water seal.

iii. Good tensile strength and easy to roll by machine to required design geometry.

iv. Provides gas tightness to store biogas

3.5.3. Materials for Digester's Gas Pipe: The materials used for the gas pipe are galvanized steel pipe, which was used inside the water jacket, and flexible plastic pipe which was used from the digester outlet to the galvanized pipe inlet at the bottom of the

water jacket. Both have the same diameter. Galvanized steel pipe was selected based on its resistance to corrosion and rigidity, flexible plastic pipe was selected based on its resistance to corrosion and flexibility.

3.6. Fabrication of Digester Parts

Having selected the materials to be used, machining of component parts was carried out using the appropriate machine and tools at the laboratory of the Department of Mechanical Engineering, Landmark University, Omu Aran.

3.7. Design and Loading of Digester

The volume of the identical anaerobic digesters was determined by the quantity of volatile solids (VS) to be digested and the RT. The total volume of each digester tank was 25 liters. The tanks were air-tight and distinctly positioned above the ground in order to have maximum access to sunlight for heating. A gas holder tank made from thin sheet metal was also used to construct the temporary biogas storage container until usage. The five different kinds of pre-treated substrates were introduced simultaneously into the respective digesters both in mono and in co-digestion with poultry droppings for a period of 20 to 30 days according to experimental design thus making ten digestion regimes in all.

3.8. Experimental Design

3.8.1 Central Composite Design (CCD)

The Central Composite Design was used in experimental design and optimization of the bioconversion of the biomass in both mono and co-digestion regimes to biogas as shown in tables 3.1 and 3.2. This tool was used because of its recorded efficiency in the improvement of bioprocessing (Betiku and Ajala, 2014; Mazza *et al.,* 2014). The Five-level-five-factors factorial design was adopted which generated a total of 50 experimental runs with an alpha value of 2.37841. The five important variables selected for the modeling and optimization are Temperature (^{o} C), pH, Retention time (days), Total solids (g/kg) and Volatile solids (g/kg) separately designated as X_1, X_2, X_3, X_4 and X_5 respectively (Tables 3.1 and 3.2). This selection was based on the need to standardize them in the AD of the substrates as this will have qualitative application in subsequent research on the same substrates especially for industrial scale production.

Table 3.1: Factors and their Levels for Central Composite Design

Variable	Symbol	Coded factor levels				
		-2	-1	0	1	2
Temperature (^{o}C)	X_1	30	32.5	35	37.5	40
pH	X_2	6.0	6.5	7.0	7.5	8.0
Retention time (days)	X_3	20	22.5	25	27.5	30
Total solids (g/kg)	X_4	4	6	8	10	12
Volatile solids (g/kg)	X_5	4	6	8	10	12

Table 3.2: Central Composite Design and ANNs Design for Biogas Generation Using Coded Values

Run	X_1	X_2	X_3	X_4	X_5
1	-1	-1	-1	-1	-1
2	1	-1	-1	-1	-1
3	-1	1	-1	-1	-1
4	1	1	-1	-1	-1
5	-1	-1	1	-1	-1
6	1	-1	1	-1	-1
7	-1	1	1	-1	-1
8	1	1	1	-1	-1
9	-1	-1	-1	1	-1
10	1	-1	-1	1	-1
11	-1	1	-1	1	-1
12	1	1	-1	1	-1
13	-1	-1	1	1	-1
14	1	-1	1	1	-1
15	-1	1	1	1	-1
16	1	1	1	1	-1
17	-1	-1	-1	-1	1
18	1	-1	-1	-1	1
19	-1	1	-1	-1	1
20	1	1	-1	-1	1
21	-1	-1	1	-1	1
22	1	-1	1	-1	1
23	-1	1	1	-1	1
24	1	1	1	-1	1
25	-1	-1	-1	1	1
26	1	-1	-1	1	1
27	-1	1	-1	1	1
28	1	1	-1	1	1
29	-1	-1	1	1	1
30	1	-1	1	1	1
31	-1	1	1	1	1
32	1	1	1	1	1
33	-2	0	0	0	0
34	2	0	0	0	0
35	0	-2	0	0	0
36	0	2	0	0	0
37	0	0	-2	0	0
38	0	0	2	0	0
39	0	0	0	-2	0
40	0	0	0	2	0
41	0	0	0	0	-2

Table 3.2: Central Composite Design and ANNs Design for Biogas Generation Using Coded Values (Cont.)

42	0	0	0	0	2
43	0	0	0	0	0
44	0	0	0	0	0
45	0	0	0	0	0
46	0	0	0	0	0
47	0	0	0	0	0
48	0	0	0	0	0
49	0	0	0	0	0
50	0	0	0	0	0

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

3.8.2 Artificial Neural Networks (ANNs)

The generated data via CCD was equally used for the ANN module employing the Neural Power version 2.5 (CPC-X software) so as to select the statistically well distributed data in the input search window. Similar to the CCD module, a total of 50 experimental data were generated and divided into sets, 32 in training set, 9 in the validation set and 9 in the test set. The Tanh transfer function at hidden layer and a linear transfer function at output layer was used. The similar transfer function has been used (Adepoju and Olawale, 2014; Betiku and Ajala, 2014).

3.9. Experimental Procedure

3.9.1. Sample Collection and Pretreatment:

The plant materials (*Tithonia diversifolia* and *Chromolaena odorata*) were collected in bulk from Landmark University Orchard and were identified at the Department of Biological Sciences, Landmark University, Omu-Aran while the remaining three biomass were collected from Landmark University Farms and the Omu-Aran market in Omu-Aran, Kwara State. Each sample was collected into clean bags and was transported to the site of the experiment. In order to avoid variation in biomass status before pretreatment, all samples were air-dried until constant weights were obtained. The poultry dropping was obtained in bulk from the Teaching and Research Farms of Landmark University and kept in the refrigerator at 4^{o} C. Cattle's rumen content which was used as inoculum was also obtained in bulk from the slaughter slab of Landmark University's Cafeteria and refrigerated until usage.

Considering the lignocellulosic nature of these five biomass and to overcome the usually encountered rate-limiting phenomenon in the hydrolysis step of AD, each of them was pretreated using the combination of mechanical and thermo-alkaline (NaOH) pretreatment earlier described (Ariunbaatar *et al.,* 2014; Cesaro and Belgiorno, 2014; Kim *et al.,* 2015; Monlau *et al.,* 2015). In carrying out the mechanical pretreatment, each dried biomass was initially milled into sizes of $\leq$ 20 mm with the aid of a hammer mill after which the obtained powdery forms were stored till further actions were taken. The biomass were later heated at 80^{o} C using the CLIFTON, 88579 water bath (Nickel-Electro Ltd.,

England). The temperature was chosen as a modification to earlier report that thermal pretreatment at higher temperature (especially ≥ 100) imparts adversely on the AD system by chemical reactions leading to the formation of complex inhibitory proteins (Rafique *et al*., 2010; Liu *et al*., 2012). In carrying out the alkaline pretreatment, each mechanical and thermally treated substrate was further treated using 3 g of sodium hydroxide (NaOH) pellets per 100 g TS at a temperature of 55^{o} C for 24 h and at a solid loading of 35 g TS L^{-1} (Monlau *et al*., 2015). The entire alkaline pretreatment was done in closed containers and NaOH was used since it has been reported as the alkali of choice for most thermo-alkaline biomass pretreatments (Li *et al*., 2015).

3.10. Digestion Regimes

Ten (10) different digestion regimes were carried out which was made up of 5 each of mono and co-digestions using cattle's rumen content as inoculum. Rumen content's usage as anaerobic inoculum is well reported (Kana *et al.,* 2012). The co-digestions involved each biomass being co-digested with Poultry dropping in 1:1 proportion (Alfa *et al.,* 2014a). Eight (8) kg of the respective pretreated sample was mixed with water to form slurry and was separately charged into each digestion tank through the provided inlet on the digester tank. For the co-digestion regimes, 4 kg of poultry dropping was mixed with 4 kg of each pretreated biomass to make the 8 kg of substrate which was then turned to slurry by the addition of water. In each case, the slurry occupied three quarter of the space in the digester leaving out one quarter head-space for the collection of produced gas which was collected through a flexible hose linking the digestion tank and the gas collection unit.

3.11. Technical Evaluation of the Anaerobic Digestion Process

The digesters were monitored for the 20-30 day retention time in the following areas:

i. Measurement of gas production,

ii. Periodic microbial succession evaluation,

iii. Feedstock and digestate analyses inorder to ascertain efficiency of the anaerobic treatment

3.12. Measurement of Gas Production

This procedure was carried out daily at 6:00 pm by computing the total gas volume of the gas holder. The gas holder is an inverted cylinder with a base diameter of 0.25 m.

The base area, $A = \prod d^2/4 = \prod \times 0.25^2 \div 4 = 0.0491 m^2$

The height of cylinder protruding above the water level was read off with the aid of the calibrated rule attached to the gas holder

If this height is denoted by x (variable),

Then, the volume of biogas (At atmospheric pressure) was obtained as the cylinder volume above the water level, that is

$$\text{Volume}, V = \prod d^2 h/4 = Ah \text{ (where h} = x)$$

Substituting for A from above,

$$\therefore V = 0.0491\, x\, m^3$$

Note that v = volume of biogas and

x = height of cylinder above water level.

3.13. Measurement of Physicochemical Parameters

Before commencement and after the anaerobic digestion process, chemical analyses were carried out in order to quantify the elements/nutrients and other physical factors. These tests were carried out on the fermenting substrates, inoculum and effluents of the digestions. Chemical parameters were evaluated in the Environmental Engineering and Soil mechanics/Geotechnics laboratories of Landmark University, Omu-Aran, Nigeria. In all samples, estimation of total carbon, total nitrogen, total phosphorus, phosphates, sulphates potassium, magnesium, calcium, iron, copper, zinc, aluminium and manganese were done using the Pallintest Advanced Digital Readout Photometer (Model 7500 PHOT.1.1.AUTO.75, Camlad, Cambridge, United Kingdom) (Dahunsi *et al.,* 2014).

Details of blanking, calibration and mode of operation of the Photometer is shown in Appendix 1 while the process is depicted in Plate 6 of Appendix section. The photometer was calibrated according to the prescribed standards (Appendix 1) and then adjusted to 0.5 absorbance and a wave length of 450 nm before analyses of samples. For the determination of Chemical Oxygen Demand (COD), the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) was adopted. Determination of Volatile fatty acids (VFAs) were done using gas chromatography (Model of GC and procedure for blanking and calibration are shown in Appendix 3) to which was attached a Flame Ionization Detector (FID) (Zhang *et al.,* 2016). For total solids (TS) analysis, samples of the substrates were dried at 105 ^{o} C to constant weight while for volatile solids (VS), a known weight of dried sample was ignited to constant weight at temperature of 575 ± 25 ^{o} C, and following prescribed standards (Montingelli *et al.,* 2016). For moisture content determination, the direct heating method of the Association of Official Analytical Chemists (AOAC, 2000) was used. Two (2) grams replicate portions of each fermenting material were weighed into different pre-weighed moisture content dishes and dried at 80^{o}C in a hot air oven until constant weight was obtained. The samples were thereafter cooled to room temperature and weighed. Moisture content was recorded as the percentage loss in weight according to the formula below:

$$\text{Moisture content} = \frac{\text{initial weight - final weight}}{\text{Initial weight}} \times 100$$

Total ash content of the samples was determined according to the dry ashing method (AOAC, 2000). Two (2) g of samples were weighed into pre-weighed incinerated cooled porcelain crucibles. Incineration of samples was then carried out in a muffle furnace at temperature of between 550^{o} C and 600^{o} C for 6 hours. After removal, they were cooled to room temperature in desiccators and weighed. The ash content was obtained from the different between the final weight and the porcelain crucible expressed as percentage of initial sample weight. All analyses were done in triplicates.

3.14. Microbial Assessment

3.14.1. Aerobic Bacteria Isolation and Identification

The microorganisms at each stage of fermentation were periodically isolated and identified. Total Aerobic Plate Count (TAPC) enumeration in the inoculum and the fermenting materials were carried out according to the method of APHA (2012). Media such as Nutrient agar, MacConkey agar, Eosin Methylene Blue agar, EMB broth, Salmonella-Shigella agar, Selenite F broth, Lactose broth and Potato dextrose agar were used. Ten (10) g of each sample was aseptically removed and diluted in 90 ml sterile physiological saline (0.1% w/v bacteriological peptone, 0.85% w/v NaCl) and homogenized. Sequential dilutions of the homogenate were obtained by plating one ml aliquot of 10^{-5}, 10^{-6} and 10^{-7} dilutions on the different media listed above. The plates were inoculated in duplicates and incubated for 24- 48 h at 37^{o} C. Distinct colonies were randomly selected and the colonies were repeatedly streaked on same agar plates until pure cultures were obtained (Harrigan and McCance, 1979).

The presumptive identification of the isolates using phenotypic characteristics was based on the various tests carried out using Bergey's Manual of Systemic Bacteriology (Sneath *et al.,* 2009). Details results of the reactions of the suspected isolates to biochemical tests are shown in Appendix 2.

a. Gram's Staining

Microscopic morphology of the bacterial isolates was carried out using the Gram staining technique following the method of (Harrigan and McCance, (1979). A thin smear of pure isolates was made on a clean grease free slide and heat fixed by passing the slide over a flame. The smear was flooded with crystal violet and allowed to stay for 60 s. The crystal violet was washed off with distilled water and the smear was flooded with Gram's iodine for 30 s, the Grams iodine were washed off with 95 % ethyl alcohol for 60 s and rinsed the slide with distilled water for a few seconds. A few drops of safranin solution was used to counter stain the smear and allowed to stay for 20 s before rinsing off with distilled water. The slides were allowed to air dry before observation under the microscope. The slide was

then allowed to air dry. Slides were observed using oil immersion lens of a light microscope at 100X magnification (Harrigan and McCance, 1979).

b. Coagulase Test

In carrying out this test, a drop of physiological saline was dropped on both ends of a sterile slide. A portion of the isolated organism was added to the two portions and smears were made. After this, a drop of rabbit serum was added to one of the smears and clumping was observed (Harrigan and McCance, 1979). A clumping showed positive test.

c. Carbohydrate Fermentation Test

This was carried out to know the ability of the isolates to metabolize different sugars with the production of acid and / or gas using lactose, maltose, sucrose glucose and mannitol. For each sugar, 2.5 g were weighed into different 500 ml conical flask, 1.5 g sodium chloride was added, and 2.5 g tryptone and 0.0004 g of phenol red were added, 250 ml of distilled water was added, swirled and dispensed into tubes containing Durham's tubes (Seeley and Van Denmark, 1972). The pH of the medium was checked. Each sugar was dispensed into labeled universal tubes. The Durham's tubes were as well filled with sugars and carefully placed into corresponding universal bottle by inversion. A drop of the organism from peptone was inoculated into each sugar, using a syringe; the tubes were incubated for 18 – 24 h and then examined for acid and gas production.

d. Catalase Test

The enzyme catalase is produced by microorganisms that are capable of breaking down hydrogen peroxide into water and oxygen with the production of gas bubbles. When the gas bubbles are formed, it is an indication of the presence of catalase enzyme.

$$2H_2O_2 \rightarrow 2\ H_2O + O_2$$

The was carried out by picking a colony of the 18 - 24 h old culture of the organisms and making a smear on a clean grease free slide. A drop of freshly prepared 3 % hydrogen peroxide solution was added to the smear and observed for gas bubbles formation which

represents positive catalase reaction while the absence of gas bubble indicates a negative reaction (Harrigan and McCance, 1979).

e. Oxidase Test

A Whatmann No 1 filter paper was used for this test. Few drops of the oxidase reagent (1% aqueous tetramethyl-p-phenyl ethylenediamine dihydrochloride) were placed on a filter paper with a sterile loop to form a spot. The test organism was put onto this spot with a sterilized wire loop. Formation of a very deep purple colour within 10 s is indication of a positive reaction. A delayed reaction or no development of purple colouration indicated a negative reaction (Seeley and Van Denmark, 1972).

f. Methyl Red Test

Glucose phosphate broth was prepared and sterilized as described by Harrigan and McCance, (1976). The test organisms were inoculated into the broth after cooling. The test mixture was incubated for 48 h after which 2 - 3drops of methyl red was added. A positive result was indicated by the production of a bright red colour while a negative test shows yellow colouration.

g. Voges-Proskauer Test

Voges proskauer medium was inoculated with a loopful of 18 - 24 h old broth culture of the isolates and incubated at 35^{o} C for 5 days and uninoculated broth served as control. To 1.0 ml of the culture of the individual isolate was tested by adding 0.5 ml of 16 % KOH and 0.5 ml of 6 % α-Naphthol. The content of each tube was shaken thoroughly and left to stand. Appearance of a red coloration within 5 - 10 minutes was indicative of a positive result while a reddish brown colour indicated a negative result (Harrigan and McCance, 1976).

h. Starch Hydrolysis

Equimolar amount of soluble starch was prepared and added to nutrient agar to compose modified agar without glucose and meat extract to give a 2 % NA-starch medium. The medium was sterilized and allowed to cool before pouring into plates. The agar was left to

set and single streaks of each isolate were made on the surface of the agar. The plates were incubated at 30^{o} C for 48 h after which they were flooded with Gram's iodine. Unhydrolysed starch gives blue-black colouration with iodine and the formation of clear zones around the region of growth of each test isolate indicates starch hydrolysis (Seeley and Van Denmark, 1972).

i. Citrate Utilization

Sterile Koser's citrate medium in scrupulously cleaned screw-capped bottles was stab-inoculated with 24 h old peptone water cultures of isolates. The bottles were incubated at 35^{o} C for 48 h. A change in the colour of the bromothymol blue indicator from green to blue indicated the utilization of citrate as a sole carbon source and negative result remained unchanged.

j. Indole Production Test

Two (2) % w/v peptone broth was prepared, 5 ml of which were dispensed into test tubes and sterilized. The isolates were inoculated into broth and incubated at 37^{o} C for 3 days. 0.5ml of kovac's reagent was then added to each tube of test and tubes were gently shaken and allowed to stand. A rosepink alcohol layer at the surface of medium indicated a positive reaction while no change in colour was recorded as negative (Seeley and Van Denmark, 1972).

k. Motility Test

The hanging drop slide was used in this test. In doing this, a toothpick was used to spread vaseline on the four corner of a clean coverslip. The bacterial culture was thoroughly mixed and a small suspension drop was aseptically placed at the centre of the coverslip with the aid of a inoculating loop. The depression slide was lowered with the concavity facing down onto the coverslip and the drop protrudes into the center of the concavity of the slide. It was gently pressed so that a seal was formed. The hanging drop slide was turned over and placed on the stage of the microscope so that the drop was over the light hole. The drop was examined by first locating its edge under low power and focusing on the drop before switching to the high-dry objective (40 X). The diaphragm was closed in

order to increase the contrast and to see the bacteria clearly. The coverslips and other contaminated slides were discarded in a container with disinfectant solution (Seeley and Van Damark, 1972).

l. Urease Test

This was carried out to determine the ability of the isolates to hydrolyse high concentration of urea to ammonia using Christenen's urea agar. The medium was sterilized at 121° C for 15 min. One ml of 2 % filter sterilized urea solution was added and mixed with the medium and the bottles were allowed to set. The bottles were then inoculated with the isolates and incubated at 25 ± 2° C for 7 days. The change in colour of the medium from yellow to red indicated the production of ammonia given a positive result (Seeley and Van Damark, 1972).

3.14.2. Fungal Identification

a. Inoculum Preparation

Fungal identification was carried out using morphological and physiological methods (Chander, 2002; Tsuneo, 2010). For the isolation, samples were cultured on Potato dextrose agar (SDA) and incubated at room temperature (29±2^{o} C) for 5 to 7 days. In preparing the fungal stock inocula, 7 to 14 day cultures grown on SDA with the addition of chloramphenicol for preventing bacterial growth were used. Sufficient fungal growth was observed after which the colonies were covered with 5 ml 0.0 % sterile saline. After this, suspensions were made by gently probing the surface of the covered colonies with the tip of a sterile Pasteur pipette. The suspension was immediately transferred to a sterile tube and allowed to settle for 15 min at room temperature after which the homogenous upper liquid was decanted and used for further experiment. For the identification, both microscopic and macroscopic features of the hyphal mass, morphology of produced spores and the nature of the fruiting bodies were considered (Tsuneo, 2010).

b. Turbidity Standard for Preparing Fungal Inoculum

The inoculum density for fungal enumeration is usually standardized using a $BaSO_4$ turbidity standard which equals a 0.5 McFarland standard or its optical equivalent.

Microscopic enumeration was used to adjust the inoculum size to be between 1.0×10^6 and 5.0×10^6 spores/ml using a haemocytometer (Neubauer chamber). In some instances, no conidia were produced and for such, small mycelia was collected and homogenized with the aid of a tissue grinder in 2 ml of sterile saline. Sterile saline was thereafter used to adjust the suspensions that resulted to the opacity of 0.5 McFarland standards. Quantification of the inocula was then done by counting of microconidia in a hematocytometer and also by plating 0.01 ml of the suspensions on SDA plates which were subsequently incubated at 28° C and checked daily for fungal growth (Indira, 2014).

3.14.3. Anaerobic Bacteria Identification

For isolation of *Clostridium* species, samples were cultured twice i.e on Reinforced Clostridia medium (RCM) (Oxoid, USA) and were later sub-cultured onto blood agar and incubated at 37º C for 7 days in an anaerobic jar. Developed colonies were counted and recorded (Guo *et al.,* 2013; Ayandiran *et al.,* 2014). Pure culture was later obtained by a series of sub-culturing of distinct colonies and the isolated pure organisms were temporarily stored on freshly prepared slant. Confirmation of the presumptive colonies were done by standard morphological and biochemical methods earlier described in section 3.13 and with the aid of corresponding rapid API kits (20300 API 20 A) in an anaerobic condition (Guo *et al.,* 2013).

Other anaerobes were isolated through the use of a basal medium according to the method of Balch *et al.* (1977). The compositions of 1 L of the medium was 1.0g ammonium chloride, 0.1g magnesium chloride, 0.4g potassium di-hydrogen phosphate, 0.4g di-potassium hydrogen phosphate, 0.0001g resazurin, 0.5g cysteine HCl, 0.5g sodium sulphide, 7g sodium bicarbonate, 10g calcium carbonate, 2g yeast extract, 10 ml vitamin solution, 10 ml mineral solution and 20g agar with a final pH of 6.7 (Balch *et al.,* 1977). The morphological and biochemical characteristics of the anaerobes were determined using tests like the Gram staining, Indole, Methyl Red, Voges-Proskauer, Citrate, Triple sugar iron, Lipid hydrolysis, Starch hydrolysis and Mannitol tests. The probable isolates were then identified using the 20300 API 20 A for anaerobes. Procedures for Gram staining, Indole, Methyl Red, Voges-Proskauer, Citrate and starch hydrolysis are already explained in section 3.14.1.

a. Triple Sugar Iron Test

The Triple Sugar Iron (TSI) is a test which has three sugars (Lactose, Sucrose and Glucose) and also Iron and it also contains agar-agar as the solidifying agent. The test was done using the semi-solid media having both slant and butt. A sterilized inoculating needle was used to touch the top of a well-isolated bacterial colony and was inoculated on the TSI agar by stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant. The cap of the tube was loosely left on and incubated at 35^{o} C for 18 to 24 h. Production of large amount of acid which turns the phenol red indicator yellow both in the butt and in the slant indicates the fermentation of lactose or sucrose. However, when glucose fermented, the oxygen deficient butt was yellow, but on the slant, the acid was oxidized to carbondioxide and water by the organism and the slant was red (Prescott *et al.,* 2008).

b. Lipid Hydrolysis

In this test, the test bacteria were grown on agar plates containing tributyrin as the lipid substrate. When dispensed in the agar, tributyrin formed an emulsion producing an opaque medium. When the bacteria hydrolysed lipid, its colonies hydrolysed the tributyrin in the medium in the areas surrounding them to soluble glycerol and fatty acids (butyric acid) while the rest of the areas of the plates contain unhydrolysed tributyrin. As a result of this, transparent clear zones were formed around the colonies because the hydrolysed products i.e. glycerol and fatty acids do not form emulsion with the agar. On the other hand, the remaining area of the plates was opaque because the unhydrolysed tributyrin formed emulsion with the agar in these areas (Balch *et al.,* 1977).

3.14.4. Identification of Methanogens

For methanogens, the enriched mineral medium described for methanogenic bacteria evaluation (Ghosh *et al.,* 2014; Manimegalai *et al.,* 2014) was compounded and used in this study. It was prepared by mixing 1 L basal medium (BM) with 10 mL supplement solution, 40 mL 1 M $NaHCO_3$, 1 mL 5% (w/v) cysteine–HCl, and 2.5 mL 36 mM $FeSO_4$ (in 50 mM H_2SO_4). The BM contained NH_4Cl (0.5 g), KH_2PO_4, (0.4 g) $MgCl_2.6H_2O$ (0.15 g), $CaCl_2.2H_2O$ (0.05 g), $NaHCO_3$ (1.0 g), trace element solution [10×] (1 mL), vitamin solution [10×] (1 mL), sodium resazurin (0.001 g), Na_2S (0.50 g), cysteine–HCl (0.50 g), and Sodium–thioglycolate (0.50 g). Double distilled water (DDW) was used to make the

volume up to 1.0 L and made to have a final neutral (7.0) pH. The vitamins that made up part of the supplement solution are cyanocobalamin (5 mg), p-aminobenzoic acid (4 mg), biotin (1 mg), nicotinic acid (10 mg), calcium pantothenate (5 mg), pyridoxamine–2HCl (15 mg) and thiamine–HCl) (10 mg). The trace elements components are HCl (1.6 mM), $FeCl_2.7H_2O$ (100 mg), $ZnCl_2$ (7 mg), $MnCl_2.4H_2O$ (10 mg), H_3BO_3 (0.6 mg), $CoCl_2.6H_2O$ (13 mg), $CuCl_2.2H_2O$ (0.2 mg), $NiCl_2.6H_2O$ (2.4 mg), $Na_2MoO_4.2H_2O$ (3.6 mg), $Na_2SeO_3.5H_2O$ (0.26 mg) and Na_2WO_4 (0.66 mg) all dissolved in double distilled water. Before compounding, the BM and $FeSO_4$ were autoclaved separately while the $NaHCO_3$ and cysteine–HCl was filter sterilized before addition to the medium according to standard method (Stieglmeier *et al.,* 2009). Nitrogen gas was continuously sparged into all the liquid media at the rate of 10 mL/min for 30 min in order to rid them of dissolved oxygen (DO). This was done until resazurin (indicator dye) turned colorless. Samples were cultured on the compounded media and sub-culturing was done until pure culture was obtained. The morphological and biochemical characteristics of the methanogens were determined using tests like the Gram staining, Indole, Methyl Red, Voges-Proskauer, Citrate, Triple sugar iron, Lipid hydrolysis, Starch hydrolysis and Mannitol tests as described in section 3.13.3. All experiments were done in an anaerobic chamber leaving a 10% headspace in the jar.

3.15. Culture preservation

The pure cultures of the aerobes, anaerobes and methanogens were maintained on nutrient and thioglycolate broths respectively and kept in refrigerator at 4 ^oC. The stock cultures were sub-cultured in appropriate broth at 30 ^oC for 24-48 h before use for further work. The organisms were maintained at -80^o C with the addition of 20% (v/v) glycerol as cryoprotective agent and for long term preservation.

3.16. Daily Monitoring of Operational Parameters

In order to study and determine the most feasible local environmental conditions to optimally operate the biogas facilities, various physicochemical parameters were periodically evaluated in order to assess the stability of the digesters. Monitoring was done daily between 0800 and 1800 h. Discussed below are the physicochemical parameters monitored:

3.16.1. Temperature

This gives the kinetic energy of atoms or molecules. It was measured to determine the feedstock influence on the temperature and consequently, the metabolism of the bacteria. Thermometers were used to measure the temperatures of the digesters and that of Omu Aran. The digester temperatures were taken twice daily, i. e. 0900hrs and 1800 h respectively while the ambient Omu Aran temperature was recorded at 1300 h daily. Black polythene nylons were used to cover the digesters at night so as to eliminate the possibilities of heat loss.

3.16.2. pH

This gives the intensity of acidic or alkalinity of a medium at a given temperature. It was measured to determine the feedstock influence on the acidity/alkalinity and consequently, the metabolism of the bacteria. Samples were analyzed at ambient temperature with a pH meter. The meter was calibrated every week and analyses were carried out immediately after sampling to avoid loss of carbon dioxide from the sample.

3.17. Gas Analysis

The methane (CH_4) and carbon dioxide (CO_2) content of biogas were determined by Gas Chromatography described in section 3.2 (Borowski and Weatherly, 2013; Dahunsi and Oranusi, 2013; Alfa *et al.,* 2014b). Details of GC-FID calibration, mode of operation and volume analysed is shown in Appendix 3.

3.18. Modeling and Statistical Data Analysis

3.18.1. Response Surface Methodology (RSM)

In order to standardize the important parameters in biogas production as this will be useful during scale-up or industrial production, the RSM was used to statistically analyze the biogas generated data. This was done in order to appropriately fit the generated quadratic polynomial equation using the version 9.0.3.1 of the Design-Expert software (Stat-Ease Inc., Minneapolis, USA). Multiple regressions were employed in order to fit the coefficient of the polynomial model of the response so as to correlate it to the independent variables. Test of significance and analysis of variance (ANOVA) were used to evaluate the quality of the fit of the model as shown below:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + e \quad (3.1)$$

Where:

Y = response factor, b_o = the intercept value, b_i (i= 1, 2,…k) = the first order model coefficient, b_{ij} = the interaction effect, b_{ii} = the quadratic coefficients of X_i, e = the random error and X_iX_j = range of independent factors.

3.18.2. QuickProp (QP) of ANNs

ANNs was also used to statistically analyse the date obtained from the CCD in order to ascertain the most appropriate tool for modeling of gas generation from the biomass used in this study. QuickProp was used as the learning algorithms while the multilayer connection type used was multilayer normal feed forward (MNFF). Meanwhile, the optimum ANNs structure was determined using mean square error (MSE) approach. The higher coefficient R^2 was determined; the variable analysis also was conducted to study the effects of variables towards the biogas yield using 3-Dimensional curvature surface plots and relative importance. A hybrid ANN model was used in conducting process optimization. The results obtained from the ANNs were compared with that of RSM.

3.18.3. Validation of Experiment

After the comparison between RSM and ANNs optimization result, validation was carried out by setting up each of the biogas digestion regime in replicates of three using the predicted values. Data were collected from all three replicates and the average was taken as the final optimized result.

3.19. Microbial Optimization of Biogas Production

This was done so as to assess the biogas producing potentials of the microorganisms isolated as against the conventional use of microbial consortia popularly used in most studies including this one. The three best substrates from the biogas production and optimization studies were the co-digeston of *Chromolaena odorata* shoot + poultry

dropping followed by the co-digestion of *Tithonia diversifolia* + poultry dropping and then the mono digestion of *Chromolaena odorata* shoot. The microbial optimization procedure was carried out by digesting each of these three substrates with different combinations of already characterized organisms (acid and methane formers from this study) which were prepared under anoxic conditions. In each case, a fresh broth culture was prepared which was inoculated using isolates from their respective stock cultures and incubated for 24 to 48 h. The predicted and validated conditions of operation were followed and the generated gas was subsequently analyzed for its methane content. For the microbial optimization of each of the chosen substrates, three different combinations were carried out and used in the anaerobic digestions as shown in Table 3.3:

Table 3.3: Different Experimental Combinations for Microbial Optimization

Code	Substrate	Presumptive Methanogen	Presumptive Acidogen
a.	*Chromolaena odorata* shoot + poultry dropping	*Methanococcus* sp.	*Clostridium* sp.
b.	*Chromolaena odorata* shoot + poultry dropping	*Methanosarcinales* sp.	*Clostridium* and *Fusobacterium* spp
c.	*Chromolaena odorata* shoot + poultry dropping	*Methanosaeta* sp.	*Clostridium, Fusobacterium* and *Porphyromonas* spp
d.	*Tithonia diversifolia* shoot + poultry dropping	*Methanococcus* sp.	*Clostridium* sp.
e.	*Tithonia diversifolia* shoot + poultry dropping	*Methanosarcinales* sp.	*Clostridium* and *Fusobacterium* spp
f.	*Tithonia diversifolia* shoot + poultry dropping	*Methanosaeta* sp.	*Clostridium, Fusobacterium* and *Porphyromonas* spp
g.	*Chromolaena odorata* shoot	*Methanococcus* sp.	*Clostridium* sp.
h.	*Chromolaena odorata* shoot	*Methanosarcinales* sp.	*Clostridium* and *Fusobacterium* spp
i.	*Chromolaena odorata* shoot	*Methanosaeta* sp.	*Clostridium, Fusobacterium* and *Porphyromonas* spp

Each experiment was done in five replicates

3.20. Biofertilizer Development Procedures

Each digestate from the digestion regimes was analyzed physic-chemically and microbiologically as stated in sections 3.12 and 3.13. Curing followed for 20 days in sterile sacks and stored in dry forms (Plate 7 of Appendix section) before further actions were taken (Alfa *et al.,* 2013a, b). Same analyses were carried out after dewatering and prior to field application.

3.21. Biofertilizers Phyto-Assessment with Maize (*Zea mays*)

The nutritive value of each newly produced fertilizer was confirmed as stated below:

3.21.1. Emphasis on Nitrogen Content

Great emphasis was placed on the Nitrogen composition of each biofertilizer preparation. This is due to the paramount importance of the element in plant growth, vigor as well as serving as good substrate for protein synthesis.

3.21.2. Application Rates

Fertilizer application followed a standard protocol (Baldotto *et al.,* 2012) of 10 kg N/ha (taking 2,000,000 kg of soil in one hectare of land as standard). The application rate then followed the following order for six different runs subsequently performed:

3.21.3. *Tithonia diversifolia* Biofertilizer as Example

Total Nitrogen (N) in the *Tithonia diversifolia* biofertilizer was 0.334 mg/g.

Conversion of this value to percentage $\left(\frac{0.334 \times 100}{1000}\right)$ which gave 0.03%

If 10 kg N/ha is the recommended rate and 5 kg of soil was used in the experiment, the quantity of *Tithonia diversifolia* biofertilizer needed was

$$\frac{100}{0.03} \times 10 \times \frac{5}{2{,}000{,}000} \times (1000)\text{g}$$

This gave 83.3 g of *Tithonia diversifolia* biofertilizer to 5 kg of soil

Assumption: 2,000,000 kg of soil is in 1 hectare of land. To convert the *Tithonia diversifolia* biofertilizer to g, it was multiplied by 1000.

The table below shows the quantity of *Tithonia diversifolia* biofertilizer that was used for applications of 10, 20, 30, 40, 50 and 60 kg N/ha respectively

Table 3.4: Quantity of *Tithonia diversifolia* Shoot Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	83.3 g
4.	20 kg N/ha	166.6 g
5.	30 kg N/ha	249.9 g
6.	40 kg N/ha	333.2 g
7.	50 kg N/ha	416.5 g
8.	60 kg N/ha	499.8 g

Table 3.5: Quantity of *Chromolaena odorata* Shoot Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	62.5 g
4.	20 kg N/ha	125.0 g
5.	30 kg N/ha	187.5 g
6.	40 kg N/ha	250.0 g
7.	50 kg N/ha	312.5 g
8.	60 kg N/ha	375.0 g

Table 3.6: Quantity of *Carica papaya* Peels Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	50.0 g
4.	20 kg N/ha	100.0 g
5.	30 kg N/ha	150.0 g
6.	40 kg N/ha	200.0 g
7.	50 kg N/ha	250.0 g
8.	60 kg N/ha	300.0 g

Table 3.7: Quantity of *Telfairia occidentalis* Peels Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	62.5 g
4.	20 kg N/ha	125.0 g
5.	30 kg N/ha	187.5 g
6.	40 kg N/ha	250.0 g
7.	50 kg N/ha	312.0 g
8.	60 kg N/ha	375.0 g

Table 3.8: Quantity of *Arachis hypogaea* Hull Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	83.3 g
4.	20 kg N/ha	166.6 g
5.	30 kg N/ha	249.9 g
6.	40 kg N/ha	333.2 g
7.	50 kg N/ha	416.5 g
8.	60 kg N/ha	499.8 g

Table 3.9: Quantity of *Tithonia diversifolia* Shoot and Poultry Dropping Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	50.0 g
4.	20 kg N/ha	100.0 g
5.	30 kg N/ha	150.0 g
6.	40 kg N/ha	200.0 g
7.	50 kg N/ha	250.0 g
8.	60 kg N/ha	300.0 g

Table 3.10: Quantity of *Chromolaena odorata* Shoot and Poultry Dropping Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	50.0 g
4.	20 kg N/ha	100.0 g
5.	30 kg N/ha	150.0 g
6.	40 kg N/ha	200.0 g
7.	50 kg N/ha	250.0 g
8.	60 kg N/ha	300.0 g

Table 3.11: Quantity of *Carica papaya* Peels and Poultry Dropping Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	62.5 g
4.	20 kg N/ha	125.0 g
5.	30 kg N/ha	187.5 g
6.	40 kg N/ha	250.0 g
7.	50 kg N/ha	312.0 g
8.	60 kg N/ha	375.0 g

Table 3.12: Quantity of *Telfairia occidentalis* Peels and Poultry Dropping Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	50.0 g
4.	20 kg N/ha	100.0 g
5.	30 kg N/ha	150.0 g
6.	40 kg N/ha	200.5 g
7.	50 kg N/ha	250.0 g
8.	60 kg N/ha	300.0 g

Table 3.13: Quantity of *Arachis hypogaea* Hull and Poultry Dropping Biofertilizer Needed

S/N	Application Rate (Kg N/ha)	Quantity needed
1.	Negative Control	No fertilizer application
2.	NPK 15: 15: 15	66.7 g
3.	10 kg N/ha	62.5 g
4.	20 kg N/ha	125.0 g
5.	30 kg N/ha	187.5 g
6.	40 kg N/ha	250.0 g
7.	50 kg N/ha	312.0 g
8.	60 kg N/ha	375.0 g

3.22. Soil Preparation: Sand-loamy soil of low nutrient (<5% Nitrogen determined via analysis) was used. This soil type was chosen to ensure effective evaluation of the potency/effectiveness of the applied biofertilizers. Five (5) kg of soil was used per pot experiment and mixing of the biofertilizer with the soil was done and allowed to incubate for two weeks before commencement of planting. Inorganic fertilizer (NPK 15-15-15) was used as positive control while an experiment without any fertilizer application was also set up as negative control. Each experiment was done in triplicate and prior to planting of maize on the prepared soil, and after harvesting, soil samples were collected for analysis as reported in sections 3.12 and 3.13.

3.23. Planting and Data Collection

The viability of the maize seeds used for this experiment were evaluated by soaking in water for 24 h and kept at a temperature of 30^{o} C after which the seeds that sank to the bottom of beaker were taken as viable and used in the experiments (El-Abady, 2014). Two (2) maize seeds were planted in each pot experiment and data was collected every 5-day after seed emergence (DAE) on the following phyto-parameters: Leaf number, Leaf area, Plant height, Stem girth, Plant biomass above soil level, Root biomass and Root length. The last 3 parameters were evaluated after the harvesting of the plants. The experimental set-up at 15 DAE is shown in plate 8 of Appendix section. The quantity of elemental nutrients (N, P, K, Ca, Mg, Cu, Zn, Fe, Al, NO_3, NH_4, PO_4, Mn and SO_4) stored in leaves, stem and roots were also evaluated after harvesting using the methods described in section 3.13.

CHAPTER FOUR

4.0 RESULTS

4.1. Feedstock Composition

The results of the physico-chemical analyses of the five substrates prior to anaerobic mono-digestion are shown in tables 4.1a. The result of chemical analyses showed that though the nutrient/elemental composition of each of the five substrates differs in concentration, shoot of *Tithonia diversifolia* was the richest in terms of major elements which includes Nitrogen (37.31 g/kg TS), Potassium (8.41 g/kg TS), Phosphorus (7.16 g/kg TS) and others while the hull of *Arachis hypogaea* was the poorest with values of 34.08 g/kg TS, 5.21 g/kg TS, 3.05 g/kg TS for the three elements respectively. In terms of total solids content, the shoot of *Chromolaena odorata* was the bulkiest (103.54 g/kg) while the peels of *Telfairia occidentalis* was the lightest with value of 71.91 g/kg TS. In terms of minor nutrients e.g Zinc, Aluminium and Copper, the peels of *Carica papaya* was the richest with values of 32.32 g/kg TS, 0.52 g/kg TS and 3.87 g/kg TS respectively. All five biomass were low in C/N ratio and values between 7 and 10 were recorded for them.

Table 4.1a: Physical and Chemical Characteristics of the Five Biomass (Without Rumen Content) Before Digestion

Parameters	***Tithonia diversifolia* Shoot**	***Chromolaena odorata* Shoot**	***Carica papaya* Peels**	***Telfairia occidentalis* Peels**	***Arachis hypogaea* Hull**
pH	6.58±0.10	6.54±0.22	6.23±1.00	5.98±0.12	6.71±0.10
Total Solids (g/kg)	88.31±0.01	103.54±0.21	94.81±1.21	71.91±1.02	93.13±0.12
Volatile Solids (g/kg)	76.08±0.00	90.05±0.01	83.23±0.22	62.71±1.02	72.61±0.20
Ash Content (%)	2.20±1.00	4.44±0.02	2.54±1.00	4.00±2.01	7.32±0.26
Moisture Content (%)	94.22±0.10	89.32±0.11	97.26±0.01	95.52±0.11	82.90±3.02
COD (mg/kg TS)	193.21±1.01	187.21±0.02	165.11±2.20	142.21±1.02	120.15±1.01
Total Carbon (g/kg TS)	286.65±1.01	230.51±2.02	252.90±4.03	243.20±3.02	342.20±2.03
Total Nitrogen (g/kg TS)	37.31±0.10	28.21±0.02	35.51±2.02	25.12±0.21	34.08±1.06
C/N	8/1	8/1	7/1	10/1	10/1
Acetate (g COD/g VS)	0.06±0.12	0.06±0.12	0.08±0.10	0.06±0.12	0.01±0.10
Propionate (g COD/g VS)	0.08±0.10	0.06±0.10	0.09±0.10	0.06±0.10	0.03±0.02
TVFAs (g COD/g VS)	0.17±0.02	0.17±0.02	0.15±0.02	0.17±0.02	0.05±0.10
Ammonia (mg/g VS)	0.09±0.11	0.08±0.11	0.09±0.01	0.08±0.11	0.73±0.01
Total Phosphorus (g/kg TS)	7.16±0.02	4.12±0.01	5.32±1.02	3.21±1.02	3.05±0.01
Potassium (g/kg TS)	8.41±1.00	5.69±1.00	7.32±2.00	5.61±0.22	5.21±0.02
Phosphate (g/kg TS)	2.10±0.01	1.98±0.12	1.03±0.11	1.81±0.10	1.80±0.40
Sulphate (g/kg TS)	111.30±2.00	91.09±1.01	112.20±3.01	101.11±1.02	101.02±3.00
Calcium (g/kg TS)	184.61±1.01	339.31±5.01	220.81±4.41	257.09±4.02	92.02±3.02
Magnesium (g/kg TS)	57.50±1.01	61.29±0.11	89.32±1.02	52.21±2.02	62.21±2.05
Manganese (g/kg TS)	0.59±0.02	0.14±0.01	0.021±1.00	0.016±0.01	0.13±0.01
Iron (g/kg TS)	1.03±0.01	0.68±0.10	1.06±0.11	0.62±1.23	0.11±0.01
Zinc (g/kg TS)	31.31±0.21	24.21±0.11	32.32±0.01	24.02±1.03	31.29±0.01
Aluminium (g/kg TS)	0.05±0.21	0.38±1.00	0.52±1.02	0.45±2.00	0.03±0.01
Copper (g/kg TS)	3.32±1.00	3.21±1.02	3.87±0.03	2.81±0.11	3.00±0.01

N = 120; COD = Chemical Oxygen Demand; C/N = Carbon: Nitrogen ratio

Table 4.1b shows the results of the physico-chemical of the five substrates after addition of rumen content. As shown in the table, the addition of the rumen content brought the pH values to more alkaline range. Also, there was increase in concentration of most elements after the addition. The shoot of *Tithonia diversifolia* was richest among the five substrates in the concentration of phosphorus (6.26 g/kg TS), potassium (8.10 g/kg TS), aluminium (0.80 g/kg TS), zinc (39.00 g/kg TS), copper (4.80 g/kg TS), manganese (0.03 g/kg TS), sulphate (136.00 g/kg TS) and phosphate (3.00 g/kg TS). The highest concentration of nitrogen (41.01 g/kg TS) was found in the peels of *Telfairia occidentalis* while the shoot of *Chromolaena odorata* recorded the highest value for both total solids (120.64 g/kg TS) and calcium (400.00 g/kg TS). The highest concentration of ammonia (2.59 g/kg TS) was found in the hull of *Arachis hypogaea*. For C/N ratio, all the five substrates recorded moderate to high (15-20) ratios after addition of rumen content with the shoot of *Chromolaena odorata* having the highest value of 20.

Table 4.1b: Physical and Chemical Characteristics of the Five Biomass after Addition of Rumen Content (Inoculum) Before Digestion

Parameters	***Tithonia diversifolia* Shoot**	***Chromolaena odorata* Shoot**	***Carica papaya* Peels**	***Telfairia occidentalis* Peels**	***Arachis hypogaea* Hull**
pH	7.55±0.12	7.80±0.12	7.70±0.02	7.65±0.01	7.75±1.02
Total Solids (g/kg)	110.68±0.11	120.64±0.11	110.97±0.11	87.58±0.12	105.46±0.01
Volatile Solids (g/kg)	95.40±0.22	94.70±0.02	96.22±3.02	76.81±0.10	88.75±1.01
Ash Content (%)	3.60±0.02	5.30±0.01	2.78±0.00	4.19±0.51	8.25±1.01
Moisture Content (%)	89.32±0.11	87.35±2.01	94.03±4.01	92.42±0.11	88.54±0.02
COD (mg/kg TS)	225.09±0.11	202.26±1.40	256.5±4.04	269.02±5.01	132.02±0.21
Total Carbon (g/kg TS)	589.10±3.11	549.22±5.22	588.90±5.03	678.60±2.01	588.70±0.04
Total Nitrogen (g/kg TS)	37.00±1.12	29.00±0.22	40.00±1.01	41.01±9.11	37.03±0.05
C/N	16/1	20/1	15/1	17/1	16/1
Acetate (g COD/g VS)	0.11±1.10	0.11±1.10	0.10±1.10	0.11±1.10	0.06±1.10
Propionate (g COD/g VS)	0.15±0.03	0.15±0.03	0.15±0.01	0.15±0.03	0.08±0.05
TVFAs (g COD/g VS)	1.21±0.10	1.21±0.10	1.22±0.10	1.21±0.10	0.22±0.10
Ammonia (mg/g VS)	2.01±1.10	2.01±1.10	2.04±1.10	2.01±1.10	2.59±0.11
Total Phosphorus (g/kg TS)	6.26±0.13	4.26±0.12	6.12±0.01	4.18±0.10	5.84±1.02
Potassium (g/kg TS)	8.10±1.03	6.60±0.11	8.00±0.11	6.10±0.11	7.80±0.21
Phosphate (g/kg TS)	3.00±0.02	2.10±0.11	3.00±0.10	2.20±0.01	2.30±1.11
Sulphate (g/kg TS)	136.00±5.01	106.00±6.10	136.00±2.03	114.00±5.10	132.00±1.00
Calcium (g/kg TS)	210.00±5.02	400.0±2.42	226.00±4.09	279.00±8.01	112.00±1.00
Magnesium (g/kg TS)	78.00±3.03	56.00±2.02	100.00±0.03	56.00±0.11	80.0±2.01
Manganese (g/kg TS)	0.03±0.02	0.018±0.04	0.028±0.00	0.022±0.01	0.020±0.01
Iron (g/kg TS)	1.02±0.00	0.80±0.03	1.16±0.21	0.80±0.12	0.104±1.01
Zinc (g/kg TS)	39.00±2.02	26.00±0.03	36.00±0.03	29.00±2.01	37.00±1.01
Aluminium (g/kg TS)	0.80±0.10	0.46±0.10	0.76±0.02	0.56±0.01	0.70±2.01
Copper (g/kg TS)	4.80±0.10	3.30±0.12	4.70±0.03	3.40±0.10	4.40±2.01

N = 120; COD = Chemical Oxygen Demand; C/N = Carbon: Nitrogen ratio

Table 4.1c shows the results of the physicochemical analyses of the mixture of the five substrates with poultry dropping for the co-digestion experiments. In all biomass, the mixture of *Carica papaya* peel and poultry dropping and the mixture of *Arachis hypogaea* hull and poultry dropping were the richest in the composition of most elements/nutrients. The mixture of *Carica papaya* peel and poultry dropping was highest in the concentrations of phosphorus (6.12 g/kg TS), iron (1.24 g/kg TS) and copper (5.00 g/kg TS). The mixture of *Arachis hypogaea* hull and poultry dropping was richest in the concentrations of carbon (698.21 g/kg TS), ammonia (5.24 g/kg TS), sulphate (142.50 g/kg TS), phosphate (3.10 g/kg TS), manganese (0.030 g/kg TS), magnesium (109.00 g/kg TS) and aluminium (0.100 g/kg TS). The highest concentrations of nitrogen (48.00 g/kg TS) and total solids (128.01 g/kg TS) were recorded in the mixture of *Telfairia occidentalis* peels and poultry dropping while the mixture of *Tithonia diversifolia* shoot and poultry dropping had the highest calcium concentration of 180.00 g/kg TS. In terms of C/N, all five substrates recorded moderate values of between 15 and 16 with the mixture of *Telfairia occidentalis* peels and poultry dropping and the mixture of *Arachis hypogaea* hull and poultry dropping having the highest ratio of 16. Overall, the mixture of each biomass with poultry dropping increased the nutrient contents over the substrates without poultry dropping. On the other hand, the mixed substrates recorded lower C/N ratios.

Table 4.1c: Physical and Chemical Characteristics of the Five Biomass (With Poultry Droppings) Before Digestion

Parameters	*Tithonia diversifolia* Shoot + Poultry dropping	*Chromolaena odorata* Shoot + Poultry dropping	*Carica papaya* Peels + Poultry dropping	*Telfairia occidentalis* Peels + Poultry dropping	*Arachis hypogaea* Hull + Poultry dropping
pH	7.70±0.12	7.60±0.12	7.65±0.20	7.55±0.20	7.70±0.20
Total Solids (g/kg)	117.63±0.22	110.48±2.01	110.87±1.02	128.01±0.02	100.00±0.02
Volatile Solids (g/kg)	68.89±4.02	97.97±4.01	97.60±1.02	99.63±2.21	96.39±0.02
Ash Content (%)	8.11±1.12	8.93±0.03	6.90±0.02	6.36±0.01	3.61±0.21
Moisture Content (%)	88.37±1.02	88.52±3.07	89.13±3.22	91.89±3.02	90.00±0.01
COD (mg/kg TS)	221.12±0.40	269±3.95	288±1.05	289.45±5.02	260.01±0.23
Total Carbon (g/kg TS)	497.40±4.10	590.10±5.08	690.23±5.12	758.32±5.00	698.21±0.02
Total Nitrogen (g/kg TS)	34.00±0.10	40.00±0.02	47.00±1.01	48.00±1.02	43.00±0.02
C/N	15/1	15/1	15/1	16/1	16/1
Acetate (g COD/g VS)	0.07±1.10	0.06±1.10	0.05±1.02	0.07±1.00	0.13±0.10
Propionate (g COD/g VS)	0.08±0.03	0.09±0.03	0.08±0.03	0.10±0.03	0.12±0.03
TVFAs (g COD/g VS)	0.15±0.10	0.16±0.11	0.17±0.11	0.17±0.11	0.29±0.10
Ammonia (mg/g VS)	1.99±1.11	1.99±1.10	1.96±1.10	1.95±1.10	5.24±0.05
Total Phosphorus (g/kg TS)	5.00±0.14	2.30±.01	6.12±0.02	4.56±0.20	6.00±0.20
Potassium (g/kg TS)	7.40±0.02	8.00±2.11	8.25±0.01	6.12±0.12	8.2±0.12
Phosphate (g/kg TS)	2.60±0.01	2.00±2.21	3.00±0.01	2.30±0.01	3.10±0.02
Sulphate (g/kg TS)	120.00±5.02	70.00±1.02	142.00±0.21	118.00±3.12	142.50±0.21
Calcium (g/kg TS)	180.00±3.90	142.50±0.01	168.00±1.20	160.00±2.11	98.80±3.01
Magnesium (g/kg TS)	76.00±2.03	96.05±0.31	100.00±2.02	70.00±1.22	109.00±0.01
Manganese (g/kg TS)	0.022±0.01	0.18±0.12	0.028±0.00	0.020±0.01	0.030±0.02
Iron (g/kg TS)	0.96±0.02	0.58±0.40	1.24±0.02	0.92±0.01	1.18±0.20
Zinc (g/kg TS)	31.00±1.13	30.90±1.01	38.00±0.12	29.00±1.20	39.00±0.22
Aluminium (g/kg TS)	0.66±0.10	0.80±0.02	0.96±0.02	0.58±0.01	0.100±0.01
Copper (g/kg TS)	4.00±0.00	3.40±0.02	5.00±0.12	3.80±0.02	4.90±0.21

N = 120; COD = Chemical Oxygen Demand; C/N = Carbon: Nitrogen ratio

4.2. Digestion Efficiency and Stability

The results of the efficiency of the digestion process and stability are shown tables 4.2 (a, b) and in Appendix 4(a-j). As shown in the tables, the chemical compositions of all the digestates that resulted from the mono and co-digestions showed elevated levels in elemental and nutrient composition than obtained from the feedstocks prior to digestion. In the mono-digestion experiments, there were increase in the values of moisture content, total nitrogen, total phosphorus, potassium, sulphate, phosphate, magnesium, manganese, iron, zinc, aluminium and copper after the digestion of the substrates while other parameters recorded reduction in values. Similarly in the co-digestions, all the parameter except total and volatile solids and calcium increased in values in the final digestates. The tables also show the physic-chemical parameter results for the poultry dropping and rumen contents used as inoculum. In the co-digestions where poultry dropping was used, it was found to be denser than the mixture of substrates and poultry dropping and inoculum in terms of total and volatile solids. Another major observation was significant reduction in values of the COD of the digestates. Values of 55.89%, 57.55%, 57.60%, 57.28% and 56.52% reductions were recorded for the shoot of *Tithonia diversifolia*, shoot of *Chromolaena odorata*, peels of *Carica papaya*, peels of *Telfairia occidentalis* and hull of *Arachis hypogaea* respectively. Meanwhile, higher COD reductions were recorded in the co-digestions than the mono-digestions and values of 52.36%, 60.60%, 61.88%, 64.10% and 58.40% were recorded for the mixture of *Tithonia diversifolia* shoot and poultry dropping, the mixture of *Chromolaena odorata* shoot and poultry dropping, mixture of *Carica papaya* peels and poultry dropping, mixture of *Telfairia occidentalis* peels and poultry dropping and the mixture of *Arachis hypogaea* hull and poultry dropping respectively. In comparison with the mono-digestions, reduction in values of total and volatile solids, calcium and COD were constant in the co-digestion experiments. The highest COD reduction was recorded in the co-digestion of *Telfairia occidentalis* and poultry dropping.

Volatile fatty acids (VFAs) are usually regarded as the substantial intermediate metabolic product of anaerobic digestion process which can be accumulated and cause inhibition to the process if they are either over-produced or under-consumed by the bacterial community. As shown in tables 4.1 (a-c), VFAs accumulation was not reported during the

early days of digestion in all mono and co-digestion experiments. However, it was observed at very low levels between the 8^{th} to 14^{th} days. In all digestion set-ups, the peak of TVFA accumulation was reached between the 12^{th} and 14^{th} days before decrease in VFA concentrations was observed. The predominant acids produced in all the systems were acetate and propionate. Same trend was recorded for ammonia (NH_3) concentration throughout the digestions. The highest NH_3 was recorded between the 11^{th} and 13^{th} days of digestion after which reduction was observed for the remaining part of the digestion in all experiments. In terms of consumption/degradation, there was a delay in the degradation of propionate which commenced almost after the complete consumption of acetate.

Due to the fact that all the co-digestion substrates recorded higher nutrient/elemental compositions than the mono-digestions, higher nutrient balance and substrate interactions were subsequently observed in the co-digestions. Overall, the digestates of the co-digestions were all richer than those obtained from the mono-digestions in terms of nutrients.

Table 4.2a: Physical and Chemical Characteristics of Digestates from the Mono-Digestions

Parameters	***Tithonia diversifolia* Shoot**	***Chromolaena odorata* Shoot**	***Carica papaya* Peels**	***Telfairia occidentalis* Peels**	***Arachis hypogaea* Hull**
pH	7.65±0.22	7.65±0.10	7.60±0.03	7.75±0.31	7.65±0.01
Total Solids (g/kg TS)	88.69±0.11	96.09±1.02	93.94±0.02	74.41±0.21	86.00±1.01
Volatile Solids (g/kg TS)	51.31±0.21	50.38±3.72	50.01±2.02	64.74±0.01	46.83±1.01
Ash Content (%)	4.69±0.01	8.62±1.02	5.49±0.03	4.26±0.10	4.17±0.01
Moisture Content (%)	93.31±0.01	90.9±2.32	96.06±1.02	94.19±0.01	93.62±1.01
COD (mg/kg TS)	87.90±0.02	90.91±0.14	83±2.01	88.30±3.20	92.09±1.01
Total Carbon (g/kg TS)	292.01±0.10	298.00±2.22	289.10±3.03	339.00±3.01	510.02±0.01
Total Nitrogen (g/kg TS)	42.00±0.11	38.00±0.21	42.60±0.11	45.60±5.10	44.70±1.00
C/N Ratio	7/1	8/1	7/1	7/1	11/1
Acetate (g COD/g VS)	0.002±0.01	0.002±0.01	0.002±0.01	0.002±0.01	0.003±0.01
Propionate (g COD/g VS)	0.002±0.01	0.003±0.02	0.002±0.02	0.003±0.02	0.001±0.02
TVFAs (g COD/g VS)	0.08±0.10	0.09±0.10	0.09±0.10	0.09±0.10	0.09±0.10
Ammonia (mg/g VS)	1.13±0.01	1.15±0.02	1.16±0.01	1.15±0.02	1.85±0.01
Total Phosphorus (g/kg TS)	7.56±0.11	5.62±0.11	7.60±1.11	6.18±1.01	6.18±1.00
Potassium (g/kg TS)	9.00±0.10	7.40±0.02	10.94±0.03	8.0±1.01	8.20±2.00
Phosphate (g/kg TS)	3.30±0.10	2.70±0.10	4.51±0.02	3.10±0.01	3.20±1.01
Sulphate (g/kg TS)	146.00±4.10	128.00±2.02	159.49±0.03	142.00±4.50	152.00±1.01
Calcium (g/kg TS)	196.00±4.02	168.00±4.09	89.06±2.00	96.00±3.10	76.00±0.01
Magnesium (g/kg TS)	85.00±3.02	82.00±1.40	200.10±5.05	100.0±0.21	110.0±0.01
Manganese (g/kg TS)	0.06±0.12	0.024±0.10	0.060±0.01	0.030±0.01	0.034±1.01
Iron (g/kg TS)	1.90±0.01	1.14±0.01	4.60±1.00	1.16±0.01	1.34±2.01
Zinc (g/kg TS)	47.00±0.13	33.00±0.01	40.94±1.22	38.00±3.00	38.00±1.00
Aluminium (g/kg TS)	1.30±0.11	0.62±0.02	0.91±0.03	0.74±0.11	0.96±2.00
Copper (g/kg TS)	5.70±0.02	3.90±0.12	5.49±0.03	4.70±0.41	4.80±1.02

N = 120; COD = Chemical Oxygen Demand; C/N = Carbon: Nitrogen ratio

Table 4.2b: Physical and Chemical Characteristics of Digestates from the Co-Digestions

Parameters	***Tithonia diversifolia* Shoot + Poultry dropping**	***Chromolaena odorata* Shoot + Poultry dropping**	***Carica papaya* Peels + Poultry dropping**	***Telfairia occidentalis* Peels + Poultry dropping**	***Arachis hypogaea* Hull + Poultry dropping**
pH	7.65±0.01	7.50±0.02	7.66±0.02	7.85±0.52	7.65±0.01
Total Solids (g/kg TS)	100.29±0.40	97.61±0.10	95.40±0.22	100.29±0.12	76.00±1.01
Volatile Solids (g/kg TS)	44.13±1.32	50.67±2.00	89.04±0.10	57.11±0.15	46.83±1.01
Ash Content (%)	7.87±0.11	6.33±0.01	6.76±0.12	6.19±0.02	4.17±0.01
Moisture Content (%)	89.71±1.10	91.94±1.00	90.00±0.12	92.11±0.12	93.62±1.01
COD (mg/kg TS)	102.17±2.21	7.50±0.02	81±3.12	76.12±2.05	585.52±0.01
Total Carbon (g/kg TS)	373.03±4.22	350.54±0.22	254.90±0.03	368.80±5.05	46.70±1.00
Total Nitrogen (g/kg TS)	39.00±1.18	47.00±1.02	52.00±0.02	61.00±2.05	112.09±1.01
C/N Ratio	10/1	7/1	5/1	6/1	12/1
Acetate (g COD/g VS)	0.002±0.01	0.003±0.01	0.001±0.01	0.001±0.01	0.005±0.01
Propionate (g COD/g VS)	0.003±0.02	0.003±0.02	0.003±0.02	0.003±0.02	0.004±0.01
TVFAs (g COD/g VS)	0.05±0.10	0.06±0.10	0.06±0.10	0.06±0.10	0.14±0.10
Ammonia (mg/g VS)	1.18±0.02	1.18±0.02	1.16±0.01	2.01±0.01	2.04±0.01
Total Phosphorus (g/kg TS)	5.90±0.03	7.06±1.02	6.44±0.03	7.60±0.03	8.20±0.20
Potassium (g/kg TS)	7.60±0.09	8.60±0.03	8.50±0.02	9.00±0.01	9.60±0.21
Phosphate (g/kg TS)	2.76±0.02	3.50±0.02	3.20±0.12	4.00±0.01	4.00±0.20
Sulphate (g/kg TS)	128.00±4.10	154.00±2.01	144.00±0.21	162.00±0.02	178.00±0.12
Calcium (g/kg TS)	92.00±0.13	52.00±1.00	60.00±0.03	60.00±0.12	68.00±0.20
Magnesium (g/kg TS)	86.00±0.11	130.00±2.01	110.00±0.10	140.00±2.02	116.00±0.12
Manganese (g/kg TS)	0.026±0.10	0.034±0.01	0.030±0.11	0.038±0.01	0.042±0.02
Iron (g/kg TS)	0.110±0.02	1.34±0.21	1.26±0.02	0.142±0.01	1.64±0.02
Zinc (g/kg TS)	35.00±1.02	44.00±0.02	39.00±0.12	52.00±0.12	51.00±0.12
Aluminium (g/kg TS)	0.70±0.32	0.64±0.01	1.02±0.02	0.68±0.01	0.94±0.02
Copper (g/kg TS)	4.30±0.11	5.50±1.01	5.10±0.12	5.50±0.12	6.40±0.02

N = 120; COD = Chemical Oxygen Demand; C/N = Carbon: Nitrogen ratio

4.3. Biogas Generation

Results for the daily biogas generation in the five mono-digestion experiments are shown in table 4.3a. Biogas generation in the mono-digestion experiments commenced at different times in all the digesters used for each experiment. In the mono-digestion of *Tithonia diversifolia* shoot, biogas production started between the 3rd and 4th days of digestion, in the mono-digestion of *Chromolaena ododrata* shoot it started between the 3rd and 5th days, in the mono-digestion of *Carica papaya* peel, it commenced between the 4th and 6th day while in the mono-digestion of both *Telfairia occidentalis* peels and *Arachis hypogaea* hull, biogas generation commenced between 3rd and 6th days of digestion. In the five experiments, gas production continued at a steady rate until when the peak was achieved between the 18 to 22 days before decreasing. In all mono-digestions, biogas generation followed the order: mono-digestion of *Chromolaena ododrata* shoot > mono-digestion of *Tithonia diversifolia* shhot > mono-digestion of *Carica papaya* peels > mono-digestion of *Telfairia occidentalis* peels > mono-digestion of *Arachis hypogaea* hull. Gas chromatographic analyses showed different results for the gas composition in the five mono-digestion regimes. In the mono-digestion of *Tithonia diversifolia* shoot, gas analysis showed 64.5 ± 1.5% methane and 26 ± 2% carbon dioxide; in the mono-digestion of *Chromolaena ododrata* shoot, it was 65.5 ± 1.5% methane and 23 ± 2% carbon dioxide; in the mono-digestion of *Carica papaya* peel, analysis showed 61.5 ± 1.5% methane and 26 ± 1% carbon dioxide; in the mono-digestion of *Telfairia occidentalis* peels, there was 66.5 ± 2.5% methane and 22 ± 2% carbon dioxide while in the mono-digestion of *Arachis hypogaea* hull, there was 59.5 ± 2.5% methane and 24 ± 1% carbon dioxide. In terms of methane content, the five mono-digestions followed the order: mono-digestion of *Telfairia occidentalis* peels > mono-digestion of *Chromolaena ododrata* shoot > mono-digestion of *Tithonia diversifolia* shhot > mono-digestion of *Carica papaya* peels > mono-digestion of *Arachis hypogaea* hull.

Table 4.3a: Daily Biogas Yield ($10^{-3}m^3$/kg VS) from the Mono-Digestion Experiments

Day	*Tithonia diversifolia* Shoot	*Chromolaena odorata* Shoot	*Carica papaya* Peels	*Telfairia occidentalis* Peels	*Arachis hypogaea* Hull
1.	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
2.	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
3.	0.001±0.01	0.002±0.01	0±0.00	0±0.00	0.002±0.01
4.	0.002±0.01	0.003±0.01	0.002±0.02	0.002±0.01	0.001±0.01
5.	0.003±0.02	0.002±0.01	0.003±0.01	0.003±0.01	0.002±0.01
6.	0.0031±0.02	0.001±0.01	0.002±0.01	0.002±0.01	0.002±0.01
7.	0.002±0.01	0.0021±0.02	0.002±0.02	0.002±0.01	0.003±0.01
8.	0.0035±0.02	0.0047±0.03	0.003±0.01	0.003±0.01	0.002±0.01
9.	0.002±0.01	0.005±0.02	0.002±0.01	0.002±0.01	0.002±0.01
10.	0.003±0.01	0.0052±0.01	0.003±0.02	0.003±0.02	0.003±0.01
11.	0.0052±0.02	0.0049±0.02	0.0042±0.03	0.0042±0.01	0.003±0.01
12.	0.0051±0.02	0.0051±0.01	0.0041±0.01	0.0041±0.02	0.0031±0.01
13.	0.006±0.02	0.0046±0.02	0.005±0.02	0.005±0.01	0.004±0.02
14.	0.0050±0.02	0.0044±0.01	0.0040±0.01	0.0050±0.03	0.004±0.01
15.	0.0051±0.01	0.0035±0.01	0.005±0.02	0.0053±0.01	0.0051±0.01
16.	0.0053±0.01	0.0031±0.01	0.005±0.02	0.005±0.02	0.0053±0.01
17.	0.0052±0.01	0.006±0.02	0.005±0.01	0.005±0.01	0.0054±0.01
18.	0.004±0.02	0.006±0.02	0.0051±0.03	0.0054±0.02	**0.0057**±0.01
19.	0.0046±0.00	**0.0071**±0.02	0.0052±0.01	0.0054±0.01	0.0041±0.01
20.	**0.0056**±0.02	0.006±0.01	0.0051±0.02	0.0053±0.01	0.0045±0.01
21.	0.005±0.02	0.002±0.02	**0.0053**±0.02	0.0054±0.03	0.003±0.01
22.	0.004±0.02	0.002±0.01	0.0047±0.01	**0.0055**±0.01	0.002±0.02
23.	0.002±0.01	0.003±0.02	0.0033±0.01	0.004±0.01	0.001±0.01
24.	0.002±0.01	0.003±0.01	0.0021±0.02	0.003±0.02	0.0021±0.01
25.	0.001±0.01	0.004±0.01	0.002±0.02	0.002±0.01	0.002±0.01
26.	0.001±0.01	0.002±0.01	0.0019±0.01	0.001±0.01	0.002±0.01
27.	0.002±0.02	0.002±0.01	0.0013±0.02	0.002±0.02	0.0033±0.01
28.	0.002±0.01	0.003±0.01	0.002±0.01	0.002±0.01	0.002±0.01
29.	0.001±0.01	0.003±0.02	0.001±0.01	0.002±0.01	0.002±0.01
30.	0.002±0.01	0.002±0.01	0.001±0.01	0.002±0.02	0.001±0.01

Values in bold represent highest value of daily yield and the day obtained

Table 4.3b shows the result of daily biogas generation from the co-digetions. Biogas generation in the co-digestions of mixture of *Tithonia diversifolia* shoot and poultry dropping, mixture of *Chromolaena odorata* shoot and poultry dropping and mixture of *Telfairia occidentalis* peels and poultry dropping commenced between the 2nd and 4th days, it started between the 3rd and 4th day in the co-digestion of *Carica papaya* and poultry dropping while in the co-digestion of *Arachis hypogaea* hull and poultry dropping, production started between the 3rd and 5th days of digestion. Steady gas production was observed in all the co-digestions till between the 21st and 23rd experimental days when peak was achieved in the various set ups and then production started diminishing. There were variations in the gas composition from the co-digestions. In the co-digestion of *Tithonia diversifolia* shoot and poultry dropping, there was 69 ± 2% methane and 24 ± 1% carbon dioxide; in the co-digestion of *Chromolaena odorata* shoot and poultry dropping, there was 70 ± 2% methane and 22 ± 2% carbon dioxide; in the co-digestion of *Carica papaya* peels and poultry dropping, there was 67 ± 1% methane and 26 ± 1% carbon dioxide; in the co-digestion of *Telfairia occidentalis* peels and poultry dropping, it was 69 ± 1% methane and 25 ± 1% carbon dioxide while in the co-digestion of *Arachis hypogaea* hull and poultry dropping, gas analysis showed 65.5 ± 1.5% methane and 26 ± 2% carbon dioxide. In terms of methane content, the order observed was the co-digestion of *Chromolaena odorata* shoot and poultry dropping > the co-digestion of *Telfairia occidentalis* peels and poultry dropping > the co-digestion of *Tithonia diversifolia* shoot and poultry dropping > the co-digestion of *Carica papaya* peels and poultry dropping > the co-digestion of *Arachis hypogaea* hull and poultry dropping. In comparison, the co-digestion experiments were better in terms of commencement of gas production (2nd day in most cases) and higher quantity of gas generation. Also, production of gas was steady and reached their peaks between the 21st and 23rd days unlike the mono-digestions where diminishing was observed between the 18th and 22nd days of experiment. The methane contents of the co-digestion experiments were also higher (65.5 to 70.5) than those of the mono-digestions (59.5 to 66.5%).

Table 4.3b: Daily Biogas Yield ($10^{-3}m^3$/kg VS) from the Co-Digestion Experiments

Day	*Tithonia diversifolia* Shoot + Poultry dropping	*Chromolaena odorata* Shoot + Poultry dropping	*Carica papaya* Peels + Poultry dropping	*Telfairia occidentalis* Peels + Poultry dropping	*Arachis hypogaea* Hull + Poultry dropping
1.	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
2.	0.002±0.02	0.002±0.02	0.002±0.02	0±0.00	0±0.00
3.	0.002±0.02	0.003±0.01	0.003±0.01	0.002±0.01	0.002±0.02
4.	0.003±0.01	0.003±0.01	0.003±0.01	0.003±0.02	0.003±0.01
5.	0.002±0.01	0.004±0.02	0.004±0.01	0.002±0.01	0.002±0.02
6.	0.001±0.01	0.001±0.02	0.001±0.01	0.001±0.02	0.001±0.02
7.	0.0021±0.01	0.0041±0.01	0.0041±0.01	0.0021±0.02	0.0021±0.01
8.	0.0047±0.21	0.0047±0.01	0.0047±0.01	0.0031±0.01	0.0031±0.01
9.	0.005±0.02	0.005±0.01	0.005±0.01	0.004±0.01	0.004±0.01
10.	0.005±0.01	0.0051±0.21	0.0051±0.03	0.005±0.01	0.005±0.01
11.	0.0049±0.01	0.0049±0.01	0.0049±0.01	0.0049±0.01	0.0049±0.02
12.	0.0051±0.01	0.0051±0.01	0.0051±0.02	0.0045±0.01	0.0045±0.02
13.	0.0066±0.01	0.0066±0.01	0.0066±0.01	0.0066±0.02	0.0056±0.01
14.	0.0064±0.02	0.007±0.01	0.006±0.01	0.0064±0.02	0.0054±0.01
15.	0.0059±0.01	0.0071±0.01	0.0061±0.01	0.0069±0.01	0.0059±0.01
16.	0.0061±0.01	0.0072±0.01	0.0069±0.01	0.0061±0.01	0.0060±0.02
17.	0.006±0.01	0.0076±0.21	0.006±0.02	0.006±0.02	0.0061±0.01
18.	0.006±0.01	0.0069±0.01	0.007±0.01	0.0062±0.01	0.0059±0.02
19.	0.0061±0.01	0.0068±0.01	0.0072±0.01	0.0061±0.01	0.0058±0.01
20.	0.007±0.01	0.0075±0.01	0.006±0.01	0.0059±0.03	0.0058±0.02
21.	**0.0074**±0.02	0.0074±0.01	0.0054±0.01	0.0058±0.01	**0.0063**±0.01
22.	0.006±0.01	**0.0079**±0.01	0.006±0.02	0.0065±0.01	0.006±0.01
23.	0.005±0.01	0.005±0.01	**0.0075**±0.02	**0.0069**±0.01	0.005±0.01
24.	0.005±0.01	0.005±0.02	0.005±0.01	0.005±0.02	0.005±0.01
25.	0.004±0.01	0.004±0.01	0.004±0.01	0.004±0.01	0.004±0.02
26.	0.005±0.01	0.005±0.01	0.005±0.01	0.004±0.01	0.005±0.01
27.	0.005±0.01	0.005±0.01	0.005±0.01	0.004±0.02	0.004±0.01
28.	0.003±0.01	0.003±0.01	0.003±0.01	0.003±0.01	0.003±0.02
29.	0.003±0.01	0.003±0.01	0.003±0.01	0.003±0.01	0.003±0.01
30.	0.002±0.01	0.002±0.02	0.002±0.02	0.002±0.02	0.002±0.01

Values in bold represent highest value of daily yield and the day obtained

4.4. Monitoring of Operational Parameters

Appendix 5 shows the results of the operational parameters i.e. pH and temperature fluctuations during the mono and co-digestion experiments. The pH of the substrates in all the mono-digestions was slightly alkaline throughout the digestion process thus falling within the experimental design range (6.5 to 8) by Response Surface. In all cases, there was an initial pH fall to slightly acidic range especially during the initial period of digestion before subsequent adjustment to slightly alkaline that was maintained throughout. Also, the temperature of all the digesters remained between 30 to 40^{o} C (mesophilic range) throughout the experiment according to the experimental design. Similarly, all pH values in the co-digestions were within experimental design range throughout the digestion period. Also, temperatures fluctuations were all within the mesophilic range of design (30 to 40^{o} C). In comparison, same pH and temperature ranges were observed in both mono and co-digestions.

4.5. Microbial Composition and Succession Pattern

Microbial analyses of the cattle's rumen content used as inoculum and the poultry dropping used in the co-digestion experiments are shown in table 4.4. For the rumen content, the isolated aerobic bacteria include species of *Bacillus, Enterococcus* and *Proteus* and *Pseudomonas aeruginosa*. The mean total aerobic plate count (TAPC) was 4.1 x 10^{12} cfu/ml. Fungal isolates in the rumen conten are *Aspergillus niger, Aspergillus flavus,* species of *Mucor*, *Rhizopus* and *Penicillum* with total fungal count (TFC) of 2.0 x 10^{10} cfu/ml. The isolated anaerobes include species of *Fusobacterium*, *Bacteroides, Clostridium, Gemella* and *Porphyromonas*. The total plate count (TPC) of anaerobes was 4.7 x 10^{14} cfu/ml. Six different genera of methanogen namely *Methanococcus, Methanosarcinales, Methanosaeta, Methanobacteriales, Methanomicrobiales* and *Aminobacteria* spp were identified. The TPC of methanogens was 5.3 x 10^{14} cfu/ml.

For the poultry dropping, TAPC was 2.4 x 10^{10} cfu/ml, aerobes isolated include species of *Bacillus*, *Staphylococcus, Enterococcus, Proteus* and *Pseudomonas aeruginosa*. TFC of 1.3 x 10^{10} cfu/ml was recorded and fungal species identified include *Aspergillus niger, Mucor*, *Rhizopus* and *Penicillum.* Methanogens were absent in the poultry droppings, however, *Clostridium* and *Bacteroides* species of anaerobes were isolated having a TPC of

1.9 x 10^{10} cfu/ml. The rumen content was found to be microbially richer than the poultry dropping in terms of population and diversity.

Table 4.4 (a-e) shows the microbial compositions of the fermenting substrates in the mono-digestion experiments and employing biochemical and morphological characteristics for identification methods. The aerobes were identified as species of *Bacillus* (45%), *Enterococcus* (18%), *Serratia* (14%), *Proteus* (9%) and *Pseudomonas aeruginosa* (14%). The anaerobes were identified as species of *Fusobacterium* (14%), *Bacteroides* (17%), *Clostridium* (41%), *Gemella* (14%) and *Porphyromonas* (14%). The methanogens were identified as species of the genera *Methnococcus* (18%), *Methanosarcinales* (16%), *Methanosaeta* (24%), *Methanobacteriales* (13%), *Methanomicrobiales* (13%), and *Aminobacteria* (16%). The fungi were identified as *Aspergillus niger* (33.3%), *Aspergillus flavus* (22.2%) and species of *Mucor* (11.1%), *Rhizopus* (11.1%) and *Penicillum* (22.2%). Their succession pattern revealed that the aerobes and fungi had their highest population during the 1st week when the pH of the medium were slightly acidic and digester environment not completely anaerobic whereas, anaerobes attained their highest population by the 4th week while methanogens reached their highest populations between the 5th and 6th weeks of digestion when the medium was alkaline and digester environment anaerobic.

In the mono-digestion of *Tithonia diversifolia* shhot, the aerobes that were isolated include species of *Bacillus, Proteus*, *Enterococcus* and *Pseudomonas aeruginosa.* Anaerobes include species of *Fusobacterium, Bacteroides, Clostridium* and *Gemella.* Fungal isolates include *Aspergillus niger* and species of *Mucor*, *Rhizopus* and *Penicillum* while members of three genera of methanogens: *Methanococcus, Methanosaeta* and *Methanomicrobiales* were isolated. The highest TAPC was 2.4 x 10^{10} cfu/ml while the lowest was 3.0 x 10^{2} cfu/ml. The highest TFC was 2.1 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml. For anaerobes, the highest TPC was 1.8 x 10^{11} cfu/ml while the lowest was 1.5 x 10^{10} cfu/ml. The highest methanogenic TPC was 2.2 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml.

In the mono-digestion of *Chromolaena odorata* shoot, the aerobes that were isolated include species of *Bacillus, Proteus, Enterococcus* and *Pseudomonas aeruginosa.* Anaerobes include species of *Bacteroides* and *Clostridium*. Fungal isolates include *Aspergillus niger* and species of *Mucor, Rhizopus* and *Penicillum* while members of three genera of methanogens: *Methanococcus, Methanosarcinales* and *Methanosaeta* were identified. The highest TAPC was 2.5 x 10^{10} cfu/ml while the lowest was 1.5 x 10^{8} cfu/ml. The highest TFC was 2.2 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml. For anaerobes, the highest and lowest TPC were 2.1 x 10^{12} cfu/ml and 1.1 x 10^{6} cfu/ml respectively. The highest methanogenic TPC was 2.3 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml.

In the mono-digestion of *Carica papaya* peels, the aerobes that were isolated include species of *Bacillus, Proteus*, *Enterococcus* and *Pseudomonas aeruginosa.* Anaerobes include species of *Fusobacterium*, *Bacteroides, Clostridium* and *Gemella.* Fungal isolates include *Aspergillus niger* and species of *Mucor*, *Rhizopus* and *Penicillum* while members of three genera of methanogens: *Methanococcus, Methanosaeta* and *Methanobacteriales* were identified. The highest TAPC was 2.4 x 10^{9} cfu/ml while the lowest was 1.0 x 10^{2} cfu/ml. The highest TFC was 1.9 x 10^{8} cfu/ml while the lowest was 1.1 x 10^{4} cfu/ml. For anaerobes, the highest TPC was 2.0 x 10^{10} cfu/ml while the lowest was 1.5 x 10^{8} cfu/ml. The highest methanogenic TPC was 2.4 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{2} cfu/ml.

In the mono-digestion of *Telfairia occideentalis* peels, the aerobes that were isolated include species of *Bacillus*, *Serratia* and *Proteus.* Anaerobes include species of *Fusobacterium*, *Bacteroides, Clostridium* and *Porphyromonas.* Fungal isolates include *Aspergillus niger* and species of *Mucor*, *Rhizopus* and *Penicillum* while three genera of methanogen: *Methanosarcinales, Methanosaeta* and *Methanobacteriales* were isolated. The highest TAPC was 2.0 x 10^{10} cfu/ml while the lowest was 2.0 x 10^{2} cfu/ml. The highest TFC was 1.4 x 10^{8} cfu/ml while the lowest was 1.4 x 10^{2} cfu/ml. For anaerobes, the highest TPC was 1.5 x 10^{10} cfu/ml while the lowest was 1.2 x 10^{6} cfu/ml. The highest TPC for methanogens was 2.1 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{5} cfu/ml.

In the mono-digestion of *Arachis hypogaea* hull, the aerobes that were isolated include species of *Bacillus* and *Proteus.* Anaerobes that were implicated include species of *Fusobacterium*, *Bacteroides, Clostridium* and *Gemella.* Fungal isolates include *Aspergillus niger* and species of *Mucor Rhizopus* and *Penicillum* while three genera of methanogen: *Methanosarcinales, Methanosaeta* and *Methanomicrobiales* were isolated. The highest TAPC was 2.1 x 10^{10} cfu/ml while the lowest was 2.1 x 10^{2} cfu/ml. The highest TFC was 1.2 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{4} cfu/ml. For anaerobes, the highest TPC was 2.4 x 10^{10} cfu/ml while the lowest was 1.1 x 10^{10} cfu/ml. The highest TPC for methanogens was 1.6 x 10^{12} cfu/ml while the lowest was 1.2 x 10^{2} cfu/ml.

Table 4.4: Microbial Composition of Cattle's Rumen Content and Poultry Dropping

Rumen content							
Aerobes		**Fungi**		**Anaerobes**		**Methanogens**	
Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
Bacillus sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	4.1×10^{12}	*Aspergillus niger* *Aspergillus flavus* *Mucor* sp. *Rhizopus* sp *Penicillum* sp.	2.0×10^{10}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp. *Porphyromonas* sp.	4.7×10^{14}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	5.3×10^{14}
Poultry Dropping							
Aerobes		**Fungi**		**Anaerobes**		**Methanogens**	
Organism	**TPC**	**Organism**	**TPC**	**Organism**	**TPC**	**Organism**	**TPC**
Bacillus sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	2.4×10^{10}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.3×10^{10}	*Clostridium* sp. *Bacteroides* sp.	1.9×10^{10}	Nil	Nil

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5a: Microbial Evaluation and Succession in the Anaerobic Digestion of *Tithonia diversifolia* Shoot

Day	Aerobes (cfu/ml)		Fungi (cfu/ml)		Anaerobes (cfu/ml)		Methanogens (cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa, Proteus* sp.	2.4×10^{10}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	2.1×10^{8}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.7×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.2×10^{10}
6	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa, Proteus* sp.	1.4×10^{8}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum*	1.0×10^{3}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.5×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.0×10^{6}
12	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	3.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.7×10^{11}	*Methanococcus* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.0×10^{3}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.8×10^{11}	*Methanococcus* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	5.0×10^{8}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	1.1×10^{8}	*Methanococcus* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.9×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	Nil	Nil	*Methanococcus* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	2.2×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5b: Microbial Evaluation and Succession in the Anaerobic Digestion of *Chromolaena odorata* Shoot

Day	Aerobes (cfu/ml)		Fungi (cfu/ml)		Anaerobes (cfu/ml)		Methanogens (cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	2.5×10^{10}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	2.2×10^{8}	*Bacteroides* sp. *Clostridium* sp.	1.8×10^{10}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	1.3×10^{10}
6	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	1.5×10^{8}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	1.0×10^{3}	*Bacteroides* sp. *Clostridium* sp.	1.3×10^{10}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	1.0×10^{8}
12	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Bacteroides* sp. *Clostridium* sp.	1.6×10^{10}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	1.0×10^{3}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Bacteroides* sp. *Clostridium* sp.	2.1×10^{12}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	1.4×10^{10}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	1.1×10^{6}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	1.8×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	1.1×10^{2}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	2.3×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5c: Microbial Evaluation and Succession in the Anaerobic Digestion of *Carica papaya* Fruit Peels

Day	Aerobes (cfu/ml)		Fungi (cfu/ml)		Anaerobes (cfu/ml)		Methanogens (cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	2.4×10^{9}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	1.9×10^{8}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.7×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.4×10^{10}
6	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	2.3×10^{4}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	1.1×10^{4}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.5×10^{8}	*Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.0×10^{6}
12	*Bacillus* sp. *Enterococcus* sp. *Pseudomonas aeruginosa* *Proteus* sp.	1.0×10^{2}	*Aspergillus niger*	1.1×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.1×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.0×10^{2}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	2.0×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.5×10^{6}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	2.0×10^{4}	*Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.8×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	2.0×10^{4}	*Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	2.4×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5d: Microbial Evaluation and Succession in the Anaerobic Digestion of *Telfairia occidentalis* Fruit Peels

Day	Aerobes (cfu/ml)		Fungi (cfu/ml)		Anaerobes (cfu/ml)		Methanogens (cfu/ml)	
	Organism	**TPC**	**Organism**	**TPC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Serratia* sp. *Proteus* sp.	2.0×10^{10}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	1.4×10^{8}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.5×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.6×10^{10}
6	*Bacillus* sp. *Serratia* sp. *Proteus* sp.	2.0×10^{6}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	1.4×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.3×10^{4}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.2×10^{8}
12	*Bacillus* sp. *Serratia* sp. *Proteus* sp.	2.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.2×10^{6}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.0×10^{5}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.5×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.2×10^{2}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp.	1.3×10^{9}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	1.5×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp.	1.1×10^{4}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	2.1×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5e: Microbial Evaluation and Succession in the Anaerobic Digestion of *Arachis hypogaea* Hull

Day	Aerobes (cfu/ml)		Fungi (cfu/ml)		Anaerobes (cfu/ml)		Methanogens (cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Proteus* sp.	2.1×10^{10}	*Aspergillus niger* *Mucor* sp. *Rhizopus* sp. *Penicillum* sp.	1.2×10^{8}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.5×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.1×10^{10}
6	*Bacillus* sp. *Proteus* sp.	2.2×10^{7}	*Aspergillus niger* *Mucor* sp. *Penicillum* sp.	1.0×10^{4}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.3×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.0×10^{10}
12	*Bacillus* sp. *Proteus* sp.	2.1×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.1×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	7.0×10^{9}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	2.2×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.1×10^{11}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	2.4×10^{3}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.4×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	2.2×10^{2}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	1.6×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

For the co-digestion regimes, the microbial composition of the substrates and their succession pattern is shown in table 4.4 (f-j). As recorded in the mono-digestions, the population of aerobic bacteria and fungi was highest during the 1st week and was drastic reduced by the 2nd week. On the other hand, population of facultative anaerobes reached their highest population by the 4th week while those of methanogens experienced initial decrease before steady increase towards the end of the experiments. In the co-digestion of *Tithonia diversifolia* shoot and poultry dropping, the aerobes that were isolated include species of *Bacillus, Proteus* and *Enterococcus.* Anaerobes include species of *Fusobacterium*, *Bacteroides, Clostridium* and *Gemella.* Fungal isolates include *Aspergillus niger, Aspergillus flavus* and species of *Mucor*, *Rhizopus* and *Penicillum* while four genera of methanogens: *Methanococcus, Methanosarcinales, Methanosaeta* and *Aminobacteria* were identified. The highest TAPC was 2.5 x 10^{10} cfu/ml while the lowest was 1.0 x 10^{2} cfu/ml. The highest TFC was 1.4 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml. For anaerobes, the highest and lowest TPC were 1.6 x 10^{10} cfu/ml and 1.1 x 10^{4} cfu/ml. The highest methanogenic TPC was 2.4 x 10^{12} cfu/ml while the lowest was 1.3 x 10^{3} cfu/ml.

In the co-digestion of *Chromolaena odorata* shoot and poultry dropping, the aerobic bacteria isolated include species of *Bacillus, Proteus* and *Enterococcus.* Anaerobes that were isolated and characterized include species of *Porphyromonas, Fusobacterium*, *Bacteroides, Clostridium* and *Gemella.* Fungal isolates include *Aspergillus niger, Aspergillus flavus* and species of *Mucor*, *Rhizopus* and *Penicillum* while five genera of methanogens: *Methanosarcinales, Methanosaeta, Methanobacteriales, Methanomicrobiales* and *Aminobacteria* were identified. The highest TAPC was 2.2 x 10^{10} cfu/ml while the lowest was 1.1 x 10^{6} cfu/ml. The highest TFC was 1.0 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{2} cfu/ml. For anaerobes, the highest TPC was 2.2 x 10^{10} cfu/ml while the lowest was 1.0 x 10^{5} cfu/ml. The highest methanogenic TPC was 2.1 x 10^{12} cfu/ml while the lowest was 1.1 x 10^{3} cfu/ml.

In the co-digestion of *Carica papaya* peels and poultry dropping, the aerobic bacteria isolated include species of *Bacillus, Proteus, Enterococcus* and *Pseudomonas aeruginosa.* Anaerobes that were isolated and characterized include species of *Porphyromonas*,

Fusobacterium, *Bacteroides* and *Clostridium*. Fungal isolates include *Aspergillus niger, Aspergillus flavus* and species of *Mucor*, *Rhizopus* and *Penicillum* while three genera of methanogens: *Methanococcus, Methanosaeta* and *Aminobacteria* were identified. The highest TAPC was 2.4 x 10^{10} cfu/ml while the lowest was 1.0 x 10^{2} cfu/ml. The highest TFC was 1.1 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{2} cfu/ml. For anaerobes, the highest TPC was 1.4 x 10^{10} cfu/ml while the lowest was 1.0 x 10^{4} cfu/ml. The highest methanogenic TPC was 2.6 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml.

In the co-digestion of *Telfairia occidentalis* peels and poultry dropping, the aerobic bacteria isolated include species of *Bacillus, Serratia* and *Proteus.* Anaerobes that were isolated and characterized include species of *Fusobacterium, Bacteroides* and *Clostridium.* Fungal isolates include *Aspergillus niger, Aspergillus flavus* and species of *Mucor*, *Rhizopus* and *Penicillum* while four genera of methanogens: *Methanosarcinales, Methanobacteriales, Methanomicrobiales* and *Aminobacteria* were identified. The highest TAPC was 2.3 x 10^{10} cfu/ml while the lowest was 1.4 x 10^{8} cfu/ml. The highest TFC was 1.0 x 10^{6} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml. For anaerobes, the highest TPC was 1.2 x 10^{8} cfu/ml while the lowest was 1.0 x 10^{4} cfu/ml. The highest methanogenic TPC was 2.7 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{5} cfu/ml.

In the co-digestion of *Arachis hypogaea* hull and poultry dropping, the aerobic bacteria isolated include species of *Bacillus*, *Serratia* and *Proteus.* Anaerobes that were isolated and characterized include species of *Bacteroides, Clostridium* and *Gemella.* Fungal isolates include *Aspergillus niger, Aspergillus flavus* and species of *Mucor*, *Rhizopus* and *Penicillum* while three genera of methanogens: *Methanococcus, Methanomicrobiales* and *Aminobacteria* were identified. The highest TAPC was 2.3 x 10^{10} cfu/ml while the lowest was 1.3 x 10^{4} cfu/ml. The highest TFC was 1.1 x 10^{8} cfu/ml while the lowest was 1.2 x 10^{2} cfu/ml. For anaerobes, the highest TPC was 1.4 x 10^{10} cfu/ml while the lowest was 1.0 x 10^{3} cfu/ml. The highest methanogenic TPC was 2.7 x 10^{12} cfu/ml while the lowest was 1.0 x 10^{4} cfu/ml.

Table 4.5f: Microbial Evaluation and Succession in the Anaerobic Co-Digestion of *Tithonia diversifolia* Shoot + Poultry Dropping

Day	Aerobes (Cfu/ml)		Fungi (Cfu/ml)		Anaerobes (Cfu/ml)		Methanogens (Cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Proteus* sp. *Enterococcus* sp.	2.5×10^{10}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum*	1.4×10^{8}	*Fusobacterium* sp. *Bacteroides fragilis* *Clostridium* sp. *Gemella* sp.	1.3×10^{8}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.3×10^{10}
6	*Bacillus* sp. *Proteus* sp. *Enterococcus* sp.	2.0×10^{7}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum*	1.0×10^{3}	*Fusobacterium* sp. *Bacteroides fragilis* *Clostridium* sp. *Gemella* sp.	1.1×10^{4}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.2×10^{7}
12	*Bacillus* sp. *Proteus* sp. *Enterococcus* sp.	1.0×10^{2}	Nil	Nil	*Fusobacterium* sp. *Bacteroides fragilis* *Clostridium* sp. *Gemella* sp.	1.0×10^{6}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.3×10^{3}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Bacteroides fragilis* *Clostridium* sp. *Gemella* sp.	1.6×10^{10}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.2×10^{10}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp. *Gemella* sp.	1.6×10^{3}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.7×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp. *Gemella* sp.	1.2×10^{2}	*Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	2.4×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5g: Microbial Evaluation and Succession in the Anaerobic Co-Digestion of *Chromolaena odorata* Shoot + Poultry Dropping

Day	Aerobes (Cfu/ml)		Fungi (Cfu/ml)		Anaerobes (Cfu/ml)		Methanogens (Cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Proteu* sp. *Enterococcus* sp.	2.2×10^{10}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.2×10^{8}	*Porphyromonas* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	1.1×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.2×10^{10}
6	*Bacillus* sp. *Proteu* sp. *Enterococcus* sp.	1.1×10^{6}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Penicillum* sp.	1.0×10^{2}	*Porphyromonas* sp. *Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	1.1×10^{6}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp. *Aminobacteria* sp.	1.1×10^{7}
12	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Porphyromonas* sp. *Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	1.0×10^{5}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp. *Aminobacteria* sp.	1.1×10^{3}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Porphyromonas* sp. *Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	2.2×10^{10}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.0×10^{10}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	2.2×10^{4}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.4×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	2.2×10^{2}	*Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	2.1×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5h: Microbial Evaluation and Succession in the Anaerobic Co-Digestion of *Carica papaya* Peels + Poultry Dropping

Day	Aerobes (Cfu/ml)		Fungi (Cfu/ml)		Anaerobes (Cfu/ml)		Methanogens (Cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Enterococcus* sp *Pseudomonas aeruginosa* *Proteus* sp.	2.4×10^{10}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum*	1.1×10^{8}	*Porphyromonas* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp.	1.4×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.3×10^{10}
6	*Bacillus* sp. *Enterococcus* sp *Pseudomonas aeruginosa* *Proteus* sp.	1.1×10^{7}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum*	1.0×10^{2}	*Porphyromonas* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp.	1.0×10^{4}	*Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.0×10^{8}
12	*Bacillus* sp. *Enterococcus* sp *Pseudomonas aeruginosa* *Proteus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Porphyromonas* sp. *Fusobacterium* sp. *Clostridium* sp.	1.0×10^{7}	*Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.0×10^{3}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Porphyromonas* sp. *Fusobacterium* sp. *Clostridium* sp.	1.4×10^{10}	*Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.0×10^{7}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	1.4×10^{3}	*Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.9×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	1.4×10^{2}	*Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	2.6×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5i: Microbial Evaluation and Succession in the Anaerobic Co-Digestion of *Telfairia occidentalis* Peels + Poultry Dropping

Day	Aerobes (Cfu/ml)		Fungi (Cfu/ml)		Anaerobes (Cfu/ml)		Methanogens (Cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Serratia* sp. *Pseudomonas aeruginosa* *Proteus* sp.	2.3×10^{10}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.0×10^{8}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.2×10^{10}	*Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.2×10^{10}
6	*Bacillus* sp. *Serratia* sp. *Pseudomonas aeruginosa* *Proteus* sp.	1.4×10^{8}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.2×10^{8}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.0×10^{6}	*Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.0×10^{8}
12	Nil	Nil	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.0×10^{3}	*Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp.	1.0×10^{4}	*Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.0×10^{5}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Porphyromonas* sp.	1.3×10^{10}	*Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.0×10^{10}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp. *Porphyromonas* sp.	1.2×10^{3}	*Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.7×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Fusobacterium* sp. *Clostridium* sp.	1.2×10^{2}	*Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	2.7×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

Table 4.5j: Microbial Evaluation and Succession in the Anaerobic Co-Digestion of *Arachis hypogaea* Hull + Poultry Dropping

Day	Aerobes (Cfu/ml)		Fungi (Cfu/ml)		Anaerobes (Cfu/ml)		Methanogens (Cfu/ml)	
	Organism	**TAPC**	**Organism**	**TFC**	**Organism**	**TPC**	**Organism**	**TPC**
0	*Bacillus* sp. *Serratia* sp. *Proteus* sp.	2.3×10^{10}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.1×10^{8}	*Clostridium* sp. *Bacteroides* sp. *Gemella* sp.	1.6×10^{10}	*Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.3×10^{10}
6	*Bacillus* sp. *Serratia* sp. *Proteus* sp.	1.3×10^{4}	*Aspergillus niger* *Aspergillus flavus* *Rhizopus* sp. *Mucor* sp. *Penicillum* sp.	1.2×10^{2}	*Clostridium* sp. *Bacteroides* sp. *Gemella* sp.	1.1×10^{7}	*Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.2×10^{7}
12	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp. *Bacteroides* sp. *Gemella* sp.	1.0×10^{3}	*Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.0×10^{4}
18	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp. *Bacteroides* sp. *Gemella* sp.	1.4×10^{10}	*Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.3×10^{10}
24	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	1.4×10^{3}	*Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.7×10^{10}
30	*Bacillus* sp.	1.0×10^{2}	*Aspergillus niger*	1.0×10^{2}	*Clostridium* sp.	1.4×10^{2}	*Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	2.7×10^{12}

TAPC = Total aerobic plate count; TFC = Total fungal count; TPC = Mean Plate Count

4.6. RSM Optimization of Biogas Data

The Central Composite Design (CCD)'s experimental design matrixes for the five-level-five-factor response surface study for biogas generation from both mono and co-digestion experiments are shown in Appendix 6 (a-j). The experimentally observed and predicted yields as well as the residual/desirability values are revealed in the tables. The effects of unexplained variability in the biogas yield response due to extraneous factors were minimized by randomizing the order of experiments. The coefficients of the full regression model equation and their statistical significance were evaluated and determined. The desirability value was considered in choosing the most desired predictions. In the mono-digestion of *Tithonia diversifolia* shoot, the highest actual gas yield was 2139.20 $10^{-3}m^3$/kg VS while the predicted yield was 2219.24 $10^{-3}m^3$/kg VS. In the mono-digestion of *Chromolaena odorata* shoot, the highest actual (experimental) biogas yield was 3554.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 3555.50 $10^{-3}m^3$/kg VS; in the mono-digestion of *Carica papaya* peels, the actual biogas yield was 1839.20 $10^{-3}m^3$/kg VS while the predicted yield was 10^{-3}1894.80 m^3/kg VS; in the mono-digestion of *Telfairia occidentalis* peels, actual biogas yield was 1639.20 $10^{-3}m^3$/kg VS while the predicted yield was 1659.90 $10^{-3}m^3$/kg VS and in the mono-digestion of *Arachis hypogaea* hull, the actual biogas yield was 1739.20 $10^{-3}m^3$/kg VS while the predicted yield was 1819.89 $10^{-3}m^3$/kg VS. Among the five digestions, values obtained for both actual and predicted biogas yield followed the order: the mono-digestion of *Chromolaena odorata* shoot > the mono-digestion of *Tithonia diversifolia* shoot > the mono-digestion of *Carica papaya* peels > the mono-digestion of *Arachis hypogaea* hull > the mono-digestion of *Telfairia occidentalis* peels.

The same trend was observed for the actual and predicted biogas yield in the five co-digestion experiments. In the co-digestion of *Tithonia diversifolia* shoot and poultry dropping, the actual biogas yield was 2984.20 $10^{-3}m^3$/kg VS while the predicted value was 3011.10 $10^{-3}m^3$/kg VS; in the co-digestion of *Chromolaena ododrata* shoot and poultry dropping, the most desired actual biogas yield was 3884.20 $10^{-3}m^3$/kg VS while the predicted yield was 4178.81 $10^{-3}m^3$/kg VS; in the co-digestion of *Carica papaya* peels and poultry dropping, the actual biogas yield was 3884.20 $10^{-3}m^3$/kg VS while the predicted

value was 3991.77 $10^{-3}m^3$/kg VS; in the co-digestion of *Telfairia occidentalis* peels and poultry dropping, the actual biogas yield was 2539.20 $10^{-3}m^3$/kg VS while the predicted value was 2614.14 $10^{-3}m^3$/kg VS. In the co-digestion of *Arachis hypogaea* hull and poultry dropping, the highest actual biogas yield was 3339.20 $10^{-3}m^3$/kg VS while the predicted value was 3903.15 $10^{-3}m^3$/kg VS. In all the experiments, the predicted values were higher than the actual values after the optimization studies and the order is as follow: the co-digestion of *Chromolaena ododrata* shoot and poultry dropping > the co-digestion of *Carica papaya* peels and poultry dropping > the co-digestion of *Arachis hypogaea* hull and poultry dropping > the co-digestion of *Tithonia diversifolia* shoot and poultry dropping > the co-digestion of *Telfairia occidentalis* peels and poultry dropping. In comparison, actual and predicted values obtained from the co-digestions are higher than those of the mono-digestions. In all, the highest values (3884.20 $10^{-3}m^3$/kg VS and 4178.81 $10^{-3}m^3$/kg VS) were obtained from experiment the co-digestion of *Chromolaena ododrata* shoot and poultry dropping while the lowest (1639.20 $10^{-3}m^3$/kg VS and 1659.90 $10^{-3}m^3$/kg VS) were obtained from the mono-digestion of *Telfairia occidentalis* peels.

4.7. Validation of RSM Predictive Ability

The results of test of significance (ANOVA) and that of the second-order response surface model fit for every regression coefficient which were carried out to validate the predictive and modeling capability of RSM are shown in Appendix 7 (a-j). The ability was judged based on the values of important model parameters like the 'Adequate precision', the 'Lack of fit' and the R^2. Based on the large F-values (the test for comparing the variance associated with all terms with the residual variance) and low corresponding p-values (the probability value that is associated with the F -value for all terms) of all the ten model terms, they are remarkably significant and have very strong effects on the biogas yield with $p < 0.05$. For the five mono-digestions, the Model F-values of 3.31, 2.96, 5.46, 4.03 and 2.95 implies the model is significant in each case. In terms of significance (value of $p < 0.05$) of model terms representing the relationship between the five variables employed in the optimization study, "X_3, X_1X_3, X_2X_5, X_3X_5, X_4X_5, X_2^2" (Appendix 7a), "X_4, X_3X_4 X_4^2" (Appendix 7b), "X_2, X_5, X_1X_4, X_1X_5, X_2X_3, X_2X_4, X_2X_5, X_5^2" (Appendix 7c), "X_4, X_1X_3, X_1X_4, and X_4^2" (Appendix 7d) and "X_4, X_2X_4" (Appendix 7e) were the most significant model

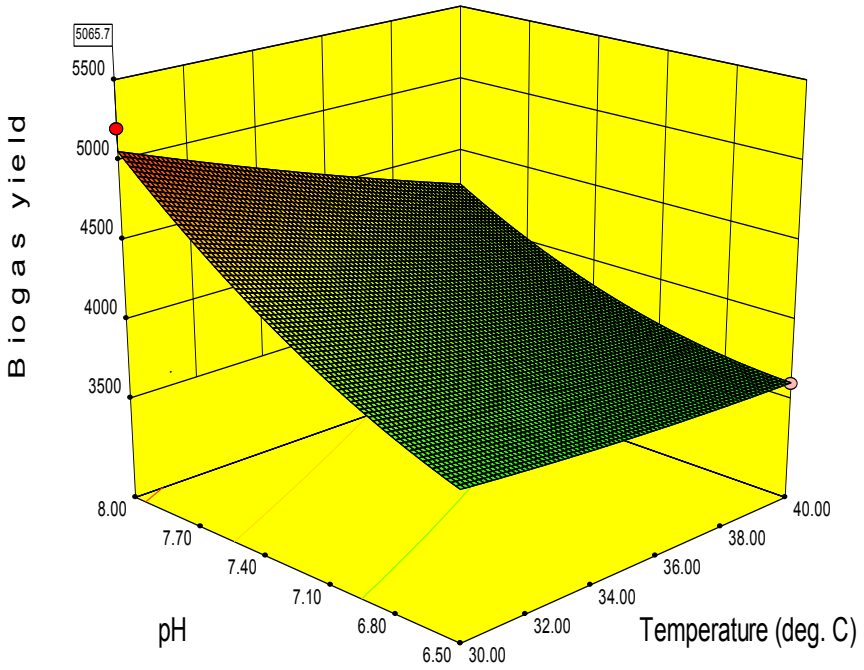
terms. In the co-digestions, the Model F-values of 2.94, 4.09, 5.05, 3.39, and 4.26 also implies significance of the model in each case. The significant ($p < 0.05$) model terms are "X_3, X_4, X_1X_3" (Appendix 7f), "X_3, X_4, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_2X_5, X_4^2" (Appendix 7g), "X_1, X_4, X_1X_4, X_2X_3, X_2X_4 (Appendix 7h), "X_1X_4, X_1X_5, X_2X_3, X_3X_4, X_3X_5" (Appendix 7i) and "X_3, X_4,X_5, X_2X_5 (Appendix 7j).

As shown in the tables, the 'Adequate Precision' values were 10.596, 7.607, 13.883, 8.009 and 10.764 for all the five mono-digestions respectively. Also, for the co-digestions, values of 9.270, 11.950, 10.461, 12.438 and 11.627 were recorded respectively. All the values obtained indicated adequate signal that the models can be used to navigate the sample designs. Furthermore, the 'goodness of fit' of the models was checked by 'Lack of fit' value. For the mono-digestions, the 'Lack of Fit' F-values of 3.33, 0.16, 0.92, 3.36 and 3.78 implies non-significance ($p > 0.05$). Also, the values of 5.51, 7.90, 0.81, 0.59 and 2.67 also implies non-significance ($p > 0.05$). Since non-significant lack of fit is good/desirable, all the models are fit for use in theoretical prediction of biogas production from the five substrates used in this study. In terms of the R^2, values of 0.8802, 0.8680, 0.9239, 0.8996 and 0.8876 were obtained for the mono-digestionsrespectively. For the co-digestions, the values were 0.8674, 0.9009, 0.9181, 0.8827 and 0.9045. In all, the co-digestion regimes recorded higher R^2 values than the mono-digestions.

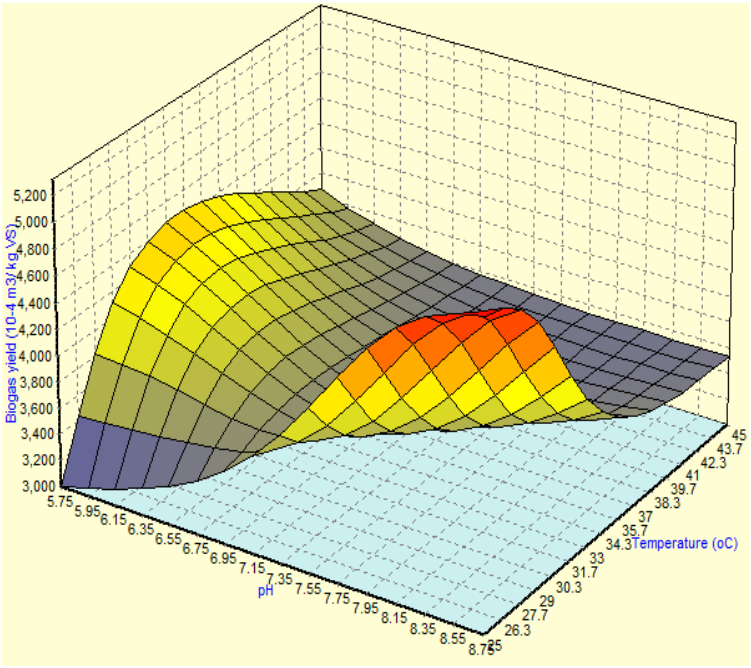
4.8. Interactions of Independent Variables

Figures 4.1a reveals the 3-dimensional response surface plots of both RSM and ANNs which are the graphical representations of the regression equation for the optimization of the five reaction variables i.e. Temperature, pH, Retention time, Total solids and Volatile solids for the mono-digestion of *Tithonia diversifolia* shoot. From figure 4.1a (i), the interactions between temperature and pH was such that maximum gas yield was achieved with increase in pH and low temperature range (30 to 33 ^oC). In figure 4.1a (ii), maximum biogas was achieved with higher retention time and at lower temperature (30^o C). From figure 4.1a (iii), increase in temperature and decreased total solids led to maximum gas yield. From figure 4.1a (iv), contribution of temperature to gas yield was constant all through with decrease in volatile solids content for achieving maximum biogas yield. From figure 4.1a (v), maximum gas yield was obtained with increase in pH and retention time of 25.7 days. From 4.1a (vi), maximum gas yield was obtained with increase in pH

while total solids fluctuated and was best at 8.53 g/kg. From 4.1a (vii), increase in both temperature and volatile solids led to the achievement of maximum gas yield. From 4.1a (viii), maximum gas yield was achieved with increase in total solids and decrease in retention time. From 4.1a (ix), maximum yield was obtained with increase in both volatile solids and retention time. From 4.1a (x), increase in total solids and decrease in volatile solids gave the maximum yield of gas as seen from the plot. In all, the RSM 3-D plots showed little to moderate interactions among the five variables (Temperature, pH, Retention time, Total solids and Volatile solids) employed in the optimization studies. On the other hand, all the ANNs 3-D plots showed pronounced interactions in their curvature natures and thus revealing that the ANN models allowed for better interactions of the five variables than RSM models.

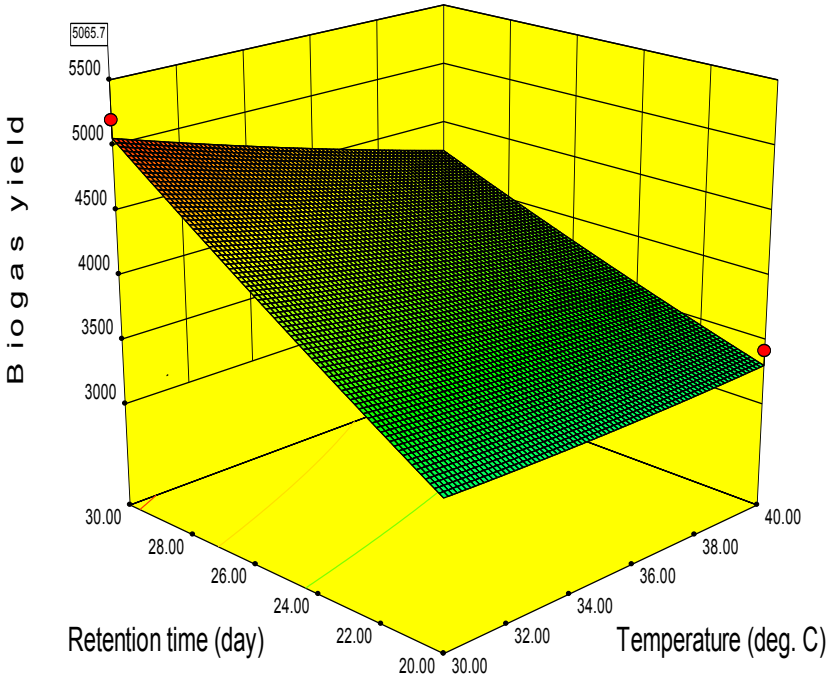


(a)

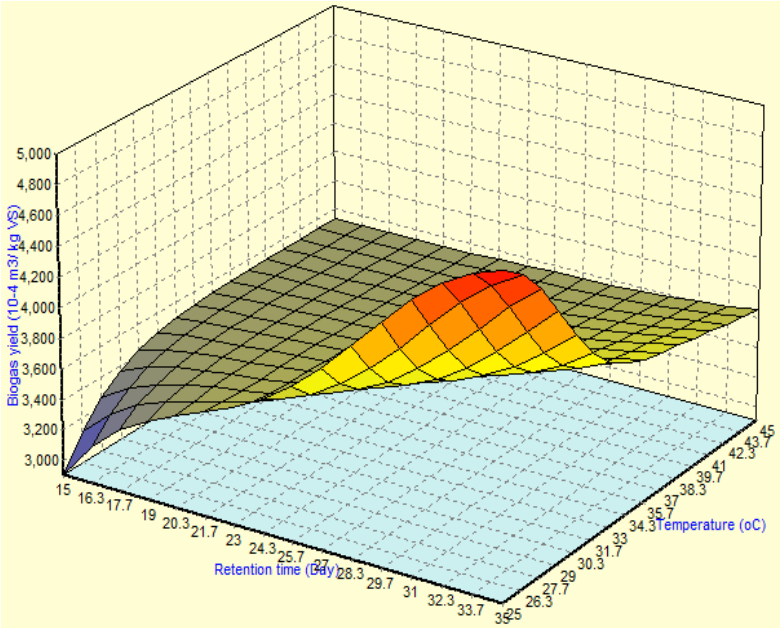


(b)

Figure 4.1a (i): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between temperature and pH for the optimization of biogas generation from *Tithonia diversifolia* shoot

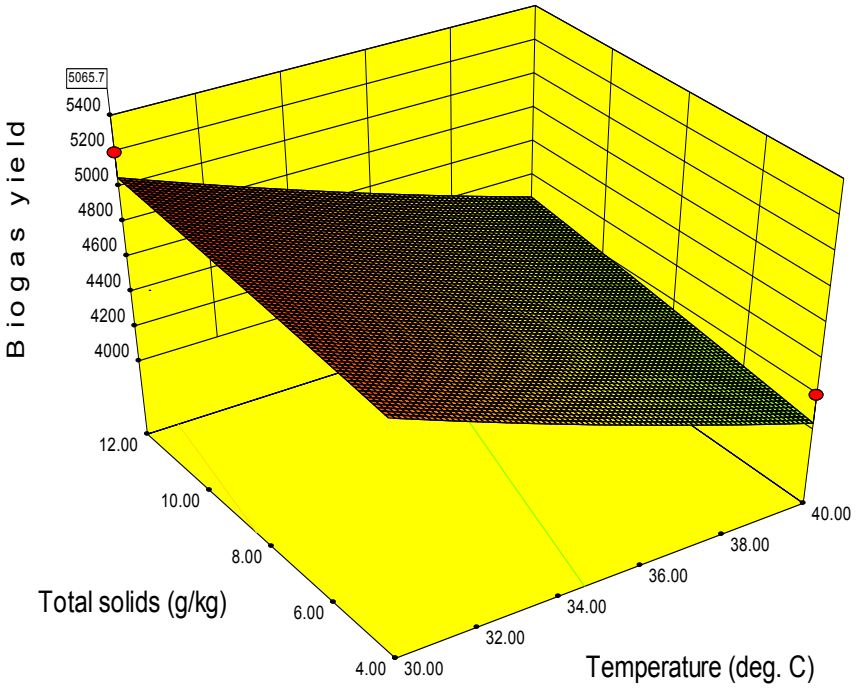


(a)

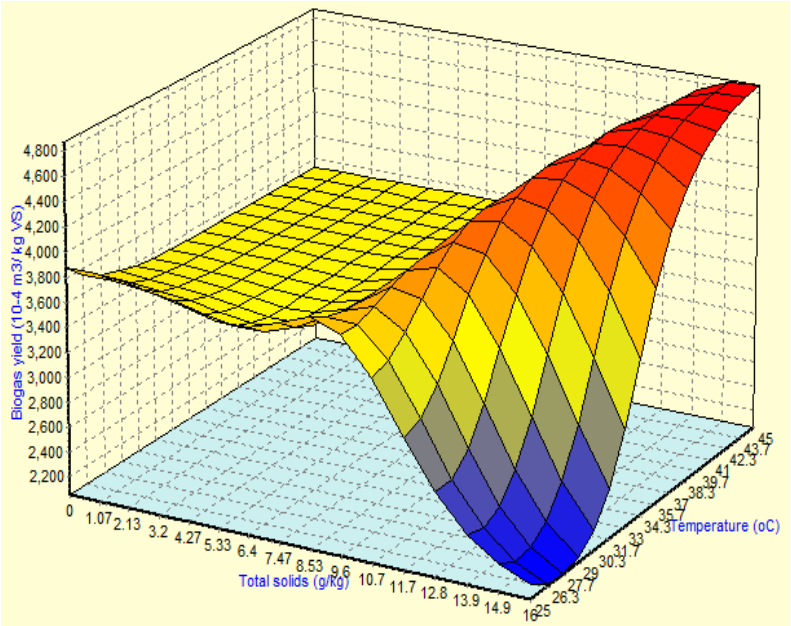


(b)

Figure 4.1a (ii): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between temperature and retention time for the optimization of biogas generation from *Tithonia diversifolia* shoot

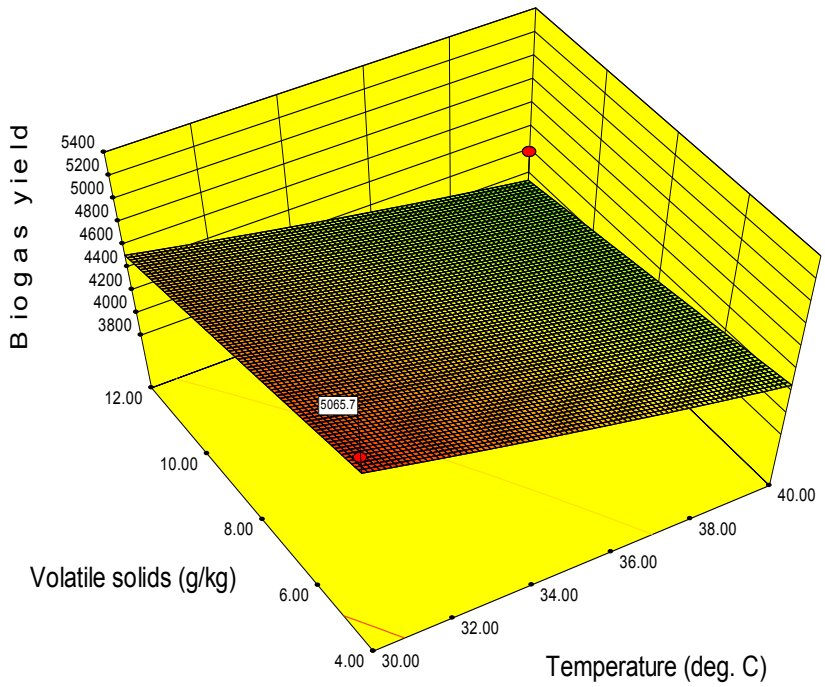


(a)

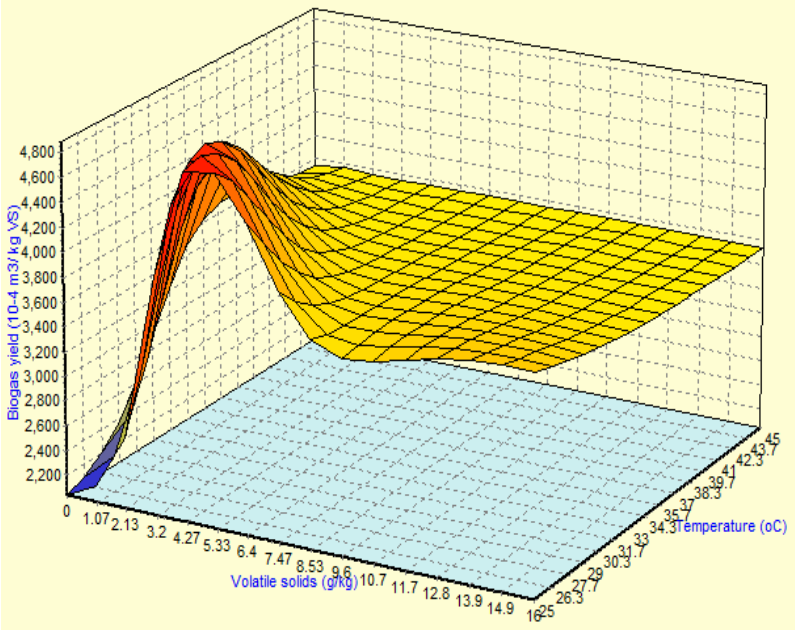


(b)

Figure 4.1a (iii): 3D Curvatures' plots of RSM (Up) and ANNs (Down) showing interaction between temperature and total solids for the optimization of biogas generation from *Tithonia diversifolia* shoot

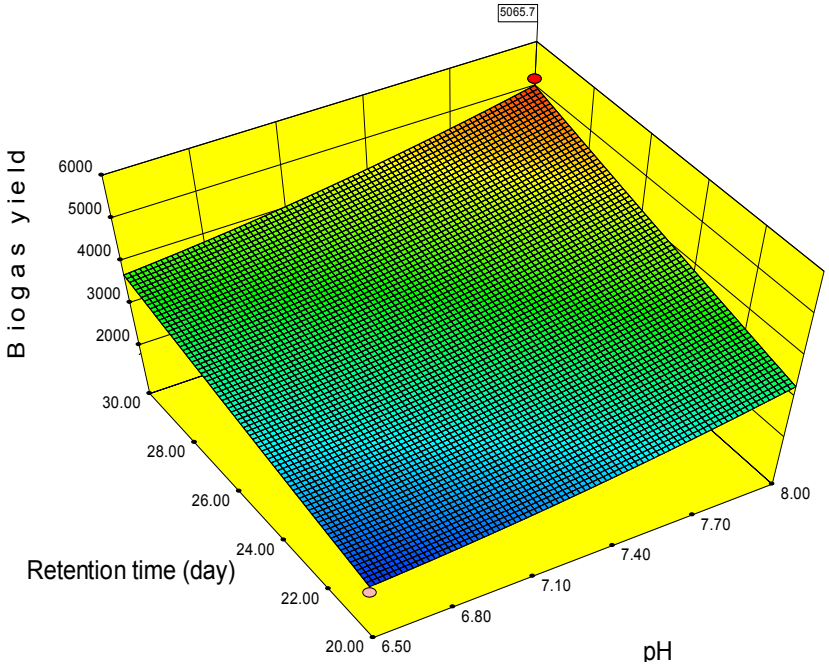


(a)

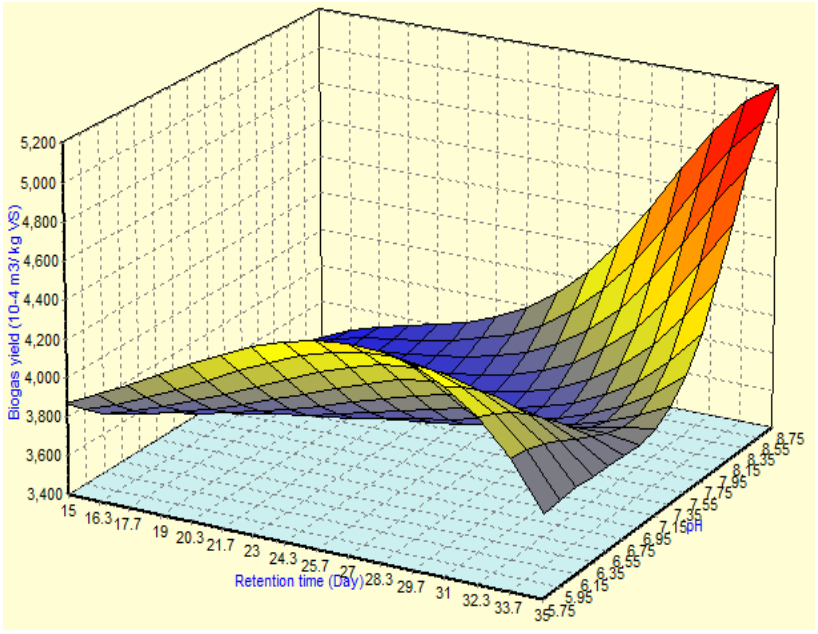


(b)

Figure 4.1a (iv): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between temperature and volatile solids for the optimization of biogas generation from *Tithonia diversifolia* shoot

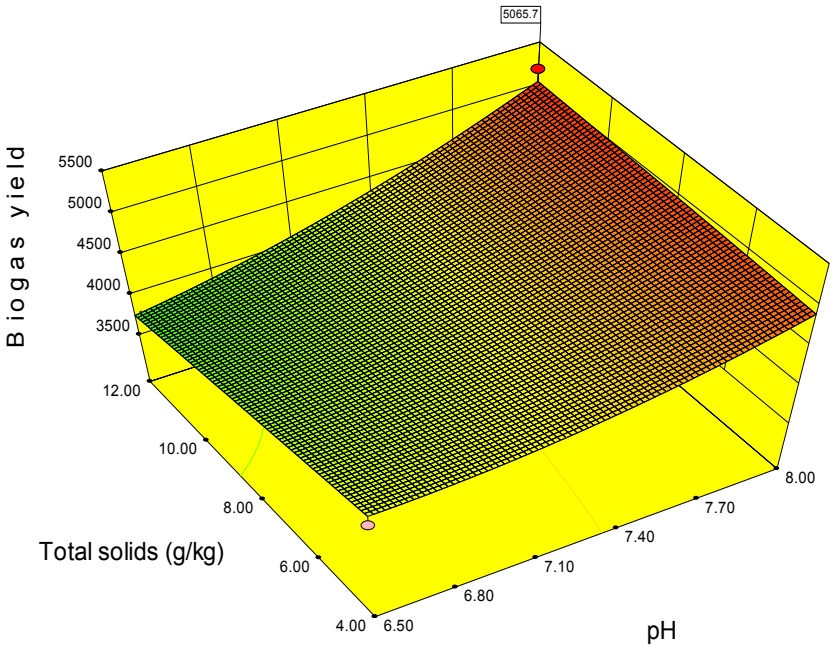


(a)

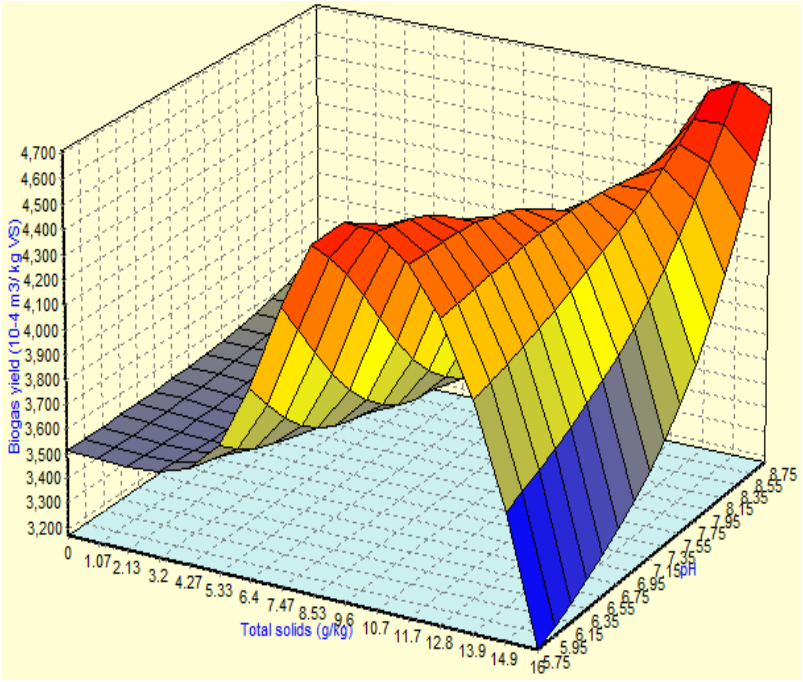


(b)

Figure 4.1a (v): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between retention time and pH for the optimization of biogas generation from *Tithonia diversifolia* shoot

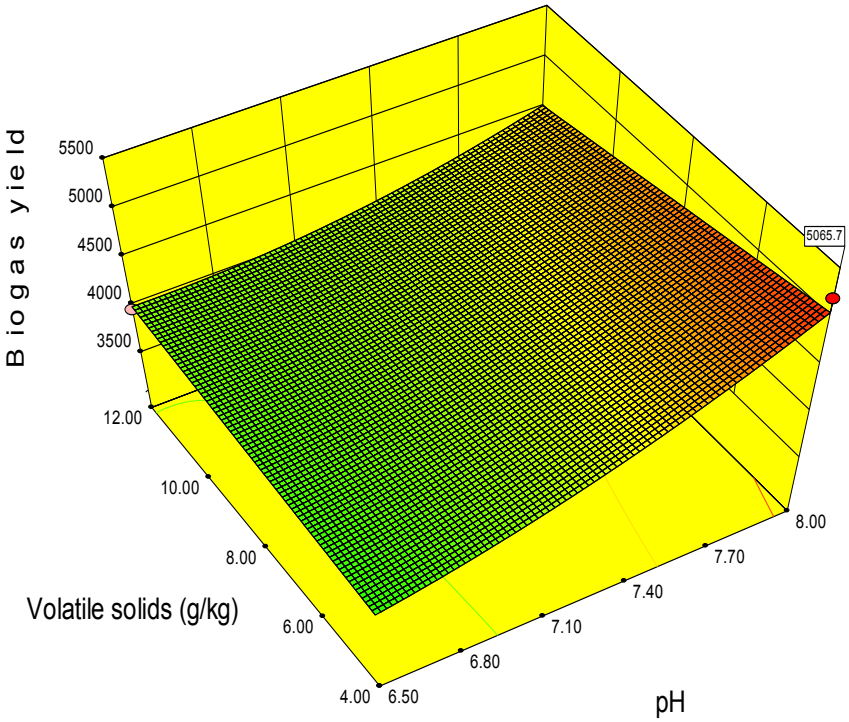


(a)

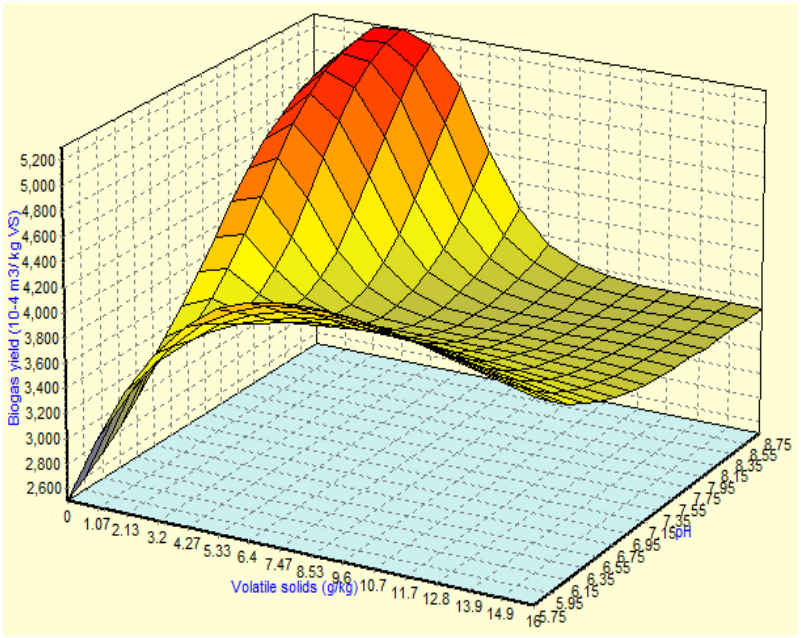


(b)

Figure 4.1a (vi): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between total solids and pH for the optimization of biogas generation from *Tithonia diversifolia* shoot

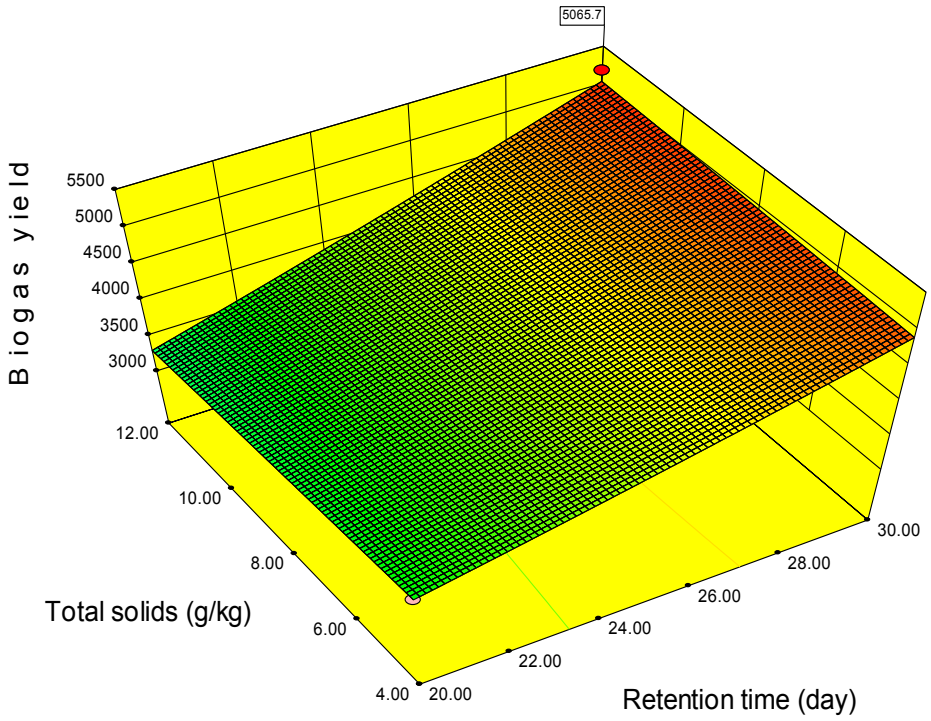


(a)

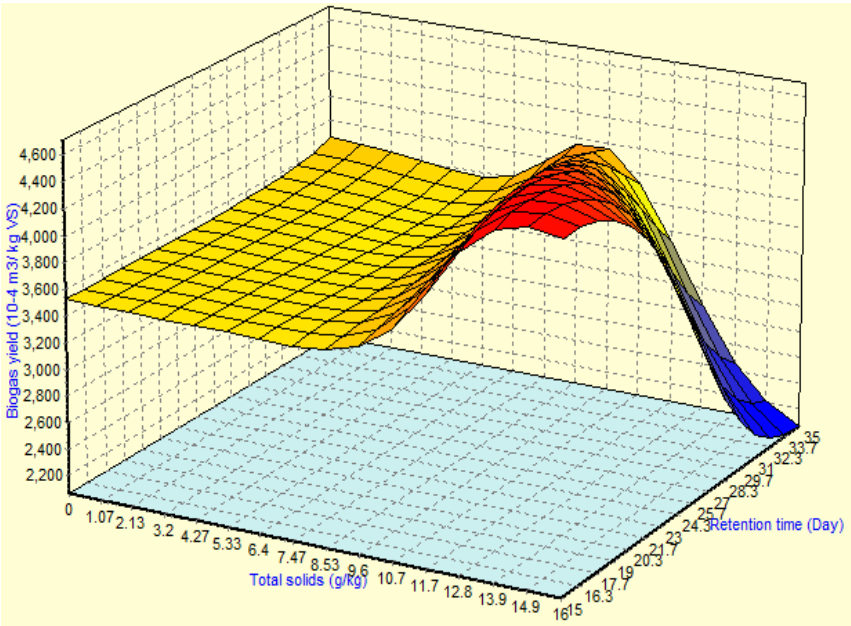


(b)

Figure 4.1a (vii): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between volatile solids and pH for the optimization of biogas generation from *Tithonia diversifolia* shoot

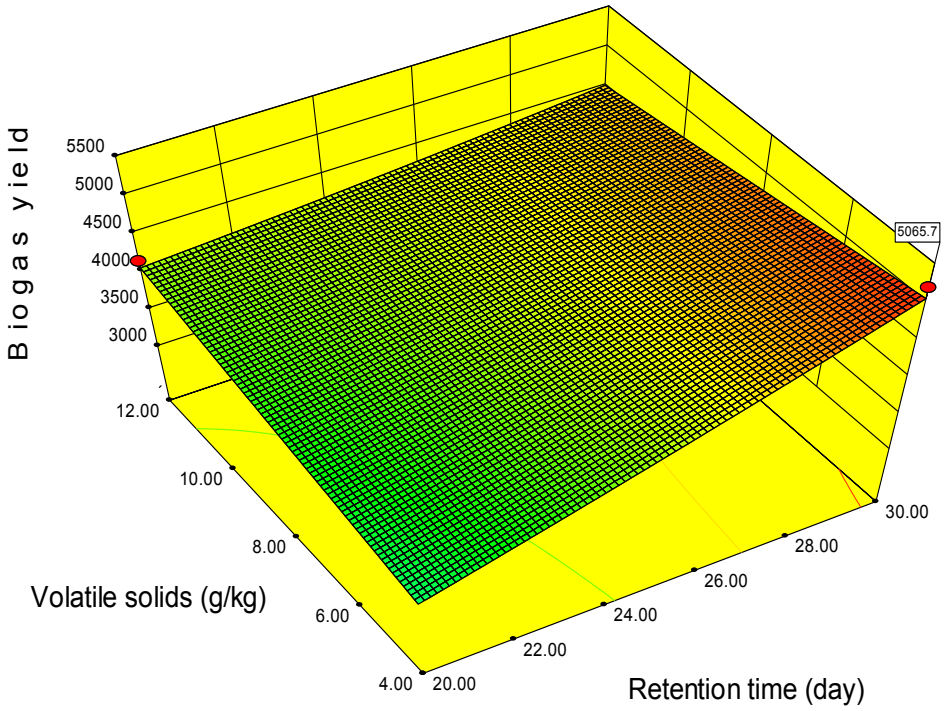


(a)

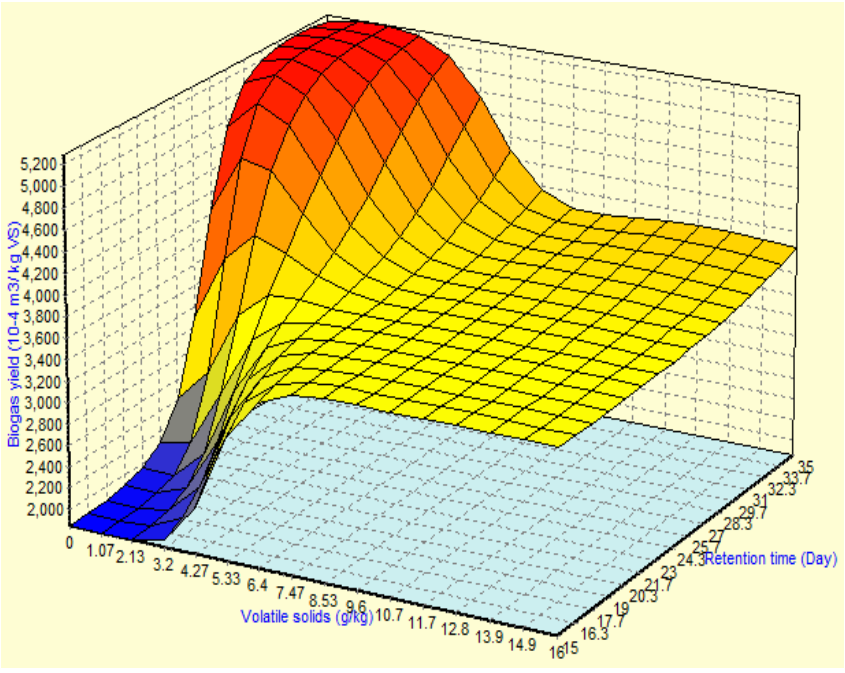


(b)

Figure 4.1a (viii): 3D Curvatures' plots of RSM (Up) and ANNs (Down) showing interaction between total solids and retention time for the optimization of biogas generation from *Tithonia diversifolia* shoot

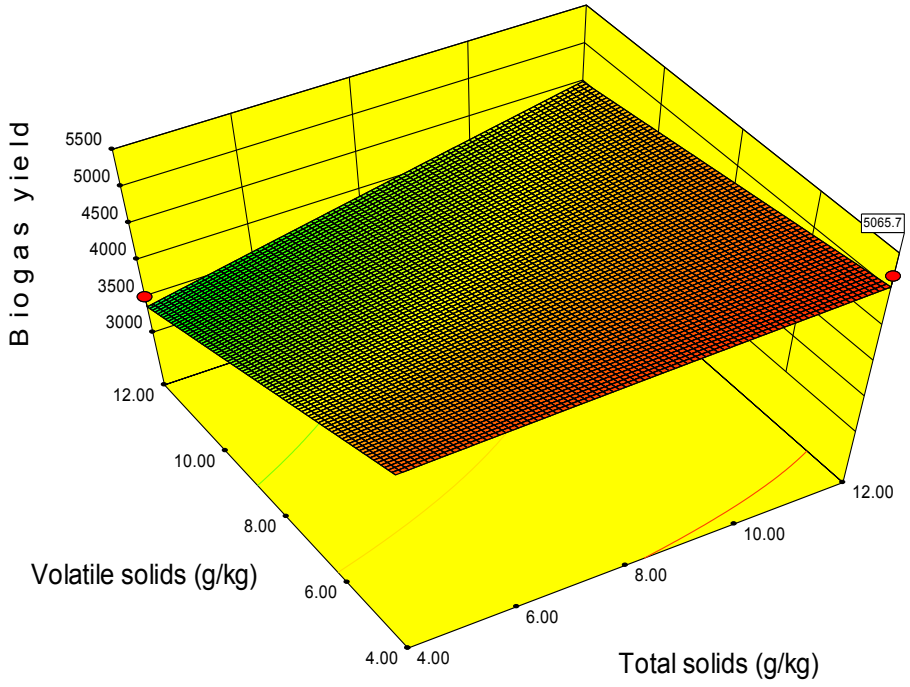


(a)

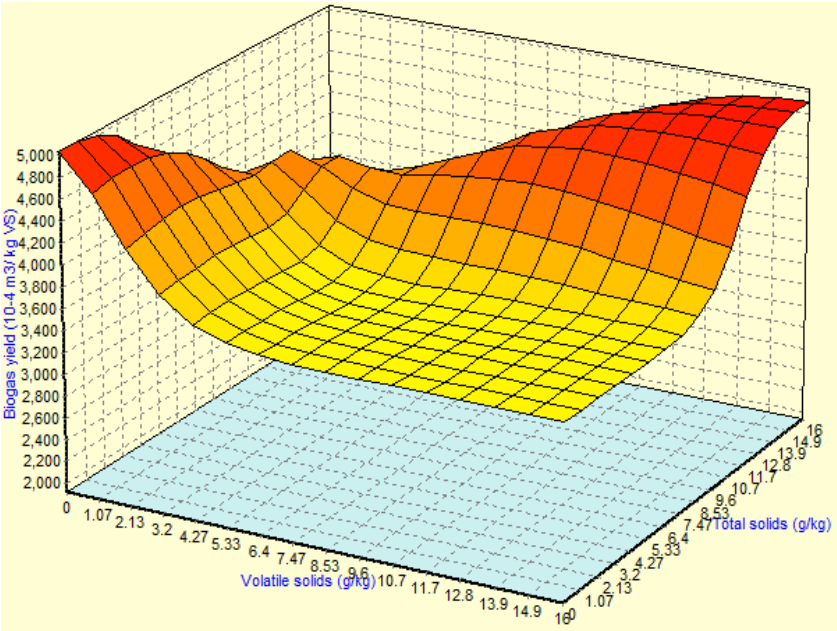


(b)

Figure 4.1a (ix): 3D Curvatures' plots of RSM (Up) and ANNs (Down) showing interaction between volatile solids and retention time for the optimization of biogas generation from *Tithonia diversifolia* shoot



(a)



(b)

Figure 4.1a (x): 3D Curvatures' plots of RSM (a) and ANNs (b) showing interaction between volatile solids and total solids for the optimization of biogas generation from *Tithonia diversifolia* shoot

Appendix 8 shows the 3-Dimensional curvature plots for all the remaining nine digestion regimes. Appendix 8a (i-x) shows the 3-Dimensional curvature nature of both RSM and ANNs plots for the optimization of biogas generation from the anaerobic digestion of *Chromolaena odorata* shoot. In figure 4.1b (i), temperature was on the increasing trend while pH was decreasing in order to ensure maximum biogas generation. From figure 4.1b (ii), temperature increase and decrease in pH values with optimal at 15 gave the maximum biogas yield. From figure 4.1b (iii), temperature was increasing while total solids increased till 6.4 g/kg to give maximum gas yield. Further increase in total solids caused decrease in gas generation. From figure 4.1b (iv), increase in temperature and decrease in volatile solids ensured the production of maximum gas yield. From figure 4.1b (v), decrease in values of retention time and neutral pH gave the maximum gas yield. From figure 4.1b (vi), increase in pH with steady increase in total solids gave rise to the maximum gas production. From figure 4.1b (vii), increase in both pH and volatile solids gave rise to maximum gas yield. From figure 4.1b (viii), higher retention time and total solids content of 8.53 g/kg gave maximum gas yield. From figure 4.1b (ix), higher retention time and increased volatile solids content (11.7 g/kg) gave rise to maximum gas yield. Lastly, from figure 4.1b (x), higher values of both total solids content (8.53) and volatile solids gave rise to maximum gas yield. In all figures, the ANNs plots were more interactive and showed pronounced interactions of the five variables than those of the RSM which showed moderate interactions.

Appendix 8b (i-x) shows the interactive plots of the five independent variable employed in the optimization of biogas generation from the anaerobic digestion of *Carica papayas* fruit peels. From figure 4.1c (i), increase in temperature and pH values gave the maximum yield of biogas. From figure 4.1c (ii), increase in temperature and decrease in retention time gave the highest yield of gas. Retention time of 15 days gave the maximum yield. From figure 4.1c (iii), increase in total solids and lower temperature produced the maximum gas yield. From figure 4.1c (iv), lower temperature with higher volatile solids content gave rise to the maximum gas yield in the plot. From figure 4.1c (v), both retention time and pH contributed to maximum gas yield at increased values. From figure 4.1c (vi), increase in pH and lower value of total solids produced the maximum gas yield.

From figure 4.1c (vii), higher pH values and decrease in volatile solids content gave rise to maximum biogas yield. From figure 4.1c (viii), lower retention time coupled with increase in values of total solids produced the maximum gas yield. From figure 4.1c (ix), higher retention time and volatile solids content of 7.47 g/kg produced the highest gas yield. From figure 4.1c (x), increase in total solids and decrease in volatile solids content gave rise to maximum gas generation. Again, higher variable interactions were recorded in the ANNs plots.

Appendix 8c (i-x) shows the 3-Dimensional curvature plots of the interactions of the five variables employed in the optimization of biogas generation from the anaerobic digestion of *Telfairia occidentalis* fruit peels. From figure 4.1d (i), lower pH value coupled with moderate temperature value (33 ^{o}C) gave rise to the maximum gas generation. From figure 4.1d (ii), higher temperature and lower retention time (15 days) ensured the production of maximum biogas. From figure 4.1d (iii), increase in temperature and decrease in total solids (5.33 g/kg) produced the maximum yield of gas. From figure 4.1d (iv), lower temperature (33 ^{o}C) and higher volatile solids concentration enhanced the maximum production of gas. From figure 4.1d (v), decrease in pH and retention time (21.7 days) gave the maximum gas yield. From figure 4.1d (vi), increase in both pH and total solids concentration resulted in maximum yield of gas. From figure 4.1d (vii), both pH and volatile solids increased in values to ensure the generation of maximum biogas. From figure 4.1d (viii), both total solids and retention time fluctuated and the values that resulted in the maximum yield of gas were 10.7 g/kg and 25.7 days respectively. From figure 4.1d (ix), increase in both volatile solids and retention time gave rise to maximum gas yield. From figure 4.1d (x), increase in total solids was commensurate with generation of maximum biogas while the contribution of volatile solids was negligible. In all, the ANNs plots showed more interactions among the five variables than the RSM plots.

Appendix 8d (i-x) shows the 3-Dimensional surface plots for the interaction of the five variables employed in the optimization of biogas from the anaerobic digestion of *Arachis hypogaea* hull. From figure 4.1e (i), low temperature (30.3 ^{o}C) and decrease in pH resulted in the production of maximum gas yield. From figure 4.1e (ii), lower temperature (30.3 ^{o}C) coupled with high retention time gave rise to maximum gas generation. From figure

4.1e (iii), low temperature (33 ^{o}C) and high total solids content gave rise to the maximum gas production. From figure 4.1e (iv), low volatile solids content contributed to the production of maximum gas yield while the impact of temperature remained negligible. From figure 4.1e (v), high retention time and low pH values gave the maximum yield of gas. From figure 4.1e (vi), increasing total solids with optimal of 4.27 g/kg and high pH resulted in the production of maximum biogas yield. From figure 4.1e (vii), both volatile solids and pH increased in values to ensure the generation of maximum biogas. From figure 4.1e (viii), high retention time with low total solids content gave the maximum biogas yield. From figure 4.1e (ix), both retention time and volatile solids were high to thus producing the maximum gas. From figure 4.1e (x), high levels of total solids and low volatile solids contributed to producing the highest biogas yield. In all, both models (RSM and ANNs) showed good interactions in the curvature nature of their plots. The ANNs however were still more pronounced and better.

Appendix 8e (i-x) shows the 3-Dimensional surface plots for the interaction of the five independent variable used in the optimization of biogas generation from the anaerobic co-digestion of *Tithonia diversifolia* and poultry dropping. From figure 4.1f (i), increase in temperature and decrease in pH (6.16) culminated into generating the maximum biogas yield from the experiment. From figure 4.1f (ii), increase in temperature coupled with decrease in retention time gave rise to the maximum gas yield. From figure 4.1f (iii), increase in temperature and decrease in total solids gave rise to the maximum gas yield. From figure 4.1f (iv), increasing temperature and decreasing volatile solids content produced the maximum biogas yield. From figure 4.1f (v), increase in both retention time and pH led to maximum gas yield. From figure 4.1f (vi), increasing total solids content coupled with decreasing pH caused maximum gas yield. From figure 4.1f (vii), increasing pH values and decreasing volatile solids (2.13) gave maximum gas yield. From figure 4.1f (viii), increasing retention time and decreasing total solids content gave rise to maximum gas production. From figure 4.1f (ix and x), increase in the trio of retention time, total and volatile solids led to generation of maximum gas. Once again, higher level of variable interactions was found in the ANNs surface plots in comparison with those of RSM.

Appendix 8f (i-x) shows the 3-Dimensional surface plots of the interactions of the five independent variables employed in the optimization of biogas generation from the anaerobic co-digestion of *Chromolaena odorata* shoot and poultry dropping. From figure 4.1g (i), increasing temperature coupled with decreasing pH values gave the maximum biogas yield. From figure 4.1g (ii), decrease in temperature (30 ^{o}C) and increase in retention time led to the generation of maximum gas. From figure 4.1g (iii), decrease in temperature (30.3 ^{o}C) and increase in total solids gave the best gas yield. From figure 4.1g (iv), increase in temperature and decrease in volatile solids gave the maximum gas yield. From figure 4.1g (v and vi), decreased pH (6.55) coupled with increase in both retention time and total solids resulted in maximum gas yield. From figure 4.1g (vii), increase in both pH and volatile solids content gave the best biogas yield. From figure 4.1g (viii and ix), increase in values of retention time, total and volatile solids all contributed to generating the maximum gas yield. From figure 4.1g (x), increase in total solids concentration and decrease in volatile solids resulted in maximum gas yield. In all, the ANNs surface plot showed pronounced interactions between the five variables as reflected in their curvature nature unlike the RSM that showed moderate interactions.

Appendix 8g (i-x) shows the 3-Dimensional surface plots of the relationship between the five independent variables employed in the optimization of biogas generation from the co-digestion of *Carica papayas* fruit peels and poultry dropping. From figure 4.1h (i and ii), decrease in both temperature and pH and increase in retention time gave rise to the maximum gas yield. From figure 4.1h (iii and iv), increase in temperature and decrease in both total and volatile solids contents resulted in maximum gas yield. From figure 4.1h (v), increase in temperature and neutral pH (7.1) gave the best gas yield. From figure 4.1h (vi and vii), increase in pH values and decrease in total and volatile solids resulted in maximum gas yield. From figure 4.1h (viii and ix), increase in retention time and decreased total and volatile solids led to maximum gas production. From figure 4.1h (x), increase in total solids and decrease in volatile solids gave the best gas yield. Again, ANNs surface plots displayed better and more pronounced variable interactions than those of the RSM in this study.

Appendix 8h (i-x) shows the RSM and ANNs' 3-Dimensional surface plots for the relationships between the five independent variables (temperature, pH, retention time, total and volatile solids) employed in the optimization of biogas generation from the anaerobic co-digestion of *Telfairia occidentalis* fruit peels and poultry dropping. From figure 4.1i (i and ii), increase temperature and retention time and decrease in pH values led to production of maximum gas. From figure 4.1i (iii), decrease in temperature and increase in total solids content produced maximum gas. From figure 4.1i (iv), increasing trend for both temperature and volatile solids gave rise to maximum biogas yield. From figure 4.1i (v, vi and vii), increase in pH and decrease in retention time, solid and volatile solids led to maximum gas yield. From figure 4.1i (viii), increase in total solids coupled with increased retention time (27 days) gave the best gas yield. Further increase in retention time led to decreased gas generation. From figure 4.1i (ix and x), increase in retention time and volatile solids with insignificant decrease in total solids content gave the best gas yield. In this experiment also, the ANNs plots showed higher variable relationships than those of the RSM.

Appendix 8i (i-x) shows the 3-Dimensional surface plots of the interactions between the five independent variables (temperature, pH, retention time, total and volatile solids) employed in the optimization of biogas generation from the anaerobic co-digestion of *Arachis hypogaea* hull and poultry dropping. From figure 4.1j (i), increase in temperature coupled with decrease I pH gave maximum gas yield. From figure 4.1j (ii and iii), decrease in temperature (31.7 ^{o}C) and increase in retention time and total solids gave the best gas yield. From figure 4.1j (iv), increase in both temperature and volatile solids content yielded maximum biogas. From figure 4.1j (v), increase in pH and constant retention time yielded highest biogas. From figure 4.1j (vi), decrease in total solids and neutral pH (7.15) gave the best gas yield. From figure 4.1j (vii), increase in pH and minimal level of volatile solids (7.47 g/kg) gave the best gas yield. From figure 4.1j (viii, ix and x), increased retention time and increase total and volatile solids yielded the best biogas. However, for 4.1j (x), volatile solids contribution was low (4 g/kg). In all, higher variable interactions were shown by the ANNs surface plots than those of the RSM.

The regression coefficient and significance of response surface quadratic for biogas generation from all the ten substrates digested in this study are shown in appendix 3 (a-j). From the RSM surface plots in figures 4.1 and Appendix 8, the developed regression model equations describing the relationship between the biogas yield (Y) and the coded values of independent factors of temperature (X_1), pH (X_2), retention time (X_3), total solids (X_4) and volatile solids (X_5) and their respective interactions are described in Equations 4.1 to 4.10 below:

For the mono-digestion of *Tithonia diversifolia* shoot, the equation is:

$$\begin{aligned} Y = {} & 3589.55 - 14.85x_1 + 76.87x_2 + 196.17x_3 + 59.06x_4 + 52.68x_5 \\ & - 58.29x_1x_2 - 196.59x_1x_3 + 22.75x_1x_4 + 46.70x_1x_5 + 47.43x_2x_3 \\ & + 179.66x_2x_4 - 193.63x_2x_5 - + 112.29x_3x_4 - 325.79x_3x_5 + 251.90x_4x_5 \\ & + 24.14x_1^2 - 147.51x_2^2 - 29.10x_3^2 - 6.38x_4^2 + 1.78x_5^2 \end{aligned} \quad (4.1)$$

For the mono-digestion of *Chromolaena odorata* shoot, the equation is:

$$\begin{aligned} Y = {} & 1709.59 + 49.24x_1 - 21.15x_2 + 66.34x_3 + 206.16x_4 - 106.58x_5 - 3.64x_1x_2 \\ & - 34.34x_1x_3 - 57.49x_1x_4 + 97.14x_1x_5 - 69.05x_2x_3 - 30.37x_2x_4 + 51.26x_2x_5 \\ & - 217.64x_3x_4 - 95.75x_3x_5 - 43.34x_4x_5 + 87.55x_1^2 + 104.06x_2^2 + 75.54x_3^2 \\ & - 247.41x_4^2 + 4.41x_5^2 \end{aligned} \quad (4.2)$$

For the mono-digestion of *Carica papaya* fruit peels, the equation is:

$$\begin{aligned} Y = {} & 1762.31 - 53.30x_1 + 95.79x_2 + 41.13x_3 + 7.29x_4 - 113.38x_5 + 38.35x_1x_2 \\ & + 85.14x_1x_3 - 180.58x_1x_4 - 114.43x_1x_5 - 105.32x_2x_3 + 135.67x_2x_4 + 119.31x_2x_5 \\ & - + 71.36x_3x_4 + 58.70x_3x_5 + 2.70x_4x_5 - 38.09x_1^2 - 11.74x_2^2 + 10.43x_3^2 \\ & + 24.45x_4^2 - 72.70x_5^2 \end{aligned} \quad (4.3)$$

For the mono-digestion of *Telfairia occidentalis* fruit peels, the equation is:

$$\begin{aligned} Y = {} & 1770.17 + 13.16x_1 - 2.51x_2 - 13.62x_3 + 50.41x_4 + 3.64x_5 + 15.19x_1x_2 + \\ & 71.23x_1x_3 + 52.31x_1x_4 + 14.24x_1x_5 - 9.47x_2x_3 - 26.60x_2x_4 - 25.73x_2x_5 \\ & + 0.23x_3x_4 + 17.33x_3x_5 - 1.79x_4x_5 + 21.42x_1^2 + 16.89x_2^2 - 20.48x_3^2 \\ & - 55.72x_4^2 + 7.04x_5^2 \end{aligned} \quad (4.4)$$

For the mono-digestion of *Arachis hypogaea* fruit pods, the equation is:

$$\begin{aligned} Y = {} & 1662.02 - 65.93x_1 + 99.71x_2 + 117.08x_3 + 162.94x_4 - 63.17x_5 \\ & - 154.68x_1x_2 - 132.15x_1x_3 - 118.16x_1x_4 + 147.50x_1x_5 + 127.53x_2x_3 \\ & + 182.63x_2x_4 - 147.86x_2x_5 + 142.30x_3x_4 - 170.05x_3x_5 - 160.59x_4x_5 \\ & + 48.78x_1^2 + 62.53x_2^2 + 65.81x_3^2 - 63.33x_4^2 + 51.80x_5^2 \end{aligned} \quad (4.5)$$

For the co-digestion of *Tithonia diversifolia* shoot and poultry dropping, the equation is:

$$\begin{aligned} Y = {} & 3545.80 - 168.76x_1 + 158.38x_2 + 215.05x_3 + 297.09x_4 + 12.61x_5 \\ & + 110.61x_1x_2 + 389.41x_1x_3 + 174.68x_1x_4 + 233.59x_1x_5 - 220.72x_2x_3 \\ & - 246.93x_2x_4 - 207.79x_2x_5 + 44.81x_3x_4 \; 28.35x_3x_5 - 143.87x_4x_5 \end{aligned} \quad (4.6)$$

$$+ 151.63x_1^2 + 160.46x_2^2 + 64.84x_3^2 + 26.97x_4^2 - 44.20x_5^2$$

For the co-digestion of *Chromolaena odorata* shoot and poultry dropping, the equation is:

$$\begin{aligned} Y = {} & 3946.63 - 70.12x_1 - 4.78x_2 + 188.22x_3 + 226.97x_4 + 68.39x_5 \\ & + 198.06x_1x_2 - 217.61x_1x_3 - 225.14x_1x_4 - 3.28x_1x_5 - 214.91x_2x_3 \\ & - 208.82x_2x_4 - 136.20x_2x_5 + 60.86x_3x_4 + 172.88x_3x_5 + 269.89x_4x_5 \\ & + 30.60x_1^2 + 48.90x_2^2 - 77.99x_3^2 - 280.98x_4^2 + 45.53x_5^2 \end{aligned} \quad (4.7)$$

For the co-digestion of *Carica papaya* fruit peels and poultry dropping, the equation is:

$$\begin{aligned} Y = {} & 3861.63 + 124.89x_1 + 9.56x_2 + 33.40x_3 + 132.94x_4 + 62.83x_5 + 36.17x_1x_2 \\ & - 85.30x_1x_3 + 270.54x_1x_4 - 2.79x_1x_5 - 182.46x_2x_3 + 206.20x_2x_4 + 50.01x_2x_5 \\ & + 72.43x_3x_4 - 50.97x_3x_5 - 49.56x_4x_5 + 0.28x_1^2 - 13.32x_2^2 - 26.71x_3^2 \\ & - 28.02x_4^2 - 51.60x_5^2 \end{aligned} \quad (4.8)$$

For the co-digestion of *Telfairia occidentalis* shoot and poultry dropping, the equation is:

$$\begin{aligned} Y = {} & 2598.05 + 66.99x_1 - 22.99x_2 + 95.31x_3 + 42.49x_4 + 26.87x_5 + 108.29x_1x_2 - \\ & 58.59x_1x_3 - 178.86x_1x_4 - 215.43x_1x_5 + 144.40x_2x_3 + 19.80x_2x_4 + 70.79x_2x_5 \\ & - 163.03x_3x_4 - 177.55x_3x_5 - 23.90x_4x_5 + 2.37x_1^2 - 27.83x_2^2 - 36.10\ x_3^2 - 2.12x_4^2 \\ & - 4.66x_5^2 \end{aligned} \quad (4.9)$$

For the co-digestion of *Arachis hypogeal* fruit peels and poultry dropping, the equation is:

$$\begin{aligned} Y = {} & 2547.12 + 60.92x_1 + 2.61x_2 + 127.22x_3 + 169.05x_4 + 109.37x_5 \\ & + 100.20x_1x_2 + 85.28x_1x_3 + 8.35x_1x_4 + 30.41x_1x_5 + 101.14x_2x_3 \\ & + 71.49x_2x_4 + 182.16x_2x_5 + 72.27x_3x_4 + 29.38x_3x_5 + 97.98x_4x_5 \\ & - 24.84x_1^2 + 79.56x_2^2 + 18.55x_3^2 - 16.48x_4^2 + 51.40x_5^2 \end{aligned} \quad (4.10)$$

Where Y = Biogas yield ($10^{-3}m^3$/kg VS)

4.9. Comparison between RSM and ANNs Models

Both the RSM and ANNs design matrix for biogas generation from the five mono-digestion experiments with the five independent variables using actual values are shown in Tables 4.6 (a-e). The optimal conditions for mono-digestion of *Tithonia diversifolia* shoot were statistically predicted as X_1 = 36.80 ^{o} C, X_2 = 7.69, X_3 = 20.23 days, X_4 = 9.64 g/kg and X_5 = 11.78 g/kg with 100% desirability. The most desirable actual biogas yield under these set conditions was 2139.20 $10^{-3}m^3$/ kg VS while the predicted biogas yield was 2219.24 $10^{-3}m^3$/ kg VS for RSM and 21.44.60 $10^{-3}m^3$/kg VS for ANNs. In the mono-digestion of *Chromolaena odorata* shoot, the optimal conditions for the process were statistically predicted as X_1 = 30.00 ^{o} C, X_2 = 7.5, X_3 = 30 days, X_4 = 12.00 g/kg and X_5 = 4.00 g/kg with the desirability of 0.958 (95.8%). The most desirable actual biogas yield

under these set conditions was 3554.20 $10^{-3}m^3$/ kg VS while the predicted biogas yield was 3565.70 $10^{-3}m^3$/kg VS for RSM and 3555.50 $10^{-3}m^3$/kg VS for ANNs.

In the mono-digestion of *Carica papaya* peels, the optimal conditions for the process were statistically predicted as X_1 = 32.00 ^{o} C, X_2 = 7.50, X_3 = 30.00 days, X_4 = 12.00 g/kg and X_5 = 12.00 g/kg with 100% desirability. The most desirable actual biogas yield under these set conditions was 1839.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 1894.80 $10^{-3}m^3$/kg VS for RSM and 1838.70 m^3/kg VS. In the mono-digestion of *Telfairia occidentalis* peels, the optimal conditions for the process were statistically predicted as X_1 = 30.02 ^{o} C, X_2 = 7.90, X_3 = 20.03 days, X_4 = 5.94 g/kg and X_5 = 4.01 g/kg with 100% desirability. The most desirable actual biogas yield was under these set conditions was 1639.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 1659.90 $10^{-3}m^3$/kg VS for RSM and 1639.50 $10^{-3}m^3$/kg VS for ANNs. In the mono-digestion of *Arachis hypogaea* hull, the optimal conditions for the process were statistically predicted as X_1 = 30.00 ^{o} C, X_2 = 7.50, X_3 = 30.00 days, X_4 = 12.00 g/kg and X_5 = 4.00 g/kg with 91% desirability. The most desirable actual biogas yield under these above set conditions was 1739.20 $10^{-3}m^3$/ kg VS while the predicted biogas yield was 1819.89 $10^{-3}m^3$/kg VS for RSM and 1743.60 $10^{-3}m^3$/kg VS for ANNs.

In order to verify the predictions of the RSM and ANNs models for all the five mono-digestions, the optimal conditions were applied to three independent replicates, and the average biogas yield obtained were 2208.82, 4040.92, 1802.98, 1642.58 and 1712.21 $10^{-3}m^3$/kg VS for the mono-digestions respectively.

Table 4.6a: Experimental Matrix for Biogas Generation from the Anaerobic Digestion of *Tithonia diversifolia* Shoot with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	36.80	7.69	20.23	9.64	11.78	2139.2	2219.24	2144.6
2	36.02	6.61	29.19	7.99	4.22	1280.9	1283.93	1277.9
3	34.94	6.71	29.97	8.56	4.30	1965.1	2196.74	1985.3
4	30.00	6.50	20.00	12.00	4.00	1073.3	1146.52	1746.8
5	30.08	6.51	27.97	10.60	4.15	2100.1	2121.93	2099.4
6	39.39	7.93	20.19	10.89	11.93	1923.1	2181.76	1746.8
7	39.96	6.50	30.00	10.86	4.02	1984.2	2186.67	1990.3
8	30.24	6.52	29.96	4.80	4.08	925.91	1086.23	924.22
9	30.01	7.93	29.91	8.51	4.03	1963.3	2085.48	1946.2
10	39.78	7.95	20.05	9.97	11.42	1951.1	2008.86	1950.1
11	36.58	6.69	29.83	7.89	4.21	1907.1	2082.26	1961.5
12	30.47	6.53	20.08	11.92	4.05	2181.0	2209.41	2170.9
13	39.98	6.61	29.93	9.53	4.73	1191.6	1190.92	1194.0
14	34.59	6.55	29.57	9.34	4.23	2151.1	2239.34	2149.1
15	32.21	6.52	28.06	11.54	4.02	1221.2	1286.02	1218.0
16	30.00	7.57	29.97	9.26	4.06	2111.9	2181.64	1746.8
17	30.18	6.67	27.33	10.17	4.01	2098.0	2244.50	2095.0
18	39.90	6.73	29.98	9.29	4.05	1732.0	1881.09	1728.4
19	39.99	8.00	20.20	8.33	11.92	1877.3	1895.44	1746.8
20	30.56	6.87	29.35	7.54	4.17	100.92	118.130	112.87
21	30.26	7.99	29.98	8.44	4.00	1900.1	1982.01	1926.4
22	30.20	7.15	29.93	9.11	4.31	1597.2	1599.69	1596.0
23	39.91	6.54	29.90	5.97	4.71	1556.1	1693.88	1555.5
24	31.59	6.52	29.66	8.92	5.99	2042.1	2112.76	1996.0
25	30.09	6.50	20.03	10.47	4.02	1988.1	2081.04	1982.5
26	30.54	7.01	29.75	9.22	4.07	1950.0	2041.08	1940.5
27	30.01	7.63	29.96	8.64	4.08	1569.0	1802.04	1567.1
28	30.07	7.19	29.88	10.31	4.23	2010.0	2081.26	1994.4
29	30.00	6.59	23.64	10.58	4.01	1400.0	1481.62	1402.8
30	39.87	6.62	29.54	6.73	4.04	1976.0	2092.90	1976.3
31	30.00	7.57	29.97	9.26	4.06	2111.9	1486.02	1218.0
32	30.18	6.67	27.33	10.17	4.01	2098.0	2281.64	1746.8
33	39.90	6.73	29.98	9.29	4.05	1732.0	2344.50	2095.0
34	39.99	8.00	20.20	8.33	11.92	1877.3	1981.09	1728.4
35	30.56	6.87	29.35	7.54	4.17	100.92	1898.44	1746.8
36	36.58	6.69	29.83	7.89	4.21	2302.2	2314.42	1950.1
37	30.47	6.53	20.08	11.92	4.05	2245.9	2970.03	1961.5
38	39.98	6.61	29.93	9.53	4.73	2670.3	2612.21	2170.9

4.6a: Experimental Matrix for Biogas Generation from the Anaerobic Digestion of *Tithonia diversifolia* Shoot with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
39	34.59	6.55	29.57	9.34	4.23	2587.1	3521.31	1194.0
40	32.21	6.52	28.06	11.54	4.02	2421.3	2491.48	2149.1
41	30.00	7.53	27.97	9.26	4.06	2509.2	2543.31	1218.0
42	30.18	6.77	27.33	10.17	4.01	2507.9	2614.15	1746.8
43	37.90	6.73	26.98	9.29	4.05	2012.1	2107.04	2095.0
44	36.99	8.00	20.20	8.33	11.92	2302.2	2311.14	1728.4
45	30.56	6.57	27.35	7.54	4.17	2378.4	2312.12	1746.8
46	30.26	7.89	26.98	8.44	4.00	2501.6	2539.03	112.87
47	30.20	7.15	2.93	9.11	4.31	2400.1	2431.23	1926.4
48	35.91	6.54	27.90	5.97	4.71	2301.4	2331.90	1596.0
49	31.59	6.51	27.66	8.92	5.99	2031.3	2141.80	1555.5
50	30.09	6.50	20.03	10.47	4.02	2231.1	2242.02	1996.0

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6b: Experimental Matrix for Biogas Generation from the Anaerobic Digestion of *Chromolaena Odorata* Shoot with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	30.00	7.50	30.00	12.00	4.00	3554.2	3565.70	3555.5
2	30.00	7.50	30.00	9.87	4.03	4200.0	4326.61	4113.2
3	30.00	7.60	30.00	9.35	4.00	3472.2	3415.44	3476.9
4	30.00	7.96	30.00	11.99	4.12	4175.4	4211.84	4171.8
5	30.00	7.50	30.01	9.34	4.00	3896.4	3901.82	3610.1
6	30.00	7.40	29.90	12.00	4.69	3600.3	3601.79	3605.2
7	30.00	7.80	29.84	8.62	4.00	3573.2	3680.67	3573.8
8	30.22	7.50	29.95	8.58	4.00	4201.1	4376.98	4009.1
9	30.00	7.38	30.00	6.33	4.00	4502.8	4561.98	4526.2
10	30.00	7.60	30.00	6.45	4.17	3500.0	3539.40	3488.9
11	30.00	7.98	30.00	5.65	4.00	3834.1	3934.47	3831.4
12	30.00	8.00	29.93	10.96	5.34	3572.2	3626.10	3575.8
13	30.02	8.00	30.00	4.00	4.00	3521.1	3609.64	3584.4
14	30.00	8.00	30.00	12.00	6.74	3591.1	3684.88	3559.6
15	30.00	7.78	30.00	11.64	4.00	3480.6	3493.12	3610.1
16	30.00	7.77	30.00	12.00	4.43	3500.0	3569.73	3507.8
17	30.00	8.00	29.86	4.21	5.03	3452.2	3504.70	3610.1
18	30.88	7.65	30.00	4.00	4.00	3861.1	3898.46	3865.0
19	30.00	8.00	30.00	12.00	11.29	3590.1	3586.44	3591.9
20	40.00	6.50	20.00	12.00	12.00	3472.9	3560.67	3474.8
21	40.00	6.50	20.00	9.70	12.00	3873.0	3941.07	4117.1
22	30.00	7.97	30.00	12.00	11.79	5200.1	5065.70	5200.2
23	30.16	8.00	30.00	12.00	12.00	3968.0	4031.32	3961.2
24	31.24	8.00	30.00	12.00	10.84	4191.0	4225.86	4199.9
25	35.08	8.00	30.00	11.99	6.18	3562.1	3522.99	3571.5
26	40.00	6.57	20.00	11.98	12.00	3594.9	3622.30	3610.1
27	39.99	6.50	20.36	9.68	12.00	3594.0	3614.03	3612.6
28	40.00	6.54	20.00	7.20	12.00	2005.7	2184.88	2007.0
29	39.97	6.50	20.75	7.36	12.00	3422.0	3449.23	3422.6
30	40.00	6.53	20.01	4.19	11.78	4432.2	4438.07	4388.7
31	35.00	8.00	22.09	5.43	10.00	3627.4	3671.31	3654.8
32	37.00	7.65	30.00	5.61	8.31	3501.1	3601.02	3554.3
33	36.00	8.00	29.93	12.00	5.34	3512.2	3521.20	3515.5
34	36.88	6.50	30.00	8.62	4.00	3300.1	3102.41	3374.8
35	37.00	6.50	30.00	8.58	6.74	3581.2	3565.31	3546.5
36	36.51	8.00	30.00	6.33	4.00	3302.2	3311.42	3305.1
37	34.09	8.00	30.00	6.45	4.43	2945.9	2970.03	2948.8
38	30.00	8.00	29.86	5.65	5.03	3670.3	3612.21	3654.4
39	30.88	7.78	30.00	10.96	4.00	3587.1	3521.31	3543.6

Table 4.6b: Experimental Matrix for Biogas Generation from the Anaerobic Digestion of *Chromolaena Odorata* Shoot with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	36.88	6.50	30.00	8.62	4.00	3300.1	3102.41	3374.8
41	40.00	8.00	20.00	12.00	12.00	2509.2	2543.31	2561.0
42	40.00	7.65	20.00	11.64	12.00	2507.9	2614.15	3065.9
43	30.00	8.00	30.00	12.00	11.79	3012.1	3107.04	2305.5
44	30.16	6.50	30.00	4.21	12.00	2302.2	2311.14	2361.5
45	31.24	6.50	30.00	4.00	10.84	2378.4	2312.12	2508.8
46	35.08	7.97	30.00	12.00	6.18	2501.6	2549.03	2451.1
47	40.00	8.00	20.00	12.00	6.54	2400.1	2461.23	2323.1
48	39.40	8.00	20.36	9.70	5.03	2301.4	2331.90	2035.6
49	38.41	7.56	20.00	11.09	4.00	2031.3	2041.80	2243.3
50	37.71	7.63	24.32	10.32	11.29	2231.1	2262.02	

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6c: Experimental Matrix for Biogas Generation from the Anaerobic Digestion of *Carica papaya* Peels with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	32.00	7.50	30.00	12.00	12.00	1839.20	1894.80	1838.70
2	32.00	7.50	30.00	11.83	12.00	1680.90	1700.30	1680.20
3	33.00	7.51	30.00	11.77	11.99	1965.10	2021.80	1967.00
4	32.00	7.50	29.63	11.93	12.00	1403.90	1490.62	1404.10
5	32.00	7.50	29.71	12.00	11.86	1700.10	1701.87	1702.40
6	31.01	7.50	30.00	12.00	11.63	1723.10	1754.92	1723.00
7	31.00	7.50	29.76	11.92	11.71	1284.20	1207.26	1284.60
8	30.00	7.50	29.33	11.99	12.00	2115.90	2204.80	2201.80
9	30.01	7.50	30.00	11.98	11.23	1963.30	2004.63	1743.20
10	33.00	7.50	29.88	10.74	12.00	1951.10	2007.50	1951.20
11	32.00	7.50	30.00	11.99	10.87	1907.10	2008.74	1909.60
12	31.00	7.50	30.00	10.53	11.92	1821.00	1902.82	1821.00
13	31.01	7.64	30.00	11.33	12.00	1591.60	1603.57	1743.20
14	30.00	6.56	30.00	12.00	10.91	1561.10	1607.20	1561.30
15	31.00	6.50	28.83	12.00	11.40	1721.20	1802.53	1718.90
16	30.02	6.50	28.10	12.00	11.97	2199.90	2199.92	2201.60
17	31.85	6.51	29.48	12.00	12.00	1728.00	1816.15	1726.80
18	30.41	6.50	27.91	11.99	12.00	1732.00	1770.82	1731.90
19	30.00	6.52	27.59	12.00	12.00	1877.30	1906.89	1869.70
20	30.00	6.52	30.00	12.00	9.85	1800.90	1850.08	1800.90
21	30.00	6.50	27.20	11.91	12.00	1900.10	2007.85	1899.90
22	32.90	6.51	30.00	12.00	12.00	1597.20	1617.86	1596.80
23	30.00	6.50	30.00	11.17	9.57	1556.10	1507.21	1555.30
24	30.05	6.72	30.00	9.91	12.00	1742.10	1700.01	1743.20
25	32.77	6.51	29.15	12.00	12.00	1688.10	1798.64	1743.20
26	30.00	6.50	30.00	10.76	9.48	1450.00	1581.74	1451.10
27	30.63	6.97	29.98	9.00	12.00	1569.00	1576.99	1569.60
28	30.00	7.09	30.00	11.98	12.00	1710.00	1874.91	1718.60
29	30.00	7.50	29.60	9.00	8.08	1800.00	2073.20	1808.70
30	30.08	7.55	28.00	11.39	12.00	1376.00	1462.92	1375.40
31	31.05	7.50	29.71	12.00	11.86	1563.30	1604.63	1751.20
32	31.01	7.50	30.00	9.00	11.63	1651.10	1607.50	1609.60
33	31.00	7.70	29.76	11.92	11.71	1607.10	1608.74	1601.00
34	30.00	7.50	29.33	10.99	12.00	1421.00	1402.82	1403.20
35	30.01	7.50	28.00	11.98	11.23	1521.60	1503.57	1500.30
36	32.00	7.60	29.88	10.74	12.00	1501.10	1507.20	1501.90
37	31.00	7.50	28.00	10.99	10.87	1521.20	1502.53	1401.60
38	31.00	7.50	30.00	10.53	11.92	2179.90	2139.92	1926.80
39	31.01	7.64	30.00	9.33	12.00	1528.00	1516.15	1501.90

Table 4.6c: Experimental Matrix for Biogas Generation from the Anaerobic Digestion of *Carica papaya* Peels with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	30.00	6.56	29.00	12.00	10.91	1732.00	1750.82	1669.70
41	31.00	6.50	28.83	12.00	11.40	1877.30	1806.89	1800.90
42	30.02	6.50	28.10	12.00	11.97	1854.90	1850.08	1809.90
43	31.85	6.51	29.48	12.00	12.00	1800.10	1787.85	1696.80
44	30.41	6.50	27.91	11.99	12.00	1597.20	1517.86	1505.30
45	30.00	6.52	27.59	12.00	12.00	1576.10	1507.21	1503.20
46	30.00	6.52	30.00	12.00	9.85	1642.10	1600.01	1583.20
47	32.00	7.50	30.00	11.99	10.87	1888.10	1798.64	1751.10
48	31.00	7.50	30.00	10.53	11.92	1546.32	1543.03	1529.60
49	31.01	7.64	30.00	11.33	12.00	1654.02	1535.21	1518.60
50	30.05	7.54	27.00	10.05	10.40	1651.50	1603.21	1608.70

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6d: Experimental Matrix for Biogas Generation from the Digestion of *Telfairia occidentalis* Peels with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	30.02	7.90	20.03	5.94	4.01	1639.2	1659.90	1639.5
2	39.98	7.90	29.88	11.45	11.83	1660.9	1690.95	1661.2
3	30.43	7.99	20.05	6.64	4.11	1865.1	1701.66	1870.4
4	39.85	6.59	25.46	11.79	11.60	1603.9	1620.58	1603.6
5	39.98	6.53	29.57	11.98	7.08	1400.1	1411.60	1400.5
6	39.52	6.52	25.39	10.86	11.51	1900.1	1911.31	1885.6
7	40.00	7.72	29.99	11.03	10.89	1841.2	1900.22	1840.3
8	39.93	7.08	29.23	11.89	9.23	1825.9	1901.59	1826.3
9	39.68	6.68	29.68	9.99	11.24	1763.3	1783.09	1763.1
10	39.56	7.41	29.89	11.42	11.77	1751.1	1801.77	1751.2
11	39.77	6.74	29.92	8.40	11.45	1607.1	1652.99	1607.1
12	30.22	7.92	20.09	7.46	4.05	1821.0	1852.25	1819.5
13	39.17	6.68	26.24	10.69	11.97	1891.6	1906.40	1890.2
14	39.96	6.63	25.40	11.30	11.62	1800.1	1816.65	1766.5
15	39.97	6.99	29.35	11.91	9.24	1721.2	1808.11	1721.6
16	39.96	6.55	27.00	11.29	10.30	1804.9	1826.54	1766.5
17	39.21	6.74	27.19	11.70	11.23	1728.0	1809.20	1766.5
18	39.97	7.74	29.72	10.86	11.42	1732.0	1801.06	1766.5
19	40.00	7.70	29.65	11.89	11.58	1877.3	1901.42	1877.1
20	30.43	7.99	20.05	6.64	4.11	1763.3	1783.09	1763.1
21	39.85	6.59	25.46	11.79	11.60	1751.1	1801.77	1751.2
22	39.98	6.53	29.57	11.98	7.08	1607.1	1652.99	1607.1
23	39.52	6.52	25.39	10.86	11.51	1821.0	1852.25	1819.5
24	40.00	7.72	29.99	11.03	10.89	1891.6	1906.40	1890.2
25	30.00	8.00	20.00	7.95	5.56	1688.1	1707.18	1689.3
26	40.00	8.00	29.82	11.05	4.01	1650.0	1687.04	1650.0
27	40.00	8.00	29.53	11.26	5.38	1869.0	1885.15	1881.5
28	40.00	8.00	29.18	9.85	5.07	1710.0	1783.16	1710.3
29	30.00	7.53	20.00	6.58	4.00	1850.0	1883.11	1850.7
30	40.00	8.00	26.91	10.30	4.45	1776.0	1782.69	1776.3
31	38.00	7.82	28.99	10.03	10.19	1801.1	1832.12	1821.3
32	37.93	7.08	29.23	11.89	9.03	1707.1	1716.03	1714.3
33	38.68	6.58	28.68	9.29	10.24	1751.0	1798.91	1743.2
34	38.56	7.41	29.89	10.42	10.17	1792.6	1801.20	1789.4
35	37.77	6.74	29.92	8.40	11.45	1810.1	1822.03	1803.8
36	36.22	7.62	20.09	7.46	4.05	1811.2	1823.09	1821.1
37	39.17	6.58	26.24	10.69	10.97	1834.9	1843.21	1840.0
38	38.96	6.63	25.40	11.30	10.62	1623.0	1700.01	1654.6
39	38.97	6.69	29.65	10.91	9.24	1662.0	1701.03	1669.7

Table 4.6d: Experimental Matrix for Biogas Generation from the Digestion of *Telfairia Occidentalis* Peels with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	37.96	6.55	27.00	10.29	10.30	1707.3	1712.09	1709.0
41	39.21	6.75	27.19	11.70	10.23	1811.9	1843.43	1824.8
42	39.97	7.74	29.42	10.86	11.42	1831.1	1876.32	1842.2
43	40.00	7.71	29.45	11.89	10.58	1697.2	1702.03	1699.9
44	39.99	7.19	29.94	11.53	9.40	1659.1	1711.02	1663.2
45	38.95	7.45	29.64	10.21	10.96	1642.1	1693.21	1656.6
46	40.00	7.55	30.00	10.57	8.57	1488.1	1501.02	1496.6
47	38.00	8.00	29.08	9.85	6.07	1750.0	1762.02	1758.8
48	30.00	7.53	20.00	6.58	4.00	1729.0	1725.31	1731.1
49	37.00	8.00	26.91	10.30	5.45	1820.0	1816.15	1819.6
50	38.00	7.52	27.59	10.03	10.89	1651.4	1661.61	1559.4

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6e: Experimental Matrix for Biogas Generation from the Digestion of *Arachis hypogaea* Hull with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	30.00	7.50	30.00	12.00	4.00	1739.2	1819.89	1743.6
2	30.00	7.99	30.00	12.00	4.00	1660.9	1810.61	1658.9
3	30.02	7.99	30.00	11.73	4.00	1851.1	2760.97	1846.0
4	30.00	8.00	29.48	11.93	4.00	1603.9	1723.79	1577.8
5	30.01	8.00	29.58	11.72	4.00	1650.1	2704.34	1640.3
6	31.10	8.00	29.93	12.00	4.00	1805.1	2653.89	1730.2
7	30.01	7.90	29.64	12.00	4.00	1841.2	2047.37	1833.2
8	30.00	7.94	30.00	12.00	4.61	1805.9	1030.20	1730.2
9	30.33	7.91	30.00	11.71	4.00	1763.3	1929.65	1782.1
10	30.04	7.83	30.00	11.98	4.00	1751.1	1821.96	1751.1
11	30.00	8.00	28.85	12.00	4.08	1607.1	1619.76	1611.8
12	30.00	7.96	30.00	10.91	4.00	1811.5	2594.78	1812.0
13	30.77	7.99	29.19	11.93	4.00	1841.6	2561.50	1842.8
14	30.00	8.00	29.70	12.00	5.30	1800.1	2521.60	1791.1
15	30.01	7.85	29.08	12.00	4.00	1721.2	2508.21	1730.2
16	31.01	8.00	30.00	12.00	4.97	1800.9	2494.76	1804.2
17	30.06	7.98	29.89	10.28	4.00	1728.3	2485.42	1763.4
18	31.34	8.00	30.00	12.00	4.96	1732.4	2452.97	1732.5
19	31.50	7.84	30.00	12.00	4.00	1877.3	2442.25	1885.1
20	32.22	8.00	29.48	12.00	4.00	1700.9	2434.01	1696.9
21	30.08	8.00	28.16	11.39	4.00	4100.1	3425.74	4099.9
22	30.02	8.00	30.00	11.94	6.18	1597.2	2393.18	1730.2
23	30.00	7.88	30.00	12.00	6.07	1556.1	2304.68	1567.6
24	30.00	8.00	26.53	12.00	4.11	1742.1	2263.54	1747.9
25	30.00	8.00	30.00	11.38	6.65	1688.1	2233.08	1692.5
26	30.00	7.90	25.68	12.00	4.00	1650.2	2059.74	1668.7
27	32.83	8.00	27.33	11.99	4.00	1869.1	2049.83	1869.5
28	30.00	8.00	28.10	12.00	6.79	1710.6	2034.89	1709.9
29	30.00	8.00	30.00	12.00	8.50	1850.4	2993.31	1804.4
30	37.92	8.00	30.00	12.00	4.00	1780.1	1808.36	1780.4
31	30.33	7.91	30.00	11.71	4.00	1841.6	2061.50	1611.8
32	30.04	7.83	30.00	11.98	4.00	1800.1	2021.60	1812.0
33	30.00	8.00	28.85	12.00	4.08	1721.2	1708.21	1742.8
34	30.00	7.96	30.00	10.91	4.00	1800.9	1894.76	1791.1
35	30.77	7.99	29.19	11.93	4.00	1728.0	1785.42	1730.2
36	30.00	8.00	29.70	12.00	5.30	1732.0	1852.97	1804.2
37	30.01	7.85	29.08	12.00	4.00	1877.3	1942.25	1763.4
38	31.01	8.00	30.00	12.00	4.97	1700.9	1734.01	1732.5
39	30.06	7.98	29.89	10.28	4.00	4100.1	4125.74	4105.1

Table 4.6e: Experimental Matrix for Biogas Generation from the Digestion of *Arachis Hypogaea* Hull with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	31.34	8.00	30.00	12.00	4.96	1597.2	1693.18	1696.9
41	31.50	7.84	30.00	12.00	4.00	1556.1	1604.68	1099.9
42	32.22	8.00	29.48	12.00	4.00	1742.1	1863.54	1730.2
43	30.08	8.00	28.16	11.39	4.00	1688.1	1733.08	1667.6
44	30.02	8.00	30.00	11.94	6.18	1650.0	1859.74	1747.9
45	30.00	7.88	30.00	12.00	6.07	1869.0	2049.83	1892.5
46	30.00	8.00	26.53	12.00	4.11	1710.0	2034.89	1668.7
47	30.00	8.00	30.00	11.38	6.65	1850.0	2023.31	1869.5
48	30.00	7.90	25.68	12.00	4.00	1650.0	2034.74	1609.9
49	32.83	8.00	27.33	11.99	4.00	1869.0	2029.83	1804.4
50	34.00	8.00	28.10	12.00	6.79	1730.0	1834.89	1718.5

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6 (f-j) also show both the RSM and ANNs design matrix for biogas generation from the five co-digestion experiments with the five independent variables using actual values For the co-digestion of *Tithonia diversifolia* shoot and poultry dropping, the optimal conditions for the process were statistically predicted as X_1 = 37.20 ^{o} C, X_2 = 7.50, X_3 = 29.95 days, X_4 = 11.97 g/kg and X_5 = 8.50 g/kg and the most desirable actual biogas yield under these set conditions was 2984.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 3111.07 $10^{-3}m^3$/kg VS for RSM and 2958.30 $10^{-3}m^3$/kg VS for ANNs with desirability of 1.00 (100%). In the co-digestion of *Chromolaena odorata* shoot and poultry dropping, the optimal conditions for the process were statistically predicted as X_1 = 30.00 ^{o} C, X_2 = 7.50, X_3 = 30.00 days, X_4 = 12.00 g/kg and X_5 = 12.00 g/kg with 100% desirability. The most desirable biogas yield under these set conditions was 3884.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 4178.81 $10^{-3}m^3$/kg VS for RSM and 4060.10 $10^{-3}m^3$/kg VS for ANNs.

In the co-digestion of *Carica papaya* peels and poultry dropping, the optimal conditions for the process were statistically predicted as X_1 = 36.84 ^{o} C, X_2 = 7.76, X_3 = 21.41 days, X_4 = 11.81 g/kg and X_5 = 11.81 g/kg with 100% desirability. The predicted biogas yield by RSM model under the above set conditions was 3991.77 $10^{-3}m^3$/kg VS while that of ANNs model was 3875.1 $10^{-3}m^3$/kg VS. In the co-digestion of *Telfairia occidentalis* peels and poultry dropping, the optimal conditions for the process were statistically predicted as X_1 = 37.00 ^{o} C, X_2 = 7.60, X_3 = 25.00 days, X_4 = 4.00 g/kg and X_5 = 4.00 g/kg with 98.3% desirability and the most desirable biogas yield under these conditions was 2539.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 2614.14 m^3/kg VS for RSM and 2540.34 $10^{-3}m^3$/kg VS for ANNs. In the co-digestion of *Arachis hypogaea* hull peels and poultry dropping, the optimal conditions for the process were statistically predicted as X_1 = 32.00 ^{o} C, X_2 = 7.62, X_3 = 30.00 days, X_4 = 12.00 g/kg and X_5 = 12.00 g/kg with 97.5% desirability and the most desirable actual biogas yield was under these conditions was 3339.20 $10^{-3}m^3$/kg VS while the predicted biogas yield was 3903.15 $10^{-3}m^3$/kg VS for RSM and 3338.30 $10^{-3}m^3$/kg VS for ANNs.

In order to verify the predictions of the RSM and ANNs models for all the five co-digestions, the optimal conditions were applied to three independent replicates, and the average biogas yield obtained were 3105.90, 4152.22, 3979.88, 2597.10 and 3986.13

m^3/kg VS for the five co-digestions respectively. All the values obtained after the validation tests are within the close range to those predicted by the models.

Table 4.6f: Experimental Matrix for Biogas Generation from the Co-Digestion of *Tithonia diversifolia* Shoot and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/ kg VS)	RSM Predicted yield ($10^{-3}m^3$/kg VS)	ANNs Predicted yield ($10^{-3}m^3$/ kg VS)
1	37.20	7.50	27.95	11.97	8.50	2984.2	3011.12	2958.3
2	37.99	6.65	27.83	11.46	11.49	4000.0	4111.20	3659.4
3	37.90	6.56	27.97	11.96	9.75	3872.2	3966.42	3915.6
4	37.30	6.52	27.83	11.82	11.23	3675.4	3659.40	3671.4
5	37.74	6.60	27.86	11.32	11.78	4796.4	4808.30	4809.2
6	40.00	7.96	29.37	11.87	4.00	3032.9	3156.90	3049.4
7	30.00	8.00	20.14	4.73	4.00	3273.0	3251.91	3272.5
8	37.00	6.52	30.00	12.00	6.58	3542.1	3642.30	3478.5
9	30.00	8.00	20.00	6.98	4.00	3568.0	3609.90	3687.1
10	40.00	6.83	30.00	12.00	8.89	3891.0	3807.41	3878.4
11	40.00	6.52	30.00	12.00	6.58	4534.1	4664.32	4537.7
12	30.00	8.00	20.00	6.98	4.00	3572.2	3653.21	3659.4
13	40.00	6.83	30.00	12.00	8.89	3521.1	3553.21	3659.4
14	30.00	8.00	20.02	11.89	4.01	4091.0	4144.31	4022.7
15	30.00	8.00	20.10	7.41	4.00	3980.6	3940.00	3987.5
16	30.01	8.00	20.00	9.41	4.00	5100.0	4939.82	5082.3
17	30.00	7.95	20.00	11.81	4.00	3852.2	3914.32	3884.1
18	30.02	7.99	20.00	6.69	4.52	4761.1	4902.81	4756.4
19	30.01	8.00	20.42	7.90	4.35	3990.1	3867.62	4020.0
20	30.00	8.00	20.00	5.61	5.54	3032.9	3156.90	3049.4
21	40.00	6.50	29.99	11.87	5.62	3273.0	3251.91	3272.5
22	37.30	6.50	29.84	12.00	8.81	3542.1	3642.30	3478.5
23	30.25	7.86	20.00	12.00	4.00	3568.0	3609.90	3687.1
24	36.39	6.50	29.51	12.00	11.99	3891.0	3807.41	3878.4
25	40.00	7.00	30.00	11.72	7.46	4562.1	4651.91	4585.5
26	40.00	8.00	30.00	12.00	6.95	3894.9	3719.71	3922.1
27	40.00	7.94	30.00	11.73	7.25	4674.8	4678.61	4618.8
28	40.00	7.66	30.00	12.00	5.74	3905.7	3956.60	3898.2
29	39.59	7.52	30.00	12.00	10.04	3522.0	3636.91	3659.4
30	40.00	7.96	29.37	11.87	4.00	3832.2	3836.11	3752.7
31	30.00	8.00	20.14	4.73	4.00	3841.6	3861.50	3771.4
32	37.00	6.52	30.00	12.00	6.58	3800.1	3821.60	3809.2
33	30.00	8.00	20.00	6.98	4.00	3721.2	3708.21	3795.7
34	40.00	6.83	30.00	12.00	8.89	3800.9	3894.76	3880.0
35	30.00	8.00	20.02	11.89	4.01	3728.0	3785.42	3703.5
36	30.00	8.00	20.10	7.41	4.00	3732.0	3852.97	3802.5
37	30.01	8.00	20.00	9.41	4.00	3877.3	3942.25	3810.1
38	30.00	7.95	20.00	11.81	4.00	3700.9	3734.01	3637.7
39	30.02	7.99	20.00	6.69	4.52	4100.1	4125.74	4109.4

Table 4.6f: Experimental Matrix for Biogas Generation from the Co-Digestion of *Tithonia diversifolia* Shoot and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/ kg VS)	RSM Predicted yield ($10^{-3}m^3$/kg VS)	ANNs Predicted yield ($10^{-3}m^3$/ kg VS)
40	30.01	8.00	20.42	7.90	4.35	3597.2	3693.18	3659.4
41	30.00	8.00	23.00	5.61	5.54	3556.1	3604.68	3622.7
42	40.00	6.50	29.99	11.87	5.62	3742.1	3863.54	3887.5
43	37.30	6.50	29.84	10.00	8.81	3688.1	3733.08	3582.3
44	30.25	7.66	20.00	10.00	4.00	3650.0	3859.74	3884.1
45	36.39	6.50	2.51	12.00	11.99	3869.0	3949.83	3856.4
46	36.00	7.00	30.00	11.72	7.46	3710.0	3734.89	3720.0
47	34.00	8.00	30.00	10.00	6.95	3850.0	3823.31	3849.4
48	34.00	7.94	30.00	11.73	7.25	3650.0	3834.74	3672.5
49	34.00	7.66	30.00	10.00	5.74	3869.0	3929.83	3878.5
50	37.59	7.52	30.00	10.00	9.04	3730.0	3834.89	3787.1

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6g: Experimental Matrix for Biogas Generation from the Co-Digestion of *Chromolaena odorata* Shoot and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values (G)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	30.00	7.50	30.00	12.00	12.00	3884.2	4178.81	4060.1
2	30.00	7.50	30.00	11.83	12.00	4000.5	4157.60	3893.3
3	30.00	7.51	30.00	11.77	11.99	3872.2	4043.30	3872.7
4	30.00	7.50	29.63	11.93	12.00	3875.4	4218.95	3875.4
5	30.00	7.50	29.71	12.00	11.86	4196.4	4512.12	4060.1
6	30.01	7.50	30.00	12.00	11.63	3900.3	4209.00	3929.4
7	30.00	7.50	29.76	11.92	11.71	3673.2	3884.49	3908.0
8	30.00	7.50	29.33	11.99	12.00	4201.1	4882.75	4210.6
9	30.01	7.50	30.00	11.98	11.23	3502.8	3832.75	3434.1
10	30.00	7.50	29.88	10.74	12.00	2600.8	2799.13	2592.6
11	30.00	7.50	30.00	11.99	10.87	3834.1	4168.09	3668.5
12	30.00	7.50	30.00	10.53	11.92	3572.2	3766.86	3583.9
13	30.01	7.64	30.00	11.33	12.00	3521.1	3743.44	3694.2
14	30.00	6.56	30.00	12.00	10.91	3991.1	4119.87	3894.3
15	30.00	6.50	28.83	12.00	11.40	3980.6	4206.31	3973.9
16	30.02	6.50	28.10	12.00	11.97	4000.9	4694.82	4060.1
17	31.85	6.51	29.48	12.00	12.00	3852.2	3924.91	3847.8
18	30.41	6.50	27.91	11.99	12.00	2861.1	2896.27	2862.5
19	30.00	6.52	27.59	12.00	12.00	4190.1	4600.42	4060.1
20	30.00	6.52	30.00	12.00	9.85	2932.9	2976.07	2932.8
21	30.00	6.50	27.20	11.91	12.00	3473.0	3551.58	3469.6
22	32.90	6.51	30.00	12.00	12.00	2300.1	2528.59	2294.1
23	30.00	6.50	30.00	11.17	9.57	3968.0	4077.18	3909.0
24	30.05	6.72	30.00	9.91	12.00	3891.0	3957.08	3892.7
25	32.77	6.51	29.15	12.00	12.00	3862.1	3851.91	3866.0
26	30.00	6.50	30.00	10.76	9.48	3294.9	3422.35	3371.0
27	30.63	6.97	29.98	12.00	12.00	6094.0	6175.93	6090.7
28	30.00	7.09	30.00	11.98	12.00	3905.7	4032.08	3905.4
29	30.00	6.50	29.60	12.00	8.08	3722.0	3753.25	3722.0
30	30.08	7.25	30.00	11.39	12.00	3832.2	3918.19	3840.5
31	30.00	7.50	29.33	11.99	12.00	3841.6	3861.50	3815.4
32	30.01	7.50	30.00	11.98	11.23	3800.1	3821.60	3860.1
33	30.00	7.50	29.88	10.74	12.00	3721.2	3708.21	3729.4
34	30.00	7.50	30.00	11.99	10.87	3800.9	3894.76	3808.0
35	30.00	7.50	30.00	10.53	11.92	3788.0	3885.42	3710.6
36	30.01	7.64	30.00	11.33	12.00	3732.0	3852.97	3734.1
37	30.00	6.56	30.00	12.00	10.91	3877.3	3942.25	3892.6
38	30.00	6.50	28.83	12.00	11.40	3710.9	3734.01	3768.5
39	30.02	6.50	28.10	12.00	11.97	4130.1	4165.74	4133.9

Table 4.6g: Experimental Matrix for Biogas Generation from the Co-Digestion of *Chromolaena odorata* Shoot and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	31.85	6.51	29.48	12.00	12.00	3597.2	3693.18	3594.2
41	30.41	6.50	27.91	11.99	12.00	3556.1	3604.68	3594.3
42	30.00	6.52	27.59	10.00	12.00	3742.1	3863.54	3873.9
43	30.00	6.52	30.00	1.00	9.85	3688.1	3733.08	3760.1
44	30.00	6.50	27.20	11.90	10.00	3650.0	3859.74	3747.8
45	32.90	6.51	30.00	12.00	12.00	3869.0	3949.83	3862.5
46	30.00	6.55	30.00	11.17	9.57	3710.0	3734.89	3760.1
47	30.05	6.72	30.00	9.91	12.00	3850.0	3823.31	3832.8
48	32.77	6.51	29.15	12.00	12.00	3650.0	3834.74	3769.6
49	30.00	6.54	30.00	10.76	9.48	3869.0	3929.83	3894.1
50	32.63	6.57	26.98	10.00	10.00	3730.0	3834.89	3809.0

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6h: Experimental Matrix for Biogas Generation from the Co-Digestion of *Carica papaya* Peels and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	36.84	7.76	21.41	11.81	11.81	3884.2	3991.77	3875.1
2	36.45	7.75	20.17	12.00	11.60	3000.0	3100.42	3000.0
3	36.78	7.60	20.78	11.97	11.78	3372.2	3384.68	3371.1
4	36.99	7.79	20.05	11.99	6.10	3475.4	3465.49	3439.1
5	36.87	7.70	20.97	11.84	8.48	3496.4	3491.2	3496.3
6	35.98	7.79	20.27	11.89	10.65	3700.3	3701.77	3739.0
7	36.31	7.96	21.29	11.97	11.49	3573.2	3663.01	3573.5
8	36.99	7.99	21.63	12.00	6.89	4201.1	4261.17	3822.7
9	36.93	7.86	20.06	11.78	9.86	3502.8	3666.8	3832.7
10	35.91	8.00	20.06	11.68	7.27	3600.9	3663.04	3798.0
11	37.50	7.96	21.59	11.98	11.98	3834.1	3970.11	3813.9
12	37.00	8.00	22.01	12.00	6.97	3572.2	3661.11	3679.9
13	36.89	7.98	20.21	11.89	6.87	3521.1	3664.36	3833.4
14	36.99	7.99	20.94	11.93	8.97	3991.1	4023.49	3980.8
15	36.97	7.85	20.67	11.81	11.80	3980.6	4064.71	3959.2
16	36.89	8.00	20.52	11.11	11.99	4600.8	4691.77	4685.8
17	36.82	7.95	20.00	12.00	11.33	3852.2	4040.01	3798.0
18	38.00	7.70	24.39	12.00	8.95	4661.1	4633.07	4709.0
19	35.91	7.94	20.00	12.00	5.84	4190.1	4219.73	4195.0
20	37.00	7.60	25.07	12.00	10.00	3932.9	4015.52	3798.0
21	37.00	7.70	23.79	12.00	6.88	3473.8	3413.87	3492.4
22	37.00	7.70	24.64	11.97	8.28	3800.1	3812.21	3844.7
23	36.74	7.80	24.56	11.98	12.00	4268.6	4302.77	4254.5
24	36.99	7.95	20.00	12.00	4.90	3891.5	3890.95	3832.6
25	36.99	7.70	25.46	12.00	4.94	3862.1	3910.99	3832.6
26	37.00	7.40	28.01	12.00	9.67	3494.9	3497.34	3495.3
27	37.00	7.50	28.08	12.00	10.25	4094.5	4191.04	3798.0
28	37.00	7.70	29.22	12.00	8.29	3905.7	3945.94	3884.8
29	37.00	7.96	26.69	12.00	4.02	3722.4	3843.47	3739.6
30	37.00	7.60	28.46	11.97	5.35	3832.2	3932.33	3836.7
31	37.50	7.45	26.67	10.23	6.49	3867.9	3871.31	3832.3
32	37.50	7.50	25.56	10.10	6.52	3771.1	3801.02	3763.2
33	36.00	7.45	26.81	9.34	5.51	3532.2	3621.20	3611.0
34	36.50	7.67	23.76	9.90	8.81	3100.1	3132.41	3112.1
35	37.00	7.64	23.49	8.19	8.01	2981.2	2965.31	2943.3
36	37.00	7.90	27.01	8.08	7.98	3102.2	3211.42	3208.3
37	37.00	7.95	26.91	10.01	6.81	2341.9	2373.03	2320.3
38	37.50	7.80	24.56	9.67	5.50	3190.3	3212.21	3210.1
39	37.50	7.75	25.59	9.88	4.90	3087.1	3121.31	3113.4

Table 4.6h: Experimental Matrix for Biogas Generation from the Co-Digestion of *Carica papaya* Peels and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	38.00	7.67	27.02	8.18	6.12	3021.3	3101.48	3100.0
41	38.00	7.87	27.08	9.67	6.21	3209.2	3243.31	3220.6
42	36.50	7.83	26.01	10.02	6.09	2987.9	3014.15	3011.1
43	36.50	7.84	24.80	11.12	7.90	3112.1	3167.04	3132.1
44	37.00	7.89	23.40	11.31	8.12	2702.2	2711.14	2689.1
45	37.00	6.95	23.21	10.90	9.03	2678.8	2712.12	2678.1
46	37.50	6.78	22.90	9.11	9.01	2871.6	2899.03	2852.0
47	38.00	7.00	24.32	10.21	8.83	2700.1	2761.23	2698.2
48	38.00	7.98	23.41	12.00	6.54	2301.8	2381.90	2358.2
49	38.50	8.00	23.56	11.21	5.03	2231.3	2241.80	2211.2
50	37.50	7.67	23.08	10.86	4.80	2431.3	2462.02	2428.8

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6i: Experimental Matrix for Biogas Generation from the Co-Digestion of *Telfairia occidentalis* Peels and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	37.00	7.60	25.00	4.00	4.00	2539.2	2614.14	2540.34
2	37.00	7.91	25.00	4.01	4.60	2480.9	2462.51	2484.25
3	36.00	7.86	27.00	4.63	4.00	2365.1	2408.07	2368.53
4	36.00	7.87	29.32	4.00	4.00	2473.3	2540.83	2459.64
5	37.50	8.00	29.98	5.59	4.00	2600.1	2612.07	2597.03
6	37.50	8.00	29.08	4.06	4.36	2523.1	2606.15	2523.52
7	38.81	8.00	30.00	4.00	4.09	2484.2	2486.71	2484.43
8	40.00	7.14	30.00	4.00	4.00	2435.9	2481.82	2435.95
9	40.00	7.52	29.36	4.00	4.43	2563.3	2572.90	2560.24
10	40.00	7.12	29.97	4.06	4.00	2851.1	2872.60	2836.16
11	30.01	6.55	20.00	12.00	12.00	2907.1	3065.62	2588.25
12	30.00	6.55	20.00	12.00	12.00	2681.0	2664.88	2588.24
13	30.00	6.57	20.00	11.95	12.00	2591.6	2608.56	2591.53
14	30.17	6.50	20.00	11.95	12.00	2551.1	2557.25	2553.74
15	30.00	6.50	20.34	12.00	12.00	2501.2	2556.30	2503.30
16	30.00	6.54	20.01	11.99	11.88	2511.9	2555.85	2509.90
17	30.00	6.61	20.00	12.00	12.00	1002.5	1054.87	1002.47
18	30.00	6.54	20.00	12.00	11.79	2732.0	2749.83	2731.58
19	40.00	8.00	30.00	5.47	5.06	2727.3	2749.38	2734.60
20	30.00	6.57	20.00	11.78	12.00	2700.9	2743.65	2700.42
21	30.00	6.72	20.00	12.00	12.00	2700.1	2733.33	2705.63
22	30.14	6.50	20.00	12.00	11.32	2597.2	2610.98	2600.50
23	30.00	6.87	20.00	12.00	12.00	2556.1	2504.64	2555.72
24	30.00	6.50	20.00	12.00	11.08	2642.1	2701.30	2643.52
25	39.98	8.00	30.00	7.62	4.08	2398.1	2377.95	2397.54
26	40.00	6.74	30.00	4.00	4.21	2350.1	2476.58	2588.23
27	30.00	6.94	20.18	11.90	12.00	2569.0	2673.56	2567.45
28	30.00	6.95	20.00	11.88	11.96	2410.0	2473.32	2404.37
29	30.00	6.50	22.12	12.00	12.00	2400.0	2457.96	2588.24
30	39.89	6.50	30.00	4.00	4.00	3456.0	3429.49	3456.33
31	30.01	6.55	20.00	12.00	12.00	2681.02	2540.83	2836.14
32	30.00	6.55	20.00	12.00	12.00	2691.62	2612.07	2588.26
33	30.00	6.57	20.00	11.95	12.00	2551.14	2606.15	2588.24
34	30.17	6.50	20.00	11.95	12.00	2601.25	2486.71	2591.52
35	30.00	6.50	20.34	12.00	12.00	2531.97	2581.82	2553.76
36	30.00	6.54	20.01	11.99	11.88	1902.58	2572.90	2503.34
37	30.00	6.61	20.00	12.00	12.00	2742.63	2872.60	2509.90
38	30.00	6.72	20.00	12.00	12.00	2350.1	2476.58	2588.23
39	30.14	6.50	20.00	12.00	11.32	2569.0	2673.56	2567.45

Table 4.6i: Experimental Matrix for Biogas Generation from the Co-Digestion of *Telfairia occidentalis* Peels and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	30.00	6.87	20.00	12.00	12.00	2410.0	2473.32	2404.37
41	30.00	6.50	20.00	12.00	11.08	2400.0	2457.96	2588.24
42	39.98	8.00	30.00	7.62	4.08	3456.0	3429.49	3456.33
43	30.00	6.87	20.00	12.00	12.00	2652.12	2585.85	2600.56
44	30.00	6.50	20.00	12.00	11.08	2693.31	2554.87	2535.75
45	39.98	8.00	30.00	7.62	4.08	2450.58	2749.83	2643.53
46	40.00	6.74	30.00	4.00	4.21	2569.34	2749.38	2497.52
47	30.00	6.94	20.18	11.90	12.00	2410.33	2743.65	2588.21
48	30.00	6.95	20.00	11.88	11.96	2400.62	2733.33	2567.44
49	30.00	6.94	20.18	11.90	12.00	3245.92	2620.98	2504.33
50	30.00	6.95	20.00	11.88	11.96	3215.42	2534.60	2553.80

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

Table 4.6j: Experimental Matrix for Biogas Generation from the Co-Digestion of *Arachis hypogaea* Hull and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
1	32.00	7.62	30.00	12.00	12.00	3339.2	3903.15	3338.3
2	39.98	7.99	30.00	12.00	11.92	3000.9	3887.25	3702.3
3	39.97	8.00	29.99	11.77	11.84	3665.1	3857.38	3666.4
4	39.84	8.00	29.97	11.88	12.00	3873.3	3881.06	3872.5
5	39.96	8.00	30.00	12.00	11.58	3600.1	3843.66	3699.7
6	40.00	8.00	30.00	12.00	11.43	3723.1	3824.98	3712.4
7	40.00	7.97	30.00	11.85	11.54	3884.2	3806.01	3883.9
8	39.99	7.93	29.99	11.64	12.00	3535.9	3808.08	3554.9
9	40.00	8.00	30.00	11.46	11.27	3763.3	3753.10	3767.5
10	39.93	7.86	30.00	12.00	11.79	3751.1	3758.88	3746.0
11	40.00	8.00	29.60	10.49	12.00	3507.1	3721.13	3507.7
12	39.57	7.88	30.00	11.97	12.00	3581.0	3786.21	3589.6
13	40.00	7.92	30.00	12.00	10.58	3591.6	3653.46	3590.1
14	39.01	7.98	30.00	11.90	12.00	4000.0	4132.28	3993.6
15	40.00	8.00	29.97	12.00	9.98	3701.2	3634.73	3701.7
16	38.75	8.00	30.00	11.12	12.00	3511.9	3756.92	3502.2
17	40.00	7.56	29.01	12.00	12.00	3602.5	3493.71	3586.7
18	39.46	7.74	30.00	10.81	12.00	3432.0	3568.03	3434.5
19	39.74	8.00	30.00	12.00	8.87	3227.3	3491.05	3219.5
20	40.00	7.91	30.00	10.64	9.69	3500.9	3433.42	3402.0
21	39.99	7.72	30.00	9.11	12.00	3200.1	3415.48	3286.7
22	40.00	7.27	29.21	12.00	12.00	3297.2	3322.08	3200.7
23	39.04	7.46	29.53	12.00	12.00	3256.1	3433.61	3255.3
24	40.00	8.00	29.12	11.99	7.27	2942.1	3248.76	3086.7
25	40.00	7.02	30.00	12.00	11.92	3298.1	3224.96	3204.1
26	40.00	6.85	30.00	11.75	12.00	3050.1	3126.99	3037.0
27	40.00	6.83	30.00	11.57	11.93	3009.0	3101.96	3014.7
28	40.00	8.00	30.00	11.99	4.96	2910.0	3090.30	2911.2
29	40.00	8.00	30.00	6.77	8.70	3000.0	3055.35	2986.7
30	40.00	8.00	21.73	9.77	12.00	2856.0	3052.25	2857.0
31	40.00	8.00	30.00	11.46	11.27	3751.1	3758.88	3889.6
32	39.93	7.86	30.00	12.00	11.79	3507.1	3721.13	3590.1
33	40.00	8.00	29.60	10.49	12.00	3581.0	3786.21	3593.6
34	39.57	7.88	30.00	11.97	12.00	3591.6	3653.46	3601.7
35	40.00	7.92	30.00	12.00	10.58	4000.0	3832.28	3802.2
36	39.01	7.98	30.00	11.90	12.00	3501.2	3634.73	3586.7
37	40.00	8.00	29.97	12.00	9.98	3511.9	3756.92	3534.5
38	38.75	8.00	30.00	11.12	12.00	3302.5	3493.71	3319.5
39	40.00	7.56	29.01	12.00	12.00	3232.0	3568.03	3502.0

Table 4.6j: Experimental Matrix for Biogas Generation from the Co-Digestion of *Arachis hypogaea* Hull and Poultry Dropping with Five Independent Variables for RSM and ANNs Using Actual Values (Cont.)

Run	X_1	X_2	X_3	X_4	X_5	Actual biogas yield ($10^{-3}m^3$/kg VS)	RSM Predicted biogas yield ($10^{-3}m^3$/kg VS)	ANNs Predicted biogas yield ($10^{-3}m^3$/kg VS)
40	39.46	7.74	30.00	10.81	12.00	3427.3	3491.05	3486.7
41	39.74	8.00	30.00	12.00	8.87	3100.9	3433.42	3200.7
42	40.00	7.91	30.00	10.64	9.69	3200.1	3415.48	3255.3
43	39.99	7.72	30.00	9.11	12.00	3297.2	3322.08	3286.7
44	40.00	7.27	29.21	12.00	12.00	2356.1	3433.61	3204.1
45	39.04	7.46	29.53	12.00	12.00	2992.1	3248.76	3237.0
46	40.00	8.00	29.12	11.99	7.27	3098.1	3224.96	3214.7
47	40.00	7.02	30.00	12.00	11.92	3150.1	3126.99	3111.2
48	40.00	6.85	30.00	11.75	12.00	3109.0	3101.96	2986.7
49	40.00	6.83	30.00	11.57	11.93	2990.0	3090.30	2857.0
50	40.00	8.00	30.00	11.99	4.96	3063.1	3012.30	3008.4

X_1 = Temperature; X_2 = pH; X_3 = Retention time; X_4 = Total solids: X_5 = Volatile solids

The prediction and estimation abilities of both RSM and ANN were critically examined so as to determine the potency of the two models. RSM and ANN were used to stimulate responses, which were then compared with actual values. The roots mean squared error (RSME), coefficient of determination (R^2) (Appendix 7) and the predicted values were used to compare the RSM and ANN. In the mono-digestion of *Tithonia* diversifolia shoot, the RSME of biogas for RSM (286.15) was much higher than that of ANN (93.452) while the R^2 for RSM (0.8802 i.e. 88.02%) were lower compare to that of ANN (0.9662 i.e. 96.62%). In the mono-digestion of *Chromolaena* odorata shoot, the RSME of biogas for RSM (2.117) was much lower than that of ANN (148.15) while the R^2 for RSM (0.8680 i.e. 86.80%) was also lower compare to that of ANN (0.9484 i.e. 94.84%).

In the mono-digestion of *Carica papaya* shoot, it was noticed that the RSME of biogas for RSM (287.26) was higher than that of ANN (50.768) while the R^2 for RSM (0.9239 i.e. 92.39%) was lower compare to that of ANN (0.9865 i.e. 98.65%). In the mono-digestion of *Telfairia occidentalis* peels, the RSME of biogas for RSM (157.52) was higher than that of ANN (14.042) while the R^2 for RSM (0.8996 i.e. 89.96%) was lower compare to that of ANN (0.9929 i.e. 99.29%). In the mono-digestion of *Arachis hypogaea* hull, the RSME of biogas for RSM (299.25) was higher than that of ANN (41.26) while the R^2 for RSM (0.8676 i.e. 69.76%) was lower compare to that of ANN (0.9894 i.e. 98.94%). In the co-digestion of *Tithonia diversifolia* shoot and poultry dropping, the RSME of biogas for RSM (105.61) was higher than that of ANN (84.65) while the R^2 for RSM (0.8674 i.e. 86.74% were lower than ANNs' (0.9930 i.e. 99.30%.

In the co-digestion of *Chromolaena odorata* shoot and poultry dropping, the RSME of biogas for RSM (237.40) was higher than that of ANN (87.03) whereas, the R^2 for RSM (0.9009 i.e. 90.09%) was lower compare to that of ANN (0.9907 i.e. 99.07%). In the co-digestion of *Carica papaya* peels and poultry dropping, the RSME of biogas for RSM (451.65) was higher than that of ANN (68.05) and the R^2 for RSM (0.9181 i.e. 91.81%) was lower compare to that of ANN (0.9828 i.e. 98.28%). In the co-digestion of *Telfairia occidentalis* peels and poultry dropping, the RSME obtained for RSM (460.03) was higher than that of ANN (83.72) whereas, the R^2 for RSM (0.8827 i.e. 88.27%) was lower compare to that of ANN (0.9724 i.e. 97.24%). In the co-digestion of *Arachis* hypogaea hull and poultry dropping, the RSME of biogas for RSM (308.11) was higher than that of

ANN (68.91) and the R^2 for RSM (0.9045 i.e. 90.45%) was lower compare to that of ANN (0.9997 i.e. 99.97%).

4.10. Microbial Optimization of Biogas Production

The three best substrates from all the ten biogas production and optimization studies were the co-digestion of *Chromolaena odorata* shoot and poultry dropping followed by the co-digestion of *Tithonia diversifolia* shoot and poultry dropping and then the mono-digestion of *Chromolaena odorata* shoot. The results of the microbial optimization in respect to gas yield and methane content from these substrates are shown in Table 4.7. From the Table, the average highest biogas yield (3474.5 $10^{-3}m^3$/kg VS) was obtained from experiment the co-digestion of *Chromolaena odorata* shoot and poultry dropping using *Clostridium* and *Fusobacterium* spp as acid formers and *Methanosarcinales* sp. as the sole methane former. The least biogas yield (2115.5 $10^{-3}m^3$/kg VS) was from the mono-digestion of *Chromolaena odorata* shoot using *Clostridium, Fusobacterium* and *Porphyromonas* spp as the acid formers and *Methanosaeta* sp. as the sole methane producer. In terms of methane content, the highest (62.7 %) was equally obtained from the co-digestion of *Chromolaena odorata* shoot and poultry dropping seeded with *Clostridium, Fusobacterium* and *Methanosarcinales* spp while the least (58 %) was obtained from the mono-digestion of *Chromolaena odorata* shoot seeded with *Methanococcus* and *Clostridium* spp.

Table 4.7: Average Biogas Yield and Methane Content of Substrate + Microorganisms Combinations Used for Microbial Optimization

S/N	Substrate + combined organisms	Average biogas yield ($10^{-3}m^3$/kg VS)	Methane Content (%)
1.	Co-digestion of *Chromolaena odorata* shoot and poultry dropping seeded with *Methanococcus* and *Clostridium* spp	3003.5	60.8
2.	Co-digestion of *Chromolaena odorata* shoot and poultry dropping seeded with *Methanosarcinales, Clostridium* and *Fusobacterium* spp	3474.5	62.7
3.	Co-digestion of *Chromolaena odorata* shoot and poultry dropping seeded with *Methanosaeta*, *Clostridium, Fusobacterium* and *Porphyromonas* spp	3041.1	61.5
4.	Co-digestion of *Tithonia diversifolia* shoot and poultry dropping seeded with *Methanococcus* and Clostridium spp	2431.2	59.6
5.	Co-digestion of *Tithonia diversifolia* shoot and poultry dropping seeded with *Methanosarcinales, Clostridium* and *Fusobacterium* spp	2946.9	58.7
6.	Co-digestion of *Tithonia diversifolia* shoot and poultry dropping seeded with *Methanosaeta*, *Clostridium, Fusobacterium* and *Porphyromonas* spp	2761.6	62.3
7.	Mono-digestion of *Chromolaena odorata* shoot seeded with *Methanococcus* and *Clostridium* spp	2150.6	58
8.	Mono-digestion of *Chromolaena odorata* shoot shoot seeded with *Methanosarcinales, Clostridium* and *Fusobacterium* spp	2312.5	60.5
9.	Mono-digestion of *Chromolaena odorata* shoot seeded with *Methanosaeta*, *Clostridium, Fusobacterium* and *Porphyromonas* spp	2115.5	59.5

Each experiment was done in five replicates

4.11. Physicochemical Compositions of Inorganic Fertilixer and Biofertilizers

Table 4.8 shows the chemical composition of the NPK 15-15-15 inorganic fertilizer used as control in the planting experiments. Also, tables 4.9a shows the nutrient and elemental (chemical) composition of each newly produced biofertilizer from the mono-digestions after dewatering and before usage in the phyto-assessment studies. Each fertilizer (inorganic and biofertilizer) was evaluated for all the 3 major plant nutrients (nitrogen, phosphorus and potassium) and 9 other major and minor nutrients/elements (aluminium, copper, calcium, iron, magnesium, manganese, phosphate, sulphate and zinc). The pH of each biofertilizer was also recorded before usage. Table 4.9b show the composition of the fertilizers produced from the co-digestions. From the tables, copper, iron, magnesium, manganese, phosphate, sulphate and phosphorus were all highest in the *Telfairia occidentalis* + poultry dropping biofertilizer with values of 1.12, 0.28, 9.40, 0.007, 0.62, 3.96 and 1.28 % respectively. Potassium, zinc and aluminium were highest in the *Tithonia diversifolia* + poultry dropping biofertilizer with values of 0.202, 0.666 and 0.016 mg/g respectively. Calcium was highest in the *Chromolaena odorata* biofertilizer with value of 17.74 % while the richest biofertilizer in terms of nitrogen composition (9.90 %) was the *Chromolaena odorata* + poultry dropping biofertilizer. All the biofertilizers produced from the anaerobic co-digestion with poultry dropping were richer than the ones without poultry dropping in terms of nutrient and elemental compositions. In the overall, the richest biofertilizer in terms of nutrient and elemental composition was the *Telfairia occidentalis* + poultry dropping biofertilizer.

Table 4.8: Composition of NPK 15-15-15 Inorganic Fertilizer

S/N	Parameter	Value
1.	Nitrogen (%)	15
2.	P_2O_3 (%)	15
3.	Soluble P_2O_3 (%)	13
4.	K_2O (%)	15
5.	Moisture (%)	2
6.	Particle size (mm)	1-4.75
7.	pH	7.1±0.12

Table 4.9a: Composition of Biofertilizers Produced from Mono-Digestions

S/N	Parameter	*Tithonia diversifolia* **shoot biofertilizer (%)**	*Chromolaena odorata* **shoot biofertilizer (%)**	*Carica papayas* **fruit peels biofertilizer (%)**	*Telfairia occidentalis* **fruit peels biofertilizer (%)**	*Arachis hypogaea* **hull biofertilizer (%)**
1.	pH	7.35±0.05	7.20±0.01	7.30±0.01	7.35±0.02	7.35±1.01
2.	Copper	0.61±0.01	0.52±0.01	0.65±0.02	0.67±0.02	0.56±0.05
3.	Calcium	16.80±0.01	17.74±1.05	14.00±0.02	13.20±0.05	13.20±0.05
4.	Iron	0.20±0.02	0.13±0.01	0.14±0.01	0.18±0.01	0.16±0.01
5.	Magnesium	5.20±0.05	5.36±0.02	4.80±0.02	5.24±0.02	3.44±0.01
6.	Manganese	0.03±0.05	0.002±0.02	0.003±0.01	0.002±0.01	0.003±0.01
7.	Phosphate	0.31±0.02	0.20±0.01	0.30±0.01	0.16±0.01	0.23±0.02
8.	Sulphate	1.80±0.02	1.48±0.02	1.94±0.05	1.84±0.01	1.62±0.01
9.	**Potassium**	**1.28**±0.01	**1.12**±0.02	**1.22**±0.02	**1.19**±0.01	**1.19**±0.01
10.	**Nitrogen**	**6.67**±0.01	**8.84**±0.05	**9.18**±0.03	**8.46**±0.05	**7.74**±0.0
11.	**Phosphorus**	**0.64**±0.01	**0.40**±0.02	**0.62**±0.01	**0.44**±0.02	**0.80**±0.02
12.	Zinc	3.52±0.01	2.09±0.01	3.46±0.01	2.38±0.02	3.60±0.02
13.	Aluminium	0.10±0.02	0.07±0.01	0.08±0.01	0.09±0.01	0.07±0.01

Each experiment was done in replicates of 5; Values in bold are for the 3 most important parameters i.e Nitrogen, Phosphorus and Potasium

Table 4.9b: Composition of Biofertilizers Produced from Co-Digestions

S/N	Parameter	*Tithonia diversifolia* **shoot + Poultry dropping biofertilizer (%)**	*Chromolaena odorata* **shoot + Poultry dropping biofertilizer (%)**	*Carica papayas* **fruit peels + Poultry dropping biofertilizer (%)**	*Telfairia occidentalis* **fruit peels + Poultry dropping biofertilizer (%)**	*Arachis hypogaea* **hull + Poultry dropping biofertilizer (%)**
1.	pH	7.55±0.05	7.55±0.02	7.45±0.05	7.95±0.05	7.40±0.05
2.	Copper	0.78±0.01	0.86±0.01	0.74±0.02	1.12±0.02	0.76±0.02
3.	Calcium	10.96±0.02	11.00±0.02	12.60±0.01	15.88±0.03	12.40±0.03
4.	Iron	0.22±0.02	0.22±0.00	0.18±0.01	0.28±0.02	0.16±0.01
5.	Magnesium	8.00±0.01	6.36±0.02	4.40±0.02	9.40±0.01	6.60±0.02
6.	Manganese	0.005±0.03	0.003±0.02	0.004±0.01	0.007±0.01	0.004±0.02
7.	Phosphate	0.52±0.05	0.47±0.01	0.45±0.02	0.62±0.02	0.40±0.01
8.	Sulphate	2.52±0.01	2.38±0.01	2.19±0.05	3.96±0.03	2.24±0.01
9.	**Potassium**	**2.02**±0.01	**1.40**±0.02	**1.37**±0.02	**1.62**±0.02	**1.30**±0.01
10.	**Nitrogen**	**9.36**±0.02	**9.90**±0.02	**7.38**±0.02	**9.36**±0.01	**7.72**±0.02
11.	**Phosphorus**	**1.13**±0.05	**0.96**±0.01	**0.91**±0.02	**1.28**±0.02	**0.81**±0.01
12.	Zinc	6.66±0.01	6.12±0.02	5.58±0.03	4.68±0.02	0.47±0.03
13.	Aluminium	0.16±0.01	0.14±0.01	0.12±0.01	0.13±0.01	0.10±0.01

Each experiment was done in replicates of 5; Values in bold are for the 3 most important parameters i.e Nitrogen, Phosphorus and Potasium

4.12. Microbial Evaluation of Anaerobic Digestates and Biofertilizers

Tables 4.10 (a-e) show the microbial composition of all the digestates from the mono-digestion regimes before dewatering and those of the dewatered solid biofertilizers. The digestates were all found to contain different populations of aerobes, fungi, anaerobes and methanogens while the dewatered biofertilizers contained all the microbial groups except methanogens. In all, the microbial populations in the digestates were much higher than those reported in the resulting biofertilizers after dewatering. From the anaerobic digestate of *Tithonia diversifolia* shoot, the total bacterial plate count (TBPC) before and after dewatering were 1.9×10^{11} cfu/ml and 1.8×10^{5} cfu/ml while the total fungal count (TFC) before and after dewatering were 1.0×10^{3} cfu/ml and 1.0×10^{2} cfu/ml respectively.

From the anaerobic digestate of *Chromolaena odorata* shoot, the TBPC before and after dewatering were 2.1×10^{11} cfu/ml and 3.1×10^{5} cfu/ml while the TFC before and after dewatering were 2.0×10^{4} cfu/ml and 1.0×10^{2} cfu/ml respectively. From the anaerobic digestate of *Carica papaya* peel, the bacterial TBPC before and after dewatering were 2.4×10^{12} cfu/ml and 3.0×10^{8} cfu/ml the TFC before and after dewatering were 2.0×10^{4} cfu/ml and 2.0×10^{2} cfu/ml respectively. From the anaerobic digestate of *Telfairia occidentalis* peels, the TBPC before and after dewatering were 2.0×10^{12} cfu/ml and 2.0×10^{6} cfu/ml while the TFC before and after dewatering were 3.0×10^{4} cfu/ml and 5.0×10^{2} cfu/ml respectively. From the anaerobic digestate *Arachis hypogaea* hull, the TBPC before and after dewatering were 2.5×10^{12} cfu/ml and 6.0×10^{6} cfu/ml while the TFC before and after dewatering were 1.1×10^{5} cfu/ml and 4.0×10^{2} cfu/ml respectively. In all, the richest digestate in terms of bacterial population and diversity was obtained for mono-digestion of *Carica papaya* peel while the richest in fungal composition was obtained from the mono-digestion of *Arachis hypogaea* hull. Among the five solid biofertilizers, the richest in bacterial population and diversity was obtained from the digestate of mono-digestion of *Carica papaya* peelswhile the richest in fungi was obtained from the mono-digestion of *Tithonia* diversifolia shoot.

Table 4.10a: Microbial Composition of *Tithonia diversifolia* Shoot Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp. *Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	1.9×10^{11}	*Bacillus* sp. *Clostridium* sp. *Gemella* sp.	1.8×10^{5}	*Aspergillus niger*	1.0×10^{3}	*Aspergillus niger*	1.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10b: Microbial Composition of *Chromolaena odorata* Shoot Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Bacteroides* sp. *Clostridium* sp. *Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp.	2.1×10^{11}	*Bacillus* sp. *Clostridium* sp.	3.1×10^{5}	*Aspergillus niger*	2.0×10^{4}	*Aspergillus niger*	1.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10c: Microbial Composition of *Carica papaya* Peels Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Fusobacterium* sp. *Bacteroides fragilis* *Clostridium* sp. *Gemella* sp. *Methanococcus* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	2.4×10^{12}	*Bacillus* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	3.0×10^{8}	*Aspergillus niger*	2.0×10^{4}	*Aspergillus niger*	2.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10d: Microbial Composition of *Telfairia occidentalis* Peels Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp.	2.0×10^{12}	*Bacillus* sp. *Fusobacterium* sp. *Clostridium* sp. *Porphyromonas* sp.	2.0×10^{6}	*Aspergillus niger*	3.0×10^{4}	*Aspergillus niger*	5.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10e: Microbial Composition of *Arachis hypogaea* Hull Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Methanomicrobiales* sp.	2.5×10^{12}	*Bacillus* sp. *Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	6.0×10^{6}	*Aspergillus niger*	1.1×10^{5}	*Aspergillus niger*	4.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10 (f-j) presents microbial profile of digestates from co-digestions. From the anaerobic digestate of *Tithonia diversifolia* and poultry dropping, the TBPC before and after dewatering were 2.5 x 10^{12} cfu/ml and 4.0 x 10^{6} cfu/ml) while the TFC were before and after dewatering were 2.0 x 10^{4} cfu/ml and 1.1 x 10^{3} cfu/ml respectively. From the anaerobic digestate of *Chromolaena odorata* shoot and poultry dropping, the TBPC before and after dewatering were 1.9 x 10^{12} cfu/ml and 5.0 x 10^{6} cfu/ml while the TFC before and after dewatering were 2.2 x 10^{4} cfu/ml and 2.0 x 10^{2} cfu/ml respectively. From the anaerobic digestate of *Carica papaya* peels and poultry dropping, the bacterial TBPC before and after dewatering were 2.5 x 10^{12} cfu/ml and 5.0 x 10^{6} cfu/ml while the TFC before and after dewatering were 2.2 x 10^{4} cfu/ml and 1.0 x 10^{2} cfu/ml respectively.

From the anaerobic digestate of *Telfairia occidentalis* peels and poultry dropping, the TBPC before and after dewatering were 2.7 x 10^{12} cfu/ml and 6.0 x 10^{6} cfu/ml while the TFC before and after dewatering were 4.0 x 10^{4} cfu/ml and 4.0 x 10^{2} cfu/ml respectively. From the anaerobic digestate *Arachis hypogaea* hull and poultry dropping, the TBPC before and after dewatering were 2.7 x 10^{11} cfu/ml and 5.0 x 10^{6} cfu/ml while the fungal TFC before and after dewatering were 2.0 x 10^{4} cfu/ml and 2.0 x 10^{2} cfu/ml respectively. Digestates from *Telfairia occidentalis* peels and poultry dropping and *Arachis hypogaea* hull and poultry dropping were both highest in bacterial and fungal population and diversity before and dewatering while those from *Chromolaena odorata* shoot and poultry dropping and *Carica papaya* peels and poultry dropping were the richest in microbial composition after dewatering. Overall, all the anaerobic digestates from the co-digestion experiments were richer than those from the mono-digestions in microbial population and diversity both before and after dewatering.

Table 4.10f: Microbial Composition of *Tithonia diversifolia* Shoot + Poultry Droppings Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TPC (cfu/ml)**	**Bacteria**	**TPC (cfu/ml)**	**Fungi**	**TPC (cfu/ml)**	**Fungi**	**TPC (cfu/ml)**
Bacillus sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp. *Methanococcus* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Aminobacteria* sp.	2.5×10^{12}	*Bacillus* sp. *Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	4.0×10^{6}	*Aspergillus niger* *Aspergillus flavus*	2.0×10^{4}	*Aspergillus niger* *Aspergillus flavus*	1.1×10^{3}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10g: Microbial Composition of *Chromolaena odorata* Shoot + Poultry Droppings Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Porphyromonas* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp. *Methanosarcinales* sp. *Methanosaeta* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	1.9×10^{12}	*Bacillus* sp. *Porphyromonas* sp. *Fusobacterium* sp. *Clostridium* sp. *Gemella* sp.	5.0×10^{6}	*Aspergillus niger* *Aspergillus flavus,*	2.2×10^{4}	*Aspergillus niger* *Aspergillus flavus,*	2.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10h: Microbial Composition of *Carica papaya* Peels + Poultry Droppings Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Porphyromonas* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Methanococcus* sp. *Methanosaeta* sp. *Aminobacteria* sp.	2.5×10^{12}	*Bacillus* sp. *Porphyromonas* sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp.	5.0×10^{6}	*Aspergillus niger* *Aspergillus flavus*	2.2×10^{4}	*Aspergillus niger* *Aspergillus flavus*	1.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10i: Microbial Composition of *Telfairia occidentalis* Peels + Poultry Droppings Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Fusobacterium* sp. *Bacteroides* sp. *Clostridium* sp. *Porphyromonas* sp. *Methanosarcinales* sp. *Methanobacteriales* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	2.7×10^{12}	*Bacillus* sp. *Fusobacterium* sp. *Clostridium* sp. *Porphyromonas* sp.	6.0×10^{6}	*Aspergillus niger* *Aspergillus flavus*	4.0×10^{4}	*Aspergillus niger* *Aspergillus flavus*	2.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

Table 4.10j: Microbial Composition of *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer

Before Dewatering		After Dewatering		Before Dewatering		After Dewatering	
Bacteria	**TBPC (cfu/ml)**	**Bacteria**	**TBPC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**	**Fungi**	**TFC (cfu/ml)**
Bacillus sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp. *Methanococcus* sp. *Methanomicrobiales* sp. *Aminobacteria* sp.	2.7×10^{11}	*Bacillus* sp. *Bacteroides* sp. *Clostridium* sp. *Gemella* sp.	5.0×10^{6}	*Aspergillus niger* *Aspergillus flavus* *Penicillum* sp.	2.0×10^{4}	*Aspergillus niger* *Aspergillus flavus*	2.0×10^{2}

TBPC = Total Bacterial Plate Count; TFC = Total Fungal Count

4.13. Phyto-Assessment

Tables 4.11 shows the results of phyto-assessment carried out with the application of NPK 15-15-15 inorganic fertilizer and that of the negative control (no fertilizer application). In the control, leaf number increases as the experiment progressed and the highest value (7.0) was obtained at 30 Day after Emergence (DAE) of seed. Leaf area also followed the same trend of increase and the highest value (45.1 cm^2) was obtained at 30 DAE. Plant height and stem girth increased progressively and the highest values of 32 cm and 0.9 cm were also obtained at 30 DAE. After harvesting, the weight of the biomass above the soil level was 6.1 g, while that of the root biomass was 6.5 g. The root length was 44 cm. From the NPK applied plot, leaf number increases as the experiment progressed and the highest value of 6.0 was obtained at both 25 and 30 DAE. Leaf area also followed the same trend of increase and the highest value of 46.2 cm^2 was obtained at 30 DAE. Plant height and stem girth increased progressively and the highest values of 35 cm and 1.3 cm were obtained at 30 DAE. After harvesting, the weight of the biomass above the soil level was 12.5 g while that of the root biomass was 8.2 g. The root length was 56 cm. Values obtained from all parameters indicated that the NPK 15-15-15 fertilizer plot performed better than the negative control plot.

Table 4.11: Phyto-Assessment with no Fertilizer and NPK 15-15-15 Fertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height (cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
No Fertilizer Application							
5 DAE	3±0.01	10.2±0.01	6.6±0.01	0.3±0.01	-	-	-
10 DAE	4±0.01	21.5±0.01	17±0.01	0.7±0.01	-	-	-
15 DAE	5±0.01	22.9±0.02	22±1.01	0.7±0.01	-	-	-
20 DAE	6±0.01	33.4±0.01	25±1.01	0.8±0.01	-	-	-
25 DAE	7±0.01	34.7±0.01	28±0.01	0.85±0.01	-	-	-
30 DAE	7±0.01	45.1±0.01	32±0.02	0.9±0.01	6.1±0.01	6.5±0.01	44±2.01
NPK Fertilizer Application (30 kg N/ha)							
5 DAE	3±0.01	11.1±0.01	6.5±0.01	0.3±0.01	-	-	-
10 DAE	4±0.01	11.9±0.01	13.5±0.05	0.5±0.01	-	-	-
15 DAE	5±0.01	23.5±0.01	18±0.01	0.7±0.01	-	-	-
20 DAE	5±0.01	34.5±0.01	24±0.02	0.9±0.01	-	-	-
25 DAE	6±0.01	34.9±0.01	29±0.03	1.1±0.01	-	-	-
30 DAE	6±0.01	46.2±0.01	35±0.03	1.3±0.01	12.5±0.05	8.2±0.01	56±2.01

DAE= Day after Emergence

The result of the phyto-assessments carried out with the application of the biofertilizers produced from the mono-digestion experiments are shown in tables 4.12 (a-e). In the *Tithonia diversifolia* biofertilizer plot, the leaf number increased progressively and the highest (9.0) was recorded in the 30 kg N/ha (25 and 30 DAE), 50 kg N/ha (30 DAE) and 60 kg N/ha (30 DAE) applications respectively. Leaf area also increased progressively with the days of the experiment and the highest value (51.9 cm^2) was recorded in the 30 kg N/ha at 30 DAE. Plant height and stem girth increased with length of experiment and the highest values (67 cm and 2.8 cm) were recorded in the 60 kg N/ha and 20 kg N/ha at 30 DAE respectively. In the *Chromolaena odorata* biofertilizer plot, all the measured parameters increased progressively with the length of the experiment. The highest leaf number (9.0) was recorded in the 30 and 60 kg N/ha experiments, the highest leaf area (50.8 cm^2) was recorded in the 30 kg N/ha, the highest plant height (60 cm) was found in the 60 kg N/ha while the highest value for stem girth (2.9 cm) was recorded in the 30 kg N/ha all at 30 DAE. The weight of biomass above the soil level was 19.5 g while that of the root biomass was 16 g obtained in the 30 kg N/ha experiment. The root length was highest in the 10 kg N/ha experiment with value of 54 cm.

In the *Carica papaya* biofertilizer experimental plot, all the measured phyto-parameters recorded increased values as the experiment progressed. Leaf number was highest in the 30 kg N/ha (25 and 30 DAE); 40 kg N/ha (30 DAE), 50 kg N/ha (30 DAE) and 60 kg N/ha (25 and 30 DAE) with a value of 9.0. Leaf area had the highest value of 50.8 cm^2 recorded in the 30 kg N/ha experiment, the highest value of plant height was 66 cm in the 60 kg N/ha while that of stem girth was 2.9 cm in the 20, 30 and 50 kg N/ha experiment all recorded at 30 DAE. The weight of biomass above soil level was 20 g while that of root biomass was 21 g recorded in the 30 kg N/ha experiment. The root length was highest in the 20 kg N/ha experiment with value of 65 cm. In the *Telfairia occidentalis* biofertilizer plot, all the parameters increased as the experiments progressed. Leaf number was highest in the 30 kg N/ha (25 and 30 DAE), 50 kg N/ha and 60 kg N/ha at 30 DAE respectively with a value of 9.0. Highest value of leaf area was 50.9 cm^2 recorded in the 30 kg N/ha experiment, highest value of plant height was 69 cm found in the 40 kg N/ha experiment while that of stem girth was 2.8 cm recorded in the 20 kg N/ha experiment and all the

values were recorded at 30 DAE. The weight of biomass above soil level was highest in the 20 kg N/ha with value of 31 g while that of root biomass was 22 g recorded in the 20 kg N/ha experiment. The highest value of root length was 55 cm found in the 30 and 40 kg N/ha experiments.

In the *Arachis hypogaea* biofertilizer plot, all phyto-parameters increased in values as the experiments progressed. Leaf number was highest (9) in the 30 kg N/ha (25 and 30 DAE) and 50 kg N/ha at 30 DAE respectively. Highest value of leaf area was 53.6 cm^2 recorded in the 30 kg N/ha experiment, highest value of plant height was 64 cm found in the 60 kg N/ha experiment while that of stem girth was 2.8 cm recorded in the 20 kg N/ha experiment and all the values were recorded at 30 DAE. The weight of biomass above soil level was highest in the 30 kg N/ha with value of 25 g while that of root biomass was 23 g recorded in the 10 kg N/ha experiment. The highest value of root length was 55 cm found in the 50 kg N/ha experiments.

In all the experiments, comparison between values obtained from the phyto experiments involving all the five biofertilizers from the mono-digestion experiments, the NPK 15-15-15 inorganic fertilizer and the negative control (No fertilizer application) showed that all the five biofertilizers produced better results in the performance of the maize plants. All the phyto-parameters evaluated recorded higher values at each recording time than the NPK 15-15-15 inorganic fertilizer and the negative control experiments except for root length where the value recorded for NPK (56 cm) was higher than the 53 cm for *Tithonia diversifolia,* 54.5 cm for *Chromolaena odorata* and 55 cm recorded for *Telfairia occidentalis* and *Arachis hypogea* biofertilizers respectively.

Table 4.12a: Phyto-Assessment with *Tithonia diversifolia* Shoot Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	10.1±0.01	10±0.05	0.4±0.01	-	-	-
10 DAE	4±0.01	10.9±0.01	19±0.02	0.6±0.01	-	-	-
15 DAE	5±0.01	20.4±0.01	24±0.02	1.1±0.01	-	-	-
20 DAE	7±0.01	30.0±0.01	28±0.02	1.3±0.01	-	-	-
25 DAE	8±0.01	30.5±0.01	34±1.01	1.5±0.01	-	-	-
30 DAE	8±0.01	30.9±0.01	38±2.01	1.9±0.01	10.2±0.05	12.1±0.04	53±0.05
20 kg N/ha Application							
5 DAE	3±0.01	10.2±0.01	10±0.02	0.4±0.01	-	-	-
10 DAE	5±0.01	20.2±0.01	23±0.03	0.6±0.01	-	-	-
15 DAE	6±0.01	30.1±0.01	31±0.05	1.1±0.01	-	-	-
20 DAE	7±0.01	30.5±0.01	38±0.03	1.8±0.01	-	-	-
25 DAE	8±0.01	30.8±0.01	44±0.02	2.4±0.02	-	-	-
30 DAE	8±0.01	40.2±0.01	48±0.03	2.8±0.02	11±0.02	12±0.03	48±0.05
30 kg N/ha Application							
5 DAE	3±0.01	10.2±0.01	10±0.02	0.4±0.01	-	-	-
10 DAE	5±0.01	20.2±0.01	23±0.03	0.6±0.01	-	-	-
15 DAE	6±0.01	30.1±0.01	31±0.05	1.1±0.01	-	-	-
20 DAE	7±0.01	30.5±0.01	38±0.03	1.8±0.01	-	-	-
25 DAE	8±0.01	30.8±0.01	44±0.02	2.4±0.02	-	-	-
30 DAE	9±0.01	51.5±0.02	54±0.05	2.4±0.01	14±0.02	13±0.02	52±0.02
40 kg N/ha Application							
5 DAE	4±0.01	10.9±0.01	11±0.01	0.5±0.01	-	-	-
10 DAE	5±0.01	30.6±0.01	23±0.03	0.9±0.01	-	-	-
15 DAE	7±0.01	30.8±0.01	36±1.01	1.6±0.01	-	-	-
20 DAE	7±0.01	40.5±0.01	48±0.02	1.7±0.01	-	-	-
25 DAE	8±0.01	40.9±0.01	54±2.01	1.9±0.01	-	-	-
30 DAE	8±0.02	50.3±0.01	59±0.02	2.1±0.01	20.2±2.01	19.4±0.05	53±0.05
50 kg N/ha Application							
5 DAE	3±0.01	20.3±0.01	9.5±0.05	0.5±0.01	-	-	-
10 DAE	5±0.01	20.6±0.01	23±1.05	0.8±0.01	-	-	-
15 DAE	7±0.01	30.3±0.01	33±1.01	1.5±0.01	-	-	-
20 DAE	8±0.01	40.4±0.01	45±1.02	1.5±0.01	-	-	-
25 DAE	8±0.01	40.6±0.01	51±0.01	1.7±0.02	-	-	-
30 DAE	9±0.05	40.9±0.01	56±0.05	1.9±0.02	14.2±0.04	12.5±0.05	51±0.05
60 kg N/ha Application							
5 DAE	3±0.01	20.3±0.01	9.5±0.01	0.4±0.01	-	-	-
10 DAE	5±0.01	30.2±0.01	24±0.05	0.8±0.01	-	-	-
15 DAE	6±0.01	40.0±0.01	36±0.03	1.6±0.01	-	-	-
20 DAE	7±0.01	40.6±0.01	53±0.03	1.8±0.01	-	-	-
25 DAE	8±0.01	40.9±0.01	57±0.05	2.2±0.01	-	-	-
30 DAE	9±0.04	50.2±0.01	67±0.03	2.5±0.01	15.5±1.02	11.5±0.02	44±1.01

DAE= Day after Emergence

Table 4.12b: Phyto-Assessment with *Chromolaena odorata* Shoot Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	10.3±0.01	9±0.01	0.4±0.01	-	-	-
10 DAE	5±0.01	10.4±0.01	17±0.01	0.7±0.01	-	-	-
15 DAE	5±0.01	20.3±0.01	26±0.01	1.0±0.01	-	-	-
20 DAE	6±0.01	30.2±0.01	29±0.02	1.3±0.01	-	-	-
25 DAE	7±0.01	30.5±0.01	34±0.05	1.5±0.01	-	-	-
30 DAE	7±0.01	30.8±0.01	38±1.02	1.9±0.01	6.5±0.05	11.1±0.02	54.5±2.01
20 kg N/ha Application							
5 DAE	4±0.01	10.3±0.01	10±2.01	0.5±0.01	-	-	-
10 DAE	4±0.01	10.6±0.01	21±0.03	0.8±0.01	-	-	-
15 DAE	5±0.01	20.4±0.01	28±0.05	0.8±0.01	-	-	-
20 DAE	7±0.01	30.0±0.01	33±2.01	1.3±0.01	-	-	-
25 DAE	8±0.01	30.2±0.01	37±0.05	1.5±0.01	-	-	-
30 DAE	8±0.01	30.4±0.01	42±2.01	1.8±0.01	7.4±0.02	8±0.03	44±2.05
30 kg N/ha Application							
5 DAE	3±0.01	10.5±0.01	10±0.01	0.5±0.01	-	-	-
10 DAE	5±0.01	20.3±0.01	25±2.02	0.8±0.01	-	-	-
15 DAE	6±0.01	20.8±0.01	34±0.01	1.0±0.01	-	-	-
20 DAE	7±0.01	40.2±0.01	38±0.05	1.5±0.01	-	-	-
25 DAE	8±0.01	50.6±0.01	45±0.05	2.4±0.01	-	-	-
30 DAE	9±0.01	50.8±0.01	49±0.05	2.9±0.01	9.5±1.01	16±0.05	51.5±2.01
40 kg N/ha Application							
5 DAE	3±0.01	10.6±0.01	8±0.01	0.4±0.01	-	-	-
10 DAE	4±0.01	30.6±0.01	16±2.01	0.6±0.01	-	-	-
15 DAE	6±0.01	30.8±0.01	26±0.02	0.8±0.01	-	-	-
20 DAE	7±0.01	40.5±0.01	31±3.01	1.3±0.01	-	-	-
25 DAE	8±0.01	40.6±0.01	35±0.02	1.8±0.02	-	-	-
30 DAE	8±0.01	50.1±0.01	39±2.01	2.3±0.01	6.5±0.01	11.5±0.02	44.3±2.05
50 kg N/ha Application							
5 DAE	4±0.01	20.5±0.01	11±0.02	0.4±0.01	-	-	-
10 DAE	5±0.01	20.6±0.01	20±1.01	0.8±0.01	-	-	-
15 DAE	5±0.01	30.5±0.01	29±0.02	0.8±0.01	-	-	-
20 DAE	6±0.01	40.6±0.01	38±1.05	1.2±0.01	-	-	-
25 DAE	7±0.01	40.8±0.01	47±0.03	1.5±0.01	-	-	-
30 DAE	8±0.01	40.9±0.01	54±0.04	1.8±0.01	8.5±2.01	7.5±1.01	46.4±3.01
60 kg N/ha Application							
5 DAE	4±0.01	20.4±0.01	11.5±0.05	0.4±0.01	-	-	-
10 DAE	4±0.01	30.3±0.01	26±0.01	0.8±0.01	-	-	-
15 DAE	6±0.01	40.2±0.01	34±0.03	1.6±0.01	-	-	-
20 DAE	7±0.01	40.6±0.01	47±0.02	1.8±0.01	-	-	-
25 DAE	8±0.01	50.3±0.01	53±0.01	2.2±0.01	-	-	-
30 DAE	9±0.01	50.6±0.01	60±3.01	2.5±0.01	7.5±0.04	8.5±1.01	34.5±2.01

DAE= Day after Emergence

Table 4.12c: Phyto-Assessment with *Carica papayas* Fruit Peels Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	4±0.01	10.1±0.01	9.5±0.01	0.4±0.01	-	-	-
10 DAE	5±0.01	10.3±0.01	28±0.03	0.8±0.01	-	-	-
15 DAE	6±0.01	20.0±0.01	37±0.04	1.2±0.01	-	-	-
20 DAE	6±0.01	30.0±0.01	39±0.04	1.4±0.01	-	-	-
25 DAE	7±0.01	30.5±0.01	42±0.05	1.7±0.01	-	-	-
30 DAE	8±0.01	30.5±0.01	46±2.01	2.2±0.01	17.5±2.01	16.2±1.01	45±3.01
20 kg N/ha Application							
5 DAE	3±0.01	10.2±0.01	9±0.05	0.5±0.01	-	-	-
10 DAE	5±0.01	20.2±0.01	24±2.01	0.7±0.01	-	-	-
15 DAE	6±0.01	30.1±0.01	36±2.01	1.1±0.01	-	-	-
20 DAE	7±0.01	30.5±0.01	43±2.01	1.5±0.01	-	-	-
25 DAE	8±0.01	30.7±0.01	47±2.01	2.4±0.01	-	-	-
30 DAE	8±0.01	40.0±0.01	52±3.01	2.9±0.01	17±1.01	19±2.01	65±0.05
30 kg N/ha Application							
5 DAE	3±0.01	20.3±0.01	12±1.01	0.5±0.01	-	-	-
10 DAE	5±0.01	30.0±0.01	24±1.02	1.1±0.01	-	-	-
15 DAE	7±0.01	30.8±0.01	46±0.03	1.4±0.01	-	-	-
20 DAE	8±0.01	50.0±0.01	52±1.04	1.8±0.01	-	-	-
25 DAE	9±0.01	50.3±0.01	58±2.05	2.4±0.01	-	-	-
30 DAE	9±0.01	50.8±0.01	63±0.04	2.9±0.01	24±2.01	21.6±0.05	45.5±2.01
40 kg N/ha Application							
5 DAE	4±0.01	10.9±0.01	15±2.01	0.7±0.01	-	-	-
10 DAE	6±0.01	30.6±0.01	32±0.05	1.1±0.01	-	-	-
15 DAE	7±0.01	30.8±0.01	46±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	40.5±0.01	52±0.04	1.8±0.01	-	-	-
25 DAE	8±1.01	40.7±0.01	59±2.01	2.0±0.01	-	-	-
30 DAE	9±1.01	50.2±0.01	65±2.01	2.4±0.01	23.5±3.01	19.5±1.01	53±2.00
50 kg N/ha Application							
5 DAE	4±0.01	10.9±0.01	15±2.01	0.7±0.01	-	-	-
10 DAE	6±0.01	30.6±0.01	32±0.05	1.1±0.01	-	-	-
15 DAE	7±0.01	30.8±0.01	46±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	40.5±0.01	52±0.04	1.8±0.01	-	-	-
25 DAE	8±0.01	40.6±0.01	54±0.01	2.7±0.01	-	-	-
30 DAE	9±0.02	50.0±0.02	62±1.04	2.9±0.01	20±0.01	16±0.01	50±0.01
60 kg N/ha Application							
5 DAE	3±0.01	20.3±0.01	12.5±1.01	0.5±0.01	-	-	-
10 DAE	6±0.01	30.2±0.01	36±0.01	1.1±0.01	-	-	-
15 DAE	7±0.01	40.0±0.01	47±0.01	1.5±0.01	-	-	-
20 DAE	8±0.01	40.5±0.01	52±0.03	1.9±0.01	-	-	-
25 DAE	9±0.02	40.7±0.01	56±3.01	2.1±0.01	-	-	-
30 DAE	9±0.03	50.1±0.02	66±4.01	2.4±0.01	21.5±3.01	17.5±2.01	46.5±2.05

DAE= Day after Emergence

Table 4.12d: Phyto-Assessment with *Telfairia occidentalis* Fruit Peels Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	10.1±0.01	10±0.04	0.4±0.01	-	-	-
10 DAE	4±0.01	10.4±0.01	19±2.03	0.6±0.01	-	-	-
15 DAE	5±0.01	20.4±0.01	24±2.01	1.1±0.01	-	-	-
20 DAE	7±0.01	30.0±0.01	25±2.01	1.2±0.01	-	-	-
25 DAE	7±0.01	30.3±0.01	34±1.05	1.5±0.01	-	-	-
30 DAE	8±0.01	30.5±0.01	36±2.01	1.8±0.01	16.2±3.01	16.1±2.01	52.5±1.01
20 kg N/ha Application							
5 DAE	3±0.01	10.2±0.01	10±2.01	0.4±0.01	-	-	-
10 DAE	5±0.01	20.2±0.01	23±2.01	0.6±0.01	-	-	-
15 DAE	6±0.01	30.1±0.01	31±3.01	1.1±0.01	-	-	-
20 DAE	7±0.01	30.5±0.01	38±3.01	1.8±0.01	-	-	-
25 DAE	8±0.01	30.8±0.01	44±4.01	2.4±0.01	-	-	-
30 DAE	8±0.01	40.2±0.01	50±1.05	2.8±0.01	31±2.01	22±3.01	54±4.01
30 kg N/ha Application							
5 DAE	4±0.01	30.0±0.01	12±0.03	0.6±0.01	-	-	-
10 DAE	5±0.01	30.3±0.01	26±0.03	0.7±0.01	-	-	-
15 DAE	7±0.01	30.8±0.01	40±0.02	1.4±0.01	-	-	-
20 DAE	8±0.01	50.2±0.01	48±0.02	1.6±0.01	-	-	-
25 DAE	9±1.01	50.5±0.01	58±0.02	2.4±0.01	-	-	-
30 DAE	9±1.01	50.9±0.01	62±2.01	2.7±0.01	20.5±5.01	19.4±2.01	55±2.03
40 kg N/ha Application							
5 DAE	4±0.01	10.9±0.01	11±1.01	0.6±0.01	-	-	-
10 DAE	5±0.01	30.6±0.01	23±0.01	0.9±0.01	-	-	-
15 DAE	7±0.01	30.8±0.01	36±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	40.5±0.01	51±0.03	1.7±0.01	-	-	-
25 DAE	8±0.01	40.9±0.01	54±2.01	1.9±0.01	-	-	-
30 DAE	8±0.01	50.3±0.01	69±2.01	2.3±0.01	25.2±2.01	21.5±3.01	55±2.03
50 kg N/ha Application							
5 DAE	3±0.01	20.1±0.01	9.5±0.01	0.5±0.01	-	-	-
10 DAE	5±0.01	20.4±0.01	23±0.05	0.8±0.01	-	-	-
15 DAE	7±0.01	30.3±0.01	33±0.02	1.5±0.01	-	-	-
20 DAE	8±0.01	40.4±0.01	45±0.01	1.5±0.01	-	-	-
25 DAE	8±0.01	40.6±0.01	56±0.05	1.7±0.01	-	-	-
30 DAE	9±0.01	40.9±0.01	58±2.01	1.8±0.01	18.5±3.01	17.5±2.02	54±2.05
60 kg N/ha Application							
5 DAE	3±0.01	20.0±0.01	9.5±2.01	0.4±0.01	-	-	-
10 DAE	5±0.01	30.2±0.01	24±1.01	0.8±0.01	-	-	-
15 DAE	6±0.01	30.6±0.01	36±0.02	1.6±0.01	-	-	-
20 DAE	7±0.01	40.3±0.01	53±2.01	1.8±0.01	-	-	-
25 DAE	8±0.01	40.6±0.01	54±0.03	2.2±0.01	-	-	-
30 DAE	9±0.01	40.7±0.01	65±3.01	2.5±0.01	15.5±2.01	11.5±0.02	54±2.01

DAE= Day after Emergence

Table 4.12e: Phyto-Assessment with *Arachis hypogaea* Hull Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	10.1±0.01	9±1.01	0.4±0.01	-	-	-
10 DAE	4±0.01	11.9±0.01	16±0.03	0.6±0.01	-	-	-
15 DAE	5±0.01	26.4±0.01	24±2.01	1.1±0.01	-	-	-
20 DAE	7±0.01	30.0±0.01	28±2.01	1.3±0.01	-	-	-
25 DAE	8±0.01	30.3±0.01	32±1.01	1.4±0.01	-	-	-
30 DAE	8±0.01	32.6±0.01	36±2.01	1.7±0.01	21.2±4.01	23.1±1.01	54±1.01
20 kg N/ha Application							
5 DAE	3±0.01	11.2±0.01	10±2.01	0.4±0.01	-	-	-
10 DAE	5±0.01	22.2±0.01	23±2.01	0.6±0.01	-	-	-
15 DAE	6±0.01	32.1±0.01	31±3.01	1.1±0.01	-	-	-
20 DAE	7±0.01	33.2±0.01	35±0.01	1.8±0.01	-	-	-
25 DAE	8±0.01	34.4±0.01	42±2.01	2.4±0.01	-	-	-
30 DAE	8±0.01	36.6±0.01	46±3.01	2.8±0.01	18±2.01	16±2.01	45±0.02
30 kg N/ha Application							
5 DAE	4±0.01	31.0±0.01	12±0.02	0.6±0.01	-	-	-
10 DAE	5±0.01	32.3±0.01	26±2.01	0.9±0.01	-	-	-
15 DAE	7±0.01	34.5±0.01	40±3.01	1.4±0.01	-	-	-
20 DAE	8±0.02	51.2±0.01	48±2.01	1.8±0.01	-	-	-
25 DAE	9±0.01	52.5±0.01	52±2.01	2.4±0.01	-	-	-
30 DAE	9±0.02	53.6±0.01	54±3.03	2.5±0.01	25.5±4.01	21.6±3.01	54±3.01
40 kg N/ha Application							
5 DAE	4.0±0.01	11.9±0.01	11±0.03	0.5±0.01	-	-	-
10 DAE	5±0.01	31.6±0.01	23±3.01	0.9±0.01	-	-	-
15 DAE	7±0.01	32.8±0.01	36±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	41.5±0.01	48±4.01	1.7±0.01	-	-	-
25 DAE	8±0.01	42.9±0.01	54±3.01	1.9±0.01	-	-	-
30 DAE	8±0.01	53.3±0.01	59±2.00	2.1±0.01	20.2±2.02	19.5±4.01	53±3.01
50 kg N/ha Application							
5 DAE	3±0.01	22.3±0.01	9.5±0.01	0.5±0.01	-	-	-
10 DAE	5±0.01	22.6±0.01	23±3.01	0.6±0.01	-	-	-
15 DAE	6±0.01	30.3±0.01	33±2.01	1.4±0.01	-	-	-
20 DAE	7±0.01	41.4±0.01	45±0.05	1.5±0.01	-	-	-
25 DAE	8±0.01	42.6±0.01	50±2.01	1.6±0.01	-	-	-
30 DAE	9±1.01	44.7±0.01	55±3.01	1.7±0.01	16.3±3.01	19.5±4.01	55±2.01
60 kg N/ha Application							
5 DAE	3±0.01	21.3±0.01	9.5±0.01	0.4±0.01	-	-	-
10 DAE	5±0.01	32.2±0.01	24±2.01	0.8±0.01	-	-	-
15 DAE	6±0.01	34.4±0.01	32±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	45.4±0.01	52±5.01	1.8±0.01	-	-	-
25 DAE	7±0.01	45.6±0.01	54±2.01	2.2±0.01	-	-	-
30 DAE	8±0.01	46.7±0.01	64±3.01	2.5±0.01	21.5±3.01	19.5±4.01	46±2.01

DAE= Day after Emergence

The result of the phyto-assessments carried out with the application of the biofertilizers produced from the co-digestion experiments are shown in tables 4.11 (f-j). From the *Tithonia diversifolia* + poultry dropping biofertilizer plot, all parameters followed an increasing trend as the experiments progressed. The highest leaf number (10.0) was recorded in the 10, 30, 40 and 60 kg N/ha experiments all at 30 DAE. The highest leaf area was 67.3 cm^2; highest plant height was 64 cm while the highest stem girth was 3.6 cm all from the 40 kg N/ha and at 30 DAE. The weight of biomass above the soil level was 27.5 g from the 40 kg N/ha while that of the root biomass was 24.5 g from the 60 kg N/ha. The root length had the highest level of 57 cm in the 60 kg N/ha experiment.

From the *Chromolaena odorata* + poultry dropping biofertilizer plot, all phyto-parameters increased in values as the experiments progressed. Leaf number was highest (10) in the 50 and 60 kg N/ha experiments at 30 DAE respectively. Highest leaf area was 58.7 cm^2, highest value of plant height was 69 cm found in the 60 kg N/ha experiment while that of stem girth was 2.9 cm recorded in both the 50 and 60 kg N/ha experiments and all the values were recorded at 30 DAE. The weight of biomass above soil level was highest in the 30 kg N/ha with value of 25.5 g while that of root biomass was 32.5 g recorded in the 50 kg N/ha experiment. The highest value of root length was 64 cm found in the 20 kg N/ha experiment. From the *Carica papaya* + poultry dropping biofertilizer plot, all values recorded increasing values as the experiments progressed. The highest leaf number (9) was recorded in the 20 and 30 kg N/kg (25 and 30 DAE), 50 and 60 kg N/ha (30 DAE). Leaf area was highest (56.6 cm^2) in the 30 kg N/ha; plant height was highest (64 cm) in the 60 kg N/ha while the stem girth was highest (2.8 cm) in the 20 kg N/ha experiment all recorded at 30 DAE. The weight of biomass above the soil level was 26.2 g; that of the root biomass was 29.5 g while root lenght was 63 cm all recorded from the 40 kg N/ha experiment.

From the *Telfairia occidentalis* + poultry dropping biofertilizer plot, all parameters followed an increasing trend as the experiments progressed. The highest leaf number (9.0) was recorded in the 30 kg N/ha (25 and 30 DAE), 40, 50 and 60 kg N/ha experiments all at 30 DAE. The highest leaf area was 54.8 cm^2; highest plant height was 69 cm while the highest stem girth was 3.0 cm from the 30, 60 and 20 kg N/ha experiments respectively

and at 30 DAE. The weight of biomass above the soil level was 27.5 g from the 30 kg N/ha while that of the root biomass was 19 g from the 20 kg N/ha. The root length had the highest level of 59.4 cm recorded in the 40 kg N/ha experiment. From the *Arachis hypogaea* + poultry dropping biofertilizer plot, all values recorded increasing values as the experiments progressed. The highest leaf number (9) was recorded in the 30, 40, 50 and 60 kg N/ha (30 DAE). Leaf area was highest (58.9 cm^2) in the 30 kg N/ha; plant height was heighest (66 cm) in the 60 kg N/ha while the stem girth was highest (2.9 cm) in the 20 and 30 kg N/ha experiments and all value were recorded at 30 DAE. The weight of biomass above the soil level was 25.5 g; that of the root biomass was 23.5 g while root length was 59 cm all recorded from the 30 kg N/ha experiment.

In all the experiments, comparison between values obtained from the phyto experiments involving all the ten biofertilizers, the NPK 15-15-15 inorganic fertilizer and the negative control (No fertilizer application) showed that all the ten biofertilizers produced better results in the performance of the maize plants than the NPK 15-15-15 inorganic fertilizer and the negative control experiments. Also, the performance of the biofertilizers from the co-digestion experiments recorded higher performance than those from the mono-digestions.

Table 4.12f: Phyto-Assessment with *Tithonia diversifolia* + Poultry Droppings Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
			10 kg N/ha Application				
5 DAE	3±0.01	11.1±0.01	9±1.01	0.4±0.01	-	-	-
10 DAE	4±0.01	11.9±0.01	16±0.03	0.6±0.01	-	-	-
15 DAE	5±0.01	20.4±0.01	24±2.01	1.1±0.01	-	-	-
20 DAE	7±0.01	31.0±0.01	28±2.01	1.3±0.01	-	-	-
25 DAE	9±0.01	33.8±0.01	52±2.01	1.7±0.01	-	-	-
30 DAE	10±2.01	43.6±0.01	58±3.02	1.9±0.01	21.2±4.01	22.1±1.01	55±1.01
			20 kg N/ha Application				
5 DAE	3±0.01	12.5±0.01	10.5±0.02	0.4±0.01	-	-	-
10 DAE	5±0.01	21.7±0.01	33±2.01	1.0±0.01	-	-	-
15 DAE	7±0.01	33.3±0.01	41±2.01	1.3±0.01	-	-	-
20 DAE	7±0.01	33.6±0.01	48±2.01	1.5±0.01	-	-	-
25 DAE	8±0.01	43.1±0.01	52±2.01	1.9±0.01	-	-	-
30 DAE	9±0.01	45.8±0.01	58±3.01	2.3±0.01	15.2±1.01	17±3.01	49.6±2.01
			30 kg N/ha Application				
5 DAE	3±0.01	31.3±0.01	12±1.01	0.4±0.01	-	-	-
10 DAE	5±0.01	32.6±0.01	29±2.01	1.0±0.01	-	-	-
15 DAE	7±0.01	34.9±0.01	44±3.01	1.5±0.01	-	-	-
20 DAE	8±0.01	44.7±0.01	48±3.01	1.8±0.01	-	-	-
25 DAE	9±1.01	55.4±0.01	55±2.04	2.6±0.01	-	-	-
30 DAE	10±2.01	58.9±0.01	61±4.01	3.1±0.01	13.5±2.01	11.5±2.01	52±1.01
			40 kg N/ha Application				
5 DAE	4±0.01	33.9±0.01	12.6±3.01	0.5±0.01	-	-	-
10 DAE	6±0.01	42.5±0.01	32±2.01	1.2±0.01	-	-	-
15 DAE	7±0.01	43.8±0.01	44.5±3.01	1.8±0.01	-	-	-
20 DAE	8±0.01	55.4±0.01	49±3.01	2.4±0.01	-	-	-
25 DAE	9±0.01	55.9±0.01	56±4.01	2.9±0.01	-	-	-
30 DAE	10±0.01	67.3±0.01	64±5.01	3.6±0.01	27.5±2.01	18±2.01	51.5±5.01
			50 kg N/ha Application				
5 DAE	3±0.01	32.8±0.01	11±3.01	0.4±0.01	-	-	-
10 DAE	5±0.01	42.0±0.01	24±4.01	0.8±0.01	-	-	-
15 DAE	6±0.01	44.3±0.01	34±2.01	1.5±0.01	-	-	-
20 DAE	7±0.01	45.5±0.01	44±4.01	1.7±0.01	-	-	-
25 DAE	8±0.01	46.6±0.01	56±3.01	1.9±0.01	-	-	-
30 DAE	9±0.01	57.1±0.01	59±3.01	2.2±0.01	13.4±3.01	9.5±2.01	52.4±5.01
			60 kg N/ha Application				
5 DAE	3±0.01	32.6±0.01	9.0±3.01	0.5±0.01	-	-	-
10 DAE	5±0.01	33.9±0.01	30±2.01	1.2±0.01	-	-	-
15 DAE	7±0.01	42.2±0.01	45.4±2.01	1.8±0.01	-	-	-
20 DAE	8±0.01	44.6±0.01	47±3.01	2.1±0.01	-	-	-
25 DAE	9±0.01	46.9±0.01	52±2.01	2.4±0.01	-	-	-
30 DAE	10±2.01	57.4±0.01	57±3.01	2.6±0.01	26.5±2.01	24.5±1.01	57.2±2.01

DAE= Day after Emergence

Table 4.12g: Phyto-Assessment with *Chromolaena odorata* + Poultry Droppings Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	11.5±0.01	10±1.01	0.4±0.01	-	-	-
10 DAE	5±0.01	12.9±0.01	22±2.01	0.7±0.01	-	-	-
15 DAE	6±0.01	23.5±0.01	32±2.01	0.9±0.01	-	-	-
20 DAE	7±0.01	35.2±0.01	38±3.01	1.2±0.01	-	-	-
25 DAE	8±0.01	34.7±0.01	43±2.01	1.6±0.01	-	-	-
30 DAE	9±0.01	44.2±0.01	48±4.01	1.9±0.01	8.5±1.01	12.2±1.01	43±2.01
20 kg N/ha Application							
5 DAE	4±0.01	14.6±0.01	13±2.01	0.5±0.01	-	-	-
10 DAE	4±0.01	22.1±0.01	26±1.01	0.6±0.01	-	-	-
15 DAE	6±0.01	31.2±0.01	42±2.01	0.9±0.01	-	-	-
20 DAE	7±0.01	32.5±0.01	48±3.01	1.3±0.01	-	-	-
25 DAE	8±0.01	34.8±0.01	54±2.01	2.4±0.01	-	-	-
30 DAE	9±0.01	45.7±0.02	58±3.01	2.8±0.01	13.3±3.01	14.5±2.01	64±3.01
30 kg N/ha Application							
5 DAE	3±0.01	31.0±0.01	9.5±1.01	0.5±0.01	-	-	-
10 DAE	5±0.01	33.3±0.01	24±2.01	1.0±0.01	-	-	-
15 DAE	7±0.01	33.4±0.01	34±3.01	1.3±0.01	-	-	-
20 DAE	8±0.01	54.2±0.01	38±2.01	1.8±0.01	-	-	-
25 DAE	9±0.01	55.3±0.01	44±3.01	2.4±0.01	-	-	-
30 DAE	9±0.01	58.7±0.01	51±3.01	2.8±0.01	25.5±2.01	17.4±3.01	50±2.01
40 kg N/ha Application							
5 DAE	4±0.01	22.9±0.01	11.5±0.01	0.7±0.01	-	-	-
10 DAE	6±0.01	33.6±0.01	33±0.01	1.3±0.01	-	-	-
15 DAE	7±0.01	34.8±0.01	46±2.01	1.8±0.01	-	-	-
20 DAE	8±0.01	45.5±0.01	58±2.01	2.2±0.01	-	-	-
25 DAE	9±0.01	46.9±0.01	61±3.01	2.5±0.01	-	-	-
30 DAE	9±0.01	56.5±0.01	65±4.01	2.8±0.01	23.5±3.01	21.5±3.01	54.5±0.05
50 kg N/ha Application							
5 DAE	3±0.01	22.5±0.01	12±1.01	0.5±0.01	-	-	-
10 DAE	5±0.01	23.8±0.02	35±3.01	1.2±0.01	-	-	-
15 DAE	8±0.01	34.4±0.01	47±2.01	1.6±0.01	-	-	-
20 DAE	9±0.01	46.4±0.01	55±3.01	1.9±0.01	-	-	-
25 DAE	9±0.01	47.6±0.01	59±3.01	2.4±0.01	-	-	-
30 DAE	10±1.01	58.2±0.01	67±2.05	2.9±0.01	21±2.01	32.5±3.01	59±3.01
60 kg N/ha Application							
5 DAE	4±0.01	24.5±0.01	12.5±2.01	0.6±0.01	-	-	-
10 DAE	6±0.01	32.2±0.01	37±1.01	1.2±0.01	-	-	-
15 DAE	8±0.01	41.0±0.01	54±2.01	1.7±0.01	-	-	-
20 DAE	8±0.01	41.6±0.01	59±2.01	2.1±0.01	-	-	-
25 DAE	9±0.01	44.9±0.01	65±2.05	2.6±0.01	-	-	-
30 DAE	10±2.01	57.6±0.01	69±4.01	2.9±0.01	22.5±2.01	17.5±2.01	42±3.01

DAE= Day after Emergence

Table 4.12h: Phyto-Assessment with *Carica papayas* + Poultry Droppings Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	12.1±0.01	10±1.01	0.4±0.01	-	-	-
10 DAE	4±0.01	12.9±0.01	19±2.01	0.6±0.01	-	-	-
15 DAE	5±0.01	21.4±0.01	24±3.01	1.1±0.01	-	-	-
20 DAE	6±0.01	33.0±0.01	28±2.01	1.3±0.01	-	-	-
25 DAE	7±0.01	34.5±0.01	34±3.01	1.5±0.01	-	-	-
30 DAE	8±0.01	34.7±0.01	37±2.01	1.6±0.01	13.2±3.01	16.1±2.01	47.5±2.01
20 kg N/ha Application							
5 DAE	4±0.01	13.6±0.01	13±2.01	0.5±0.01	-	-	-
10 DAE	4±0.01	23.1±0.01	26±1.01	0.6±0.01	-	-	-
15 DAE	6±0.01	35.2±0.01	42±2.01	0.9±0.01	-	-	-
20 DAE	7±0.01	36.5±0.01	48±3.01	1.3±0.01	-	-	-
25 DAE	8±0.01	36.8±0.01	54±2.01	2.4±0.01	-	-	-
30 DAE	9±0.01	43.7±0.02	58±3.01	2.8±0.01	13.4±1.01	15.8±2.01	53.9±1.01
30 kg N/ha Application							
5 DAE	3±0.01	32.0±0.01	9.5±1.01	0.5±0.01	-	-	-
10 DAE	5±0.01	33.3±0.01	24±2.01	1.0±0.01	-	-	-
15 DAE	7±0.01	34.4±0.01	34±3.01	1.3±0.01	-	-	-
20 DAE	8±0.01	52.2±0.01	37±2.01	1.8±0.01	-	-	-
25 DAE	9±0.01	54.3±0.01	45±3.01	2.4±0.01	-	-	-
30 DAE	9±0.01	56.6±0.01	53±3.01	2.7±0.01	18.2±1.01	20.4±1.01	47.9±1.01
40 kg N/ha Application							
5 DAE	4±0.01	12.9±0.01	11±1.01	0.4±0.01	-	-	-
10 DAE	5±0.01	31.6±0.01	23±1.01	0.6±0.01	-	-	-
15 DAE	6±0.01	33.8±0.01	36±2.01	1.4±0.01	-	-	-
20 DAE	7±0.02	43.5±0.01	48±4.01	1.7±0.01	-	-	-
25 DAE	8±0.01	44.9±0.01	51±2.01	1.7±0.01	-	-	-
30 DAE	8±0.00	55.3±0.01	56±3.01	1.9±0.01	26.2±2.02	29.5±2.01	63±3.01
50 kg N/ha Application							
5 DAE	3±0.01	21.3±0.01	10±2.01	0.6±0.01	-	-	-
10 DAE	5±0.01	22.6±0.01	23±2.01	0.8±0.01	-	-	-
15 DAE	6±0.01	33.3±0.01	33±3.01	1.4±0.01	-	-	-
20 DAE	7±0.01	41.4±0.01	43±2.02	1.5±0.01	-	-	-
25 DAE	8±0.01	42.5±0.01	51±4.01	1.6±0.01	-	-	-
30 DAE	9±0.01	44.8±0.01	54±3.01	1.7±0.01	14.6±2.01	12.9±3.01	58±3.01
60 kg N/ha Application							
5 DAE	3±0.01	22.3±0.01	9.5±0.01	0.4±0.01	-	-	-
10 DAE	5±0.01	33.2±0.01	24±3.01	0.8±0.01	-	-	-
15 DAE	6±0.01	44.0±0.01	36±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	45.6±0.01	53±3.01	1.8±0.01	-	-	-
25 DAE	7±0.01	46.8±0.01	55±3.02	2.2±0.01	-	-	-
30 DAE	9±0.01	56.0±0.01	64±4.01	2.4±0.01	19.5±3.01	14.5±2.01	47±2.01

DAE= Day after Emergence

Table 4.12i: Phyto-Assessment with *Telfairia occidentalis* + Poultry Droppings Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	12.1±0.01	11±1.01	0.4±0.01	-	-	-
10 DAE	4±0.01	13.9±0.01	19±3.01	0.6±0.01	-	-	-
15 DAE	5±0.01	23.4±0.01	24±2.01	1.1±0.01	-	-	-
20 DAE	6±0.01	32.2±0.01	28±1.05	1.3±0.01	-	-	-
25 DAE	7±0.01	34.6±0.01	34±2.01	1.6±0.01	-	-	-
30 DAE	8±0.01	35.9±0.01	48±3.01	1.9±0.01	15.8±2.01	18.1±2.01	57±2.01
20 kg N/ha Application							
5 DAE	3±0.01	13.2±0.01	12±2.01	0.4±0.01	-	-	-
10 DAE	5±0.01	23.2±0.01	23±1.01	0.6±0.01	-	-	-
15 DAE	6±0.01	31.3±0.01	31±2.01	1.4±0.01	-	-	-
20 DAE	7±0.01	32.5±0.01	38±2.01	1.8±0.01	-	-	-
25 DAE	8±0.01	32.8±0.01	44±3.01	2.6±0.01	-	-	-
30 DAE	8±0.01	44.4±0.01	50±5.01	3.0±0.01	17±3.01	19±2.01	49.6±3.01
30 kg N/ha Application							
5 DAE	4±0.01	32.0±0.01	12±2.00	0.5±0.01	-	-	-
10 DAE	5±0.01	33.3±0.01	26±2.01	1.0±0.01	-	-	-
15 DAE	7±0.01	41.0±0.01	40±3.00	1.6±0.01	-	-	-
20 DAE	8±0.01	51.3±0.01	48±2.01	1.8±0.01	-	-	-
25 DAE	9±0.01	53.5±0.01	57±2.01	2.6±0.01	-	-	-
30 DAE	9±0.01	54.8±0.01	58±3.01	2.9±0.01	27.5±2.01	18.8±2.01	54±2.01
40 kg N/ha Application							
5 DAE	4.0±0.01	13.9±0.01	11±3.01	0.5±0.01	-	-	-
10 DAE	5.0±0.01	33.6±0.01	23±3.01	0.9±0.01	-	-	-
15 DAE	6.0±0.01	34.8±0.01	36±2.02	1.7±0.01	-	-	-
20 DAE	7.0±0.01	42.5±0.01	48±4.01	1.8±0.01	-	-	-
25 DAE	8.0±0.01	41.9±0.01	54±4.01	2.1±0.01	-	-	-
30 DAE	9.0±0.01	53.6±0.01	59±3.01	2.4±0.01	26±2.01	17.5±3.01	59.4±2.01
50 kg N/ha Application							
5 DAE	3±0.01	22.3±0.01	9.5±2.01	0.6±0.01	-	-	-
10 DAE	5±0.01	23.6±0.01	23±2.01	0.9±0.01	-	-	-
15 DAE	7±0.01	31.3±0.01	33±1.01	1.5±0.01	-	-	-
20 DAE	8±0.01	41.4±0.01	45±2.01	1.5±0.01	-	-	-
25 DAE	8±0.01	42.6±0.01	54±3.01	1.7±0.01	-	-	-
30 DAE	9±0.01	52.0±0.01	58±3.01	2.1±0.01	17.9±3.01	16.9±3.01	58±2.01
60 kg N/ha Application							
5 DAE	3±0.01	22.3±0.01	9.5±0.05	0.6±0.01	-	-	-
10 DAE	5±0.01	33.2±0.01	24±3.01	0.8±0.01	-	-	-
15 DAE	6±0.01	42.0±0.01	36±3.01	1.6±0.01	-	-	-
20 DAE	7±0.01	43.6±0.01	54±2.05	1.8±0.01	-	-	-
25 DAE	8±0.01	42.5±0.01	57±2.01	2.5±0.01	-	-	-
30 DAE	9±0.01	54.4±0.01	69±2.01	2.8±0.01	18.5±3.01	17.5±2.01	49.6±3.01

DAE= Day after Emergence

Table 4.12j: Phyto-Assessment with *Arachis hypogea* Hull + Poultry Droppings Biofertilizer Applications Using Maize (*Zea mays*) as Test Plant

Day	Leaf number	Leaf area (cm^2)	Plant height cm)	Stem girth (cm)	Biomass above soil level (g)	Root biomass (g)	Root length (cm)
10 kg N/ha Application							
5 DAE	3±0.01	13.1±0.01	12±2.01	0.4±0.01	-	-	-
10 DAE	4±0.01	13.9±0.01	18±3.01	0.6±0.01	-	-	-
15 DAE	5±0.01	23.4±0.01	27±3.01	1.3±0.01	-	-	-
20 DAE	7±0.01	32.0±0.01	29±3.01	1.4±0.01	-	-	-
25 DAE	8±0.01	32.5±0.01	36±4.01	1.7±0.01	-	-	-
30 DAE	8±0.01	34.9±0.01	43±3.01	2.3±0.01	16±3.01	15.1±3.01	51.1±2.01
20 kg N/ha Application							
5 DAE	3±0.01	13.5±0.01	12±1.01	0.5±0.01	-	-	-
10 DAE	5±0.01	21.2±0.01	23±3.01	0.6±0.01	-	-	-
15 DAE	6±0.01	31.4±0.01	33±3.01	1.3±0.01	-	-	-
20 DAE	7±0.01	33.5±0.01	38±2.01	1.8±0.01	-	-	-
25 DAE	8±0.01	34.8±0.01	44±2.01	2.7±0.01	-	-	-
30 DAE	8±0.01	42.4±0.01	54±4.01	2.9±0.01	17±2.01	18±3.01	47±3.01
30 kg N/ha Application							
5 DAE	4±0.01	32.0±0.01	11±2.01	0.6±0.01	-	-	-
10 DAE	5±0.01	33.3±0.01	26±3.01	0.9±0.01	-	-	-
15 DAE	7±0.01	35.8±0.01	44±3.01	1.6±0.01	-	-	-
20 DAE	8±0.01	55.2±0.01	48±2.01	1.8±0.01	-	-	-
25 DAE	8±0.01	56.6±0.01	54±4.01	2.6±0.01	-	-	-
30 DAE	9±1.01	58.9±0.01	65±3.01	2.9±0.01	25.5±3.01	23.5±2.01	59±3.01
40 kg N/ha Application							
5 DAE	4.0±0.01	13.9±0.01	11±3.01	0.5±0.01	-	-	-
10 DAE	5.0±0.01	32.6±0.01	23±3.01	0.9±0.01	-	-	-
15 DAE	6.0±0.01	33.8±0.01	36±2.02	1.7±0.01	-	-	-
20 DAE	7.0±0.01	43.5±0.01	48±4.01	1.8±0.01	-	-	-
25 DAE	8.0±0.01	45.9±0.01	54±4.01	2.1±0.01	-	-	-
30 DAE	9.0±0.01	55.6±0.01	59±3.01	2.4±0.01	24.7±2.01	19.5±2.01	44.5±2.01
50 kg N/ha Application							
5 DAE	3±0.01	24.3±0.01	9.5±2.01	0.6±0.01	-	-	-
10 DAE	5±0.01	25.6±0.01	23±2.01	0.9±0.01	-	-	-
15 DAE	7±0.01	35.3±0.01	33±1.01	1.5±0.01	-	-	-
20 DAE	8±0.01	44.4±0.01	45±2.01	1.5±0.01	-	-	-
25 DAE	8±0.01	45.6±0.01	54±3.01	1.7±0.01	-	-	-
30 DAE	9±0.01	55.0±0.01	58±3.01	2.1±0.01	20.9±1.01	16.5±2.01	54.5±2.01
60 kg N/ha Application							
5 DAE	3±0.01	25.3±0.01	9.5±1.01	0.5±0.01	-	-	-
10 DAE	5±0.01	34.2±0.01	24±2.01	0.6±0.01	-	-	-
15 DAE	6±0.01	45.1±0.01	35±2.01	1.6±0.01	-	-	-
20 DAE	7±0.01	46.6±0.01	53±3.01	1.8±0.01	-	-	-
25 DAE	8±0.01	46.9±0.01	57±2.01	2.3±0.01	-	-	-
30 DAE	9±0.01	57.3±0.01	66±4.01	2.6±0.01	17.5±3.01	14.5±2.01	49.4±2.01

DAE= Day after Emergence

4.14. Nutrient Bioavailability and Accessibility

Tables 4.13 (a-b) shows the results of nutrients bioavailability and accessibility to the maize plants and the accumulation/concentration of each nutrient/element in different organs (leaves, stems and roots) of the plants used in the phyto experiments with the application of NPK 15-15-15 inorganic fertilizer and the control experiments. From the negative control (No fertilizer application) plot, the highest value of nitrogen was 18.5 mg/L; that of phosphorus was 2.15 mg/L while that of potassium was 3.5 mg/L all recorded in the roots. All other nutrients/elements also had their highest accumulations in the root except for calcium (165 mg/L), magnesium (56 mg/L) and copper (5.5 mg/L) recorded in the stem. From the NPK 15-15-15 inorganic fertilizer plot, the highest level of nitrogen accumulation was 16.8 mg/L; highest level of phosphorus was 1.9 mg/L while that of potassium was 3.3 mg/L all found in the plant stem. All other nutrients and elements also had their highest accumulation in the stem except for calcium (120 mg/L) and magnesium (24 mg/L) which recorded their highest levels in the leaves and roots respectively.

Table 4.13a: Nutrient Bioavailability and Accessibility to Plant Organs with No Fertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16±1.02	17.1±2.02	18.5±2.01
Phosphorus (P) mg/L	1.29±0.01	1.9±0.03	2.15±0.02
Potassium (K) mg/L	3.0±0.01	3.2±0.02	3.5±0.01
Calcium (Ca) mg/L	100±5.01	165.0±5.04	105.0±4.01
Magnesium (Mg) mg/L	23±1.01	56.0±0.01	37±2.01
Copper (Cu) mg/L	1.35±0.11	5.55±0.21	1.8±0.01
Zinc (Zn) mg/L	8.0±0.03	10.5±2.01	14.0±1.02
Iron (Fe) mg/L	2.3±0.03	2.8±0.02	3.3±0.01
Aluminium (Al) mg/L	0.15±0.01	0.23±0.01	0.3±0.01
Nitrate (NO_3) mg/L	1.2±0.02	1.4±0.01	1.54±0.01
Ammonium (NH_4) mg/L	0.23±0.10	0.24±0.01	0.28±0.02
Phosphate (PO_4) mg/L	49.2±2.01	61.8±2.01	80.4±3.01
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	44.0±3.01	50.0±3.02	59.0±3.01

Table 4.13b: Nutrient Bioavailability and Accessibility to Plant Organs with NPK 15-15-15 Fertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.5±0.11	16.8±0.11	15.8±1.01
Phosphorus (P) mg/L	1.58±0.10	1.9±0.02	1.82±0.01
Potassium (K) mg/L	2.9±0.01	3.3±0.02	3.2±0.01
Calcium (Ca) mg/L	120±0.10	95±2.01	155±5.01
Magnesium (Mg) mg/L	17±0.21	23±1.01	24±2.01
Copper (Cu) mg/L	1.05±0.01	1.65±0.11	1.5±0.11
Zinc (Zn) mg/L	9.8±1.01	11.5±0.11	10±2.01
Iron (Fe) mg/L	1.9±0.12	2.6±0.02	2.55±0.11
Aluminium (Al) mg/L	0.11±0.10	0.18±0.01	0.19±0.01
Nitrate (NO_3) mg/L	1.22±0.02	1.4±0.11	1.26±0.11
Ammonium (NH_4) mg/L	0.19±0.01	0.25±0.01	0.22±0.01
Phosphate (PO_4) mg/L	68.8±0.01	76±3.01	72.2±4.01
Manganese (Mn) mg/L	0.006±0.00	0.007±0.01	0.007±0.01
Sulphate (SO_4) mg/L	39±0.12	50±3.01	46±2.01

Tables 4.14a (i-vi) shows the results of nutrients bioavailability and accessibility to the maize plants and the accumulation/concentration of each nutrient/element in different organs (leaves, stems and roots) of the plants used in the phyto experiments with the application of *Tithonia diversifolia* shoot biofertilizer. From the experiment, the highest accumulation for nitrogen (29 mg/L) and ammonium (0.57 mg/L) were recorded in the root and in the 20 kg N/ha experiment. Magnesium and manganese had their highest levels (42 mg/L and 0.023 mg/L) both in the stem and in the 20 kg N/ha experiment. The highest values for phosphorus (4.7 mg/L recorded in the 20 kg N/ha), potassium (5.3 mg/L recorded in the 20 kg N/ha), copper (3.10 mg/L recorded in the 10 kg N/ha), zinc (35 mg/L recorded in the 20 kg N/ha), iron (7.2 mg/L recorded in the 20 kg N/ha), aluminium (0.52 mg/L recorded in the 20 kg N/ha), nitrates (2.7 mg/L recorded in the 30 kg N/ha) and sulphates (102 mg/L recorded in the 20 kg N/ha) were all recorded in the leaves. Calcium and phosphate had their highest accumulation levels of 195 mg/L and 101.8 mg/L in both the leaves and roots and in the 50 kg N/ha and 30 kg N/ha experiments respectively. In all, majority of the plant nutrients and elements recorded their highest accumulation levels in the leaves and the experiment with the highest level of nutrient supply to the tested plants was the 20 kg N/ha *Tithonia diversifolia* biofertilizer.

Table 4.14a (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Tithonia diversifolia* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.0±4.01	14.0±3.01	15.5±2.01
Phosphorus (P) mg/L	2.35±0.02	2.41±0.03	2.3±0.01
Potassium (K) mg/L	3.3±1.01	3.2±0.02	3.2±0.01
Calcium (Ca) mg/L	70.0±2.01	55.0±3.01	70.0±3.01
Magnesium (Mg) mg/L	14.0±0.01	13.0±3.01	14.0±2.01
Copper (Cu) mg/L	1.10±0.01	1.2±0.01	2.65±0.01
Zinc (Zn) mg/L	13.0±3.02	12.0±2.01	20.0±3.00
Iron (Fe) mg/L	2.8±1.11	2.3±0.01	4.9±0.03
Aluminium (Al) mg/L	0.17±0.01	0.14±0.01	0.42±0.01
Nitrate (NO_3) mg/L	1.4±0.02	1.3±0.01	2.3±0.02
Ammonium (NH_4) mg/L	0.25±0.01	0.23±0.01	0.35±0.01
Phosphate (PO_4) mg/L	70.1±3.03	73.1±2.01	99.8±3.02
Manganese (Mn) mg/L	0.006±0.01	0.007±0.01	0.018±0.01
Sulphate (SO_4) mg/L	39.0±3.02	40.0±4.01	48.0±3.01

Table 4.14a (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Tithonia diversifolia* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	22.0±2.01	22.0±2.05	29.0±3.01
Phosphorus (P) mg/L	4.7±1.01	2.72±1.01	3.87±0.03
Potassium (K) mg/L	5.3±1.02	3.7±0.01	4.1±0.01
Calcium (Ca) mg/L	55.0±3.01	76±3.05	65.0±4.01
Magnesium (Mg) mg/L	40.0±1.01	42.0±4.02	22.0±4.02
Copper (Cu) mg/L	2.5±0.01	2.05±0.01	2.8±0.01
Zinc (Zn) mg/L	35.0±3.02	15.5±3.03	28.0±3.01
Iron (Fe) mg/L	7.2±0.02	3.9±0.01	5.3±1.01
Aluminium (Al) mg/L	0.52±0.01	0.35±0.03	0.32±0.01
Nitrate (NO_3) mg/L	2.5±0.01	1.96±0.01	2.3±0.01
Ammonium (NH_4) mg/L	0.37±0.01	0.35±0.01	0.57±0.01
Phosphate (PO_4) mg/L	81.5±0.04	100.0±6.01	80.0±4.40
Manganese (Mn) mg/L	0.012±0.01	0.012±0.01	0.018±0.01
Sulphate (SO_4) mg/L	102.0±3.04	62.0±3.20	84.0±5.03

Table 4.14a (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Tithonia diversifolia* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	23.5±4.03	15.6±3.01	14.8±2.01
Phosphorus (P) mg/L	3.51±0.21	2.08±0.01	2.06±0.01
Potassium (K) mg/L	4.2±1.01	3.2±0.11	3.5±0.01
Calcium (Ca) mg/L	60.0±0.03	120.0±7.01	160.0±10.01
Magnesium (Mg) mg/L	28.0±4.01	30.0±5.03	28.0±3.01
Copper (Cu) mg/L	2.7±0.02	1.8±0.02	1.5±0.01
Zinc (Zn) mg/L	21.5±0.01	12.5±3.01	11.5±3.02
Iron (Fe) mg/L	4.6±0.03	2.45±1.01	2.75±0.01
Aluminium (Al) mg/L	0.20±0.01	0.24±0.01	0.20±0.01
Nitrate (NO_3) mg/L	2.7±0.01	1.4±0.01	1.44±0.03
Ammonium mg/L	0.22±0.01	0.28±0.03	0.23±0.01
Phosphate mg/L	101.8±6.01	52.8±4.11	101.8±6.01
Manganese mg/L	0.018±0.01	0.008±0.01	0.010±0.01
Sulphate mg/L	51.0±4.11	58.0±4.02	52.0±4.04

Table 4.14a (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Tithonia diversifolia* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.0±3.05	19.0±4.02	15.8±3.01
Phosphorus (P) mg/L	1.93±0.03	2.21±0.01	2.2±0.02
Potassium (K) mg/L	3.4±1.01	3.5±0.01	3.3±0.01
Calcium (Ca) mg/L	170.0±9.40	115.0±6.03	125.0±4.01
Magnesium (Mg) mg/L	18.0±3.01	33.0±3.01	28.0±3.01
Copper (Cu) mg/L	1.45±0.01	1.95±0.03	1.7±1.01
Zinc (Zn) mg/L	18.5±3.02	12.5±0.01	10.5±3.01
Iron (Fe) mg/L	2.45±0.01	3.3±0.02	2.85±1.01
Aluminium (Al) mg/L	0.14±0.01	0.28±0.01	0.23±0.01
Nitrate (NO_3) mg/L	1.86±0.01	1.44±0.03	1.32±0.01
Ammonium mg/L	0.26±0.01	0.3±0.02	0.23±0.01
Phosphate mg/L	70.8±4.01	62.8±4.00	99.8±8.01
Manganese mg/L	0.012±0.01	0.010±0.01	0.008±0.01
Sulphate mg/L	49.0 ±5.02	58.0±5.00	52.0±6.01

Table 4.14a (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Tithonia diversifolia* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.1±3.03	14.4±3.01	17.4±2.02
Phosphorus (P) mg/L	1.23±0.01	1.73±1.01	2.12±1.01
Potassium (K) mg/L	3.1±1.01	3.2±0.01	3.6±0.01
Calcium (Ca) mg/L	195.0±8.51	135.0±7.02	195.0±10.01
Magnesium (Mg) mg/L	25.0±2.01	20.0±3.01	30.0±2.01
Copper (Cu) mg/L	1.4±0.02	1.8±1.01	1.7±0.01
Zinc (Zn) mg/L	10.5±2.01	10.5±2.01	13.0±3.01
Iron (Fe) mg/L	2.5±0.01	2.4±1.01	2.7±0.01
Aluminium (Al) mg/L	0.13±0.01	0.17±0.01	0.23±0.02
Nitrate (NO_3) mg/L	1.3±1.01	1.14±0.01	1.28±0.04
Ammonium mg/L	0.22±0.01	0.29±0.01	0.22±0.01
Phosphate mg/L	58.1±4.01	77.1±5.01	57.7±3.03
Manganese mg/L	0.007±0.01	0.008±0.01	0.008±0.01
Sulphate mg/L	35.0±3.01	52.0±4.01	53.0±4.01

Table 4.14a (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Tithonia diversifolia* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	14.6±2.01	18.5±3.11	16.5±4.01
Phosphorus (P) mg/L	1.24±1.01	2.56±1.01	2.15±2.01
Potassium (K) mg/L	3.0±1.01	3.6±1.01	3.5±0.01
Calcium (Ca) mg/L	180.0±10.01	85.0±4.02	76.0±3.06
Magnesium (Mg) mg/L	17.0±3.01	34.0±2.01	40.0±4.01
Copper (Cu) mg/L	1.05±0.02	2.0±1.01	1.8±0.05
Zinc (Zn) mg/L	8.2±2.01	15.5±4.01	13.5±4.01
Iron (Fe) mg/L	2.5±0.03	3.3±1.01	3.5±0.03
Aluminium (Al) mg/L	0.11±0.01	0.26±0.03	0.28±0.01
Nitrate (NO_3) mg/L	0.98±0.04	1.48±1.01	1.46±0.04
Ammonium mg/L	0.18±0.01	0.31±0.01	0.36±0.01
Phosphate mg/L	79.3±3.05	68.4±4.01	61.6±4.03
Manganese mg/L	0.007±0.01	0.010±0.01	0.010±0.01
Sulphate mg/L	44.0±4.01	57.0±4.01	59.0±5.01

Tables 4.14b (i-vi) shows the results of the nutrient bioavailability and accessibility of nutrient to maize plant with the application of *Chromolaena odorata* biofertilizer. From the experiment, the highest accumulation of nitrogen, (19.8 mg/L recorded in the 60 kg N/ha), calcium (210 mg/L recorded in the 60 kg N/ha) and phosphate (90 mg/L recorded in the 30 kg N/ha) were recorded in the leaves. The highest values of phosphorus (2.53 mg/L recorded in the 60 kg N/ha) potassium (3.8 mg/L recorded in the 60 kg N/ha), zinc (15 mg/L recorded in the 50 kg N/ha), aluminium (0.32 mg/L recorded in the 60 kg N/ha) and nitrates (1.72 mg/L recorded in the 50 kg N/ha) were all found in the stem of the plants. In the same vein, the highest accumulations of magnesium (35 mg/L recorded in the 10 kg N/ha), copper (1.9 mg/L recorded in the 60 kg N/ha), iron (3.5 mg/L recorded in the 20 kg N/ha), ammonium (0.40 mg/L recorded in the 60 kg N/ha), manganese (0.011 mg/L recorded in the 20 kg N/ha) and sulphate (59.5 mg/L recorded in the 20 kg N/ha) were all recorded in the roots of the tested plants. In all, the plant organ that showed the highest accumulation ability in this plot was the roots and the experimental set up that showed the highest level of nutrient availability to the tested plants was the 60 kg N/ha.

Table 4.14b (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Chromolaena odorata* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.9±3.03	16.4±4.01	17.4±3.01
Phosphorus (P) mg/L	1.33±0.01	1.81±2.01	2.48±0.01
Potassium (K) mg/L	3.0±1.01	3.2±0.01	3.4±1.01
Calcium (Ca) mg/L	125.0±8.05	125.0±10.01	90.0±6.01
Magnesium (Mg) mg/L	19.0±3.02	27.0±3.01	35.0±3.01
Copper (Cu) mg/L	1.2±0.01	1.55±0.01	1.75±1.01
Zinc (Zn) mg/L	8.0±2.01	11.0±3.01	12.5±3.02
Iron (Fe) mg/L	2.25±0.03	2.7±1.01	3.4±1.01
Aluminium (Al) mg/L	0.15±0.01	0.21±0.01	0.25±0.01
Nitrate (NO_3) mg/L	1.18±1.01	1.32±0.03	1.44±0.01
Ammonium (NH_4) mg/L	0.15±0.01	0.22±0.01	0.27±0.02
Phosphate (PO_4) mg/L	66.8±4.02	62.5±5.01	83.7±3.01
Manganese (Mn) mg/L	0.005±0.01	0.008±0.01	0.010±0.01
Sulphate (SO_4) mg/L	41.0±3.02	51.0±3.01	57.0±0.02

Table 4.14b (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Chromolaena odorata* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.6±1.01	18.4±0.01	18.8±3.00
Phosphorus (P) mg/L	1.34±0.03	1.86±0.01	2.43±1.03
Potassium (K) mg/L	3.2±0.01	3.4±0.11	3.6±0.03
Calcium (Ca) mg/L	123.0±2.02	129.0±3.02	99.2±4.01
Magnesium (Mg) mg/L	21±0.02	26.0±0.05	33.0±1.01
Copper (Cu) mg/L	1.2±0.02	1.58±0.02	1.74±0.03
Zinc (Zn) mg/L	8.4±0.04	13.0±1.01	12.9±0.01
Iron (Fe) mg/L	2.35±0.01	2.9±0.20	3.5±0.02
Aluminium (Al) mg/L	0.17±0.05	0.19±0.01	0.25±0.01
Nitrate (NO_3) mg/L	1.19±0.01	1.39±1.01	1.46±0.02
Ammonium (NH_4) mg/L	0.18±0.01	0.23±0.03	0.25±0.03
Phosphate (PO_4) mg/L	68.1±1.05	64.5±1.00	85.2±1.02
Manganese (Mn) mg/L	0.004±0.01	0.009±0.01	0.011±0.01
Sulphate (SO_4) mg/L	42.5±1.01	53.0±2.00	59.5±0.05

Table 4.14b (iii): Results of Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Chromolaena odorata* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.6±4.01	16.3±3.21	17.6±4.02
Phosphorus (P) mg/L	1.61±1.01	1.78±1.01	2.36±1.01
Potassium (K) mg/L	3.1±1.01	3.3±0.21	3.4±2.01
Calcium (Ca) mg/L	160.0±8.02	160.0±10.21	64.0±3.01
Magnesium (Mg) mg/L	23.0±1.01	26.0±3.01	31.0±4.01
Copper (Cu) mg/L	1.4±1.01	1.5±0.11	1.75±0.02
Zinc (Zn) mg/L	9.4±2.01	11.5±3.01	13.0±2.01
Iron (Fe) mg/L	2.6±0.01	2.8±2.01	3.2±0.01
Aluminium (Al) mg/L	0.16±0.01	0.22±0.01	0.25±0.02
Nitrate (NO_3) mg/L	1.26±0.03	1.32±1.01	1.46±1.01
Ammonium (NH_4) mg/L	0.19±0.02	0.20±0.01	0.28±0.01
Phosphate (PO_4) mg/L	90.3±7.01	81.4±4.00	88.1±6.04
Manganese (Mn) mg/L	0.007±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	45.0±3.02	51.0±2.01	56.0±5.02

Table 4.14b (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Chromolaena odorata* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.3±4.01	17.4±4.01	18.7±3.01
Phosphorus (P) mg/L	1.82±1.01	2.48±1.01	2.16±0.01
Potassium (K) mg/L	3.1±0.03	3.5±1.02	3.5±1.01
Calcium (Ca) mg/L	200.0±11.01	80.0±6.01	58.0±4.02
Magnesium (Mg) mg/L	27.0±2.01	32.0±4.01	28.0±3.01
Copper (Cu) mg/L	1.45±1.01	1.65±0.03	1.7±0.01
Zinc (Zn) mg/L	10.5±2.02	13.0±0.02	11.5±2.01
Iron (Fe) mg/L	2.65±1.03	2.9±1.01	3.0±1.02
Aluminium (Al) mg/L	0.18±0.01	0.29±0.01	0.26±0.02
Nitrate (NO_3) mg/L	1.34±1.01	1.64±1.01	1.48±0.02
Ammonium (NH_4) mg/L	0.21±0.02	0.28±0.01	0.32±0.01
Phosphate (PO_4) mg/L	56.8±2.01	67.1±6.01	64.0±2.01
Manganese (Mn) mg/L	0.007±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	49.0±3.01	53.0±4.02	54.0±3.01

Table 4.14b (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Chromolaena odorata* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.2±1.02	18.7±2.01	18.7±3.01
Phosphorus (P) mg/L	1.90±0.02	2.52±2.01	2.16±2.01
Potassium (K) mg/L	3.2±0.01	3.6±0.01	3.5±1.00
Calcium (Ca) mg/L	205.0±5.51	82.0±2.01	58.0±3.01
Magnesium (Mg) mg/L	28.0±1.01	33.0±1.01	28.0±4.01
Copper (Cu) mg/L	1.55±0.21	1.68±1.01	1.7±2.01
Zinc (Zn) mg/L	12.9±1.01	15.0±1.01	11.5±3.01
Iron (Fe) mg/L	2.68±1.01	2.6±0.01	3.0±2.01
Aluminium (Al) mg/L	0.21±0.02	0.28±0.00	0.26±0.02
Nitrate (NO_3) mg/L	1.44±1.15	1.72±0.01	1.48±1.01
Ammonium (NH_4) mg/L	0.20±0.01	0.29±0.01	0.32±0.03
Phosphate (PO_4) mg/L	57.1±1.11	69.1±2.01	64.0±4.01
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	52.0±2.00	54.6±2.01	53.1±3.01

Table 4.14b (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Chromolaena odorata* Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.8±0.21	18.1±2.01	18.5±2.02
Phosphorus (P) mg/L	1.86±1.01	2.53±0.02	2.19±1.02
Potassium (K) mg/L	3.6±1.01	3.8±2.01	3.7±1.01
Calcium (Ca) mg/L	210.0±4.50	84.0±2.51	59.0±1.01
Magnesium (Mg) mg/L	29.5±1.01	33.0±2.00	31.0±4.01
Copper (Cu) mg/L	1.55±2.01	1.67±1.02	1.9±0.01
Zinc (Zn) mg/L	12.1±2.01	14.0±1.01	12.4±0.04
Iron (Fe) mg/L	2.85±1.01	3.1±2.01	3.4±0.01
Aluminium (Al) mg/L	0.21±0.01	0.32±0.01	0.28±1.01
Nitrate (NO_3) mg/L	1.45±0.02	1.66±1.01	1.52±0.03
Ammonium (NH_4) mg/L	0.25±0.03	0.32±0.01	0.40±0.02
Phosphate (PO_4) mg/L	58.4±2.00	68.5±2.01	66.0±2.01
Manganese (Mn) mg/L	0.010±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	53.0±2.01	54.0±2.01	56.0±1.01

Tables 4.14c (i-vi) shows the results of the nutrient bioavailability and accessibility to maize plants with the application of *Carica papaya* biofertilizer. From the experiment, the highest accumulation of nitrogen, (19.7 mg/L recorded in the 50 kg N/ha experiment), ammonium (0.29 mg/L recorded in the 60 kg N/ha experiment) and and phosphate (82.8 mg/L recorded in the 30 kg N/ha experiment) were recorded in the leaves. The highest values of phosphorus (2.36 mg/L recorded in the 30 kg N/ha experiment), potassium (3.6 mg/L recorded in the 60 kg N/ha experiment), calcium (185 mg/L recorded in the 40 kg N/ha experiment), magnesium (36 mg/L recorded in the 60 kg N/ha experiment), copper (1.8 mg/L recorded in the 60 kg N/ha experiment), zinc (13 mg/L recorded in the 60 kg N/ha experiment) and aluminium (0.27 mg/L recorded in the 60 kg N/ha experiment) were all found in the stem of the plants. However, the highest accumulations of iron (3.3 mg/L recorded in the 30 kg N/ha experiment), and nitrates (1.54 mg/L recorded in the 50 kg N/ha experiment), manganese (0.010 mg/L recorded in the 40 kg N/ha experiment) and sulphate (59 mg/L recorded in the 40 kg N/ha experiment) were all recorded in the roots of the tested plants. In all, the plant organ that showed the highest accumulation ability in this plot was the stem and the experimental set up that showed the highest level of nutrient availability to the tested plants was the 60 kg N/ha experiment.

Table 4.14c (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Carica papayas* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.0±5.02	16.1±4.02	16.2±3.05
Phosphorus (P) mg/L	1.76±1.01	1.51±0.01	2.05±1.01
Potassium (K) mg/L	3.1±2.01	3.1±0.01	3.4±2.01
Calcium (Ca) mg/L	155.0±7.01	180.0±6.01	90.0±6.21
Magnesium (Mg) mg/L	24.0±4.00	25.0±4.01	35.0±3.02
Copper (Cu) mg/L	1.3±1.01	1.4±1.01	1.65±1.01
Zinc (Zn) mg/L	10.5±3.01	9.0±3.01	12.5±3.03
Iron (Fe) mg/L	3.0±2.01	2.4±0.03	3.10±2.01
Aluminium (Al) mg/L	0.17±0.03	0.19±0.01	0.24±0.02
Nitrate (NO_3) mg/L	1.38±1.03	1.42±0.02	1.52±1.01
Ammonium (NH_4) mg/L	0.2±0.02	0.20±0.01	0.25±5.02
Phosphate (PO_4) mg/L	64.5±5.01	66.7±6.01	56.8±0.01
Manganese (Mn) mg/L	0.007±0.01	0.007±0.01	0.009±0.01
Sulphate (SO_4) mg/L	44.0±4.02	47.0±4.01	56.0±4.01

Table 4.14c (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Carica papayas* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.0±3.03	17.1±2.02	17.4±3.01
Phosphorus (P) mg/L	1.77±0.12	1.52±0.11	2.05±1.00
Potassium (K) mg/L	3.2±1.01	3.3±1.01	3.5±0.01
Calcium (Ca) mg/L	157.0±3.01	181.0±9.03	96.0±8.03
Magnesium (Mg) mg/L	23.0±2.01	25.2±3.02	35.5±3.01
Copper (Cu) mg/L	1.3±1.00	1.4±1.01	1.66±1.01
Zinc (Zn) mg/L	11.5±0.01	9.0±0.01	12.6±2.05
Iron (Fe) mg/L	3.0±0.02	2.4±2.01	3.11±1.01
Aluminium (Al) mg/L	0.17±0.01	0.20±0.01	0.24±0.01
Nitrate (NO_3) mg/L	1.39±1.01	1.43±0.03	1.52±1.05
Ammonium (NH_4) mg/L	0.2±0.22	0.20±0.01	0.24±5.01
Phosphate (PO_4) mg/L	65.5±5.01	66.6±3.02	55.8±0.00
Manganese (Mn) mg/L	0.008±0.01	0.007±0.01	0.009±0.01
Sulphate (SO_4) mg/L	44.0±4.01	48.0±1.02	57.0±5.11

Table 4.14c (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Carica papayas* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.9±3.01	19.0±5.01	17.5±3.04
Phosphorus (P) mg/L	1.62±0.02	2.36±0.01	1.89±1.01
Potassium (K) mg/L	3.0±1.01	3.5±1.01	3.4±0.02
Calcium (Ca) mg/L	66.0±6.01	135.0±4.04	90.0±8.03
Magnesium (Mg) mg/L	21.0±3.01	26.0±2.01	28.0±4.01
Copper (Cu) mg/L	1.3±1.01	1.7±1.02	1.7±1.01
Zinc (Zn) mg/L	9.6±2.01	12.5±3.03	11.5±3.02
Iron (Fe) mg/L	2.2±1.02	3.2±1.01	3.3±1.01
Aluminium (Al) mg/L	0.14±0.02	0.23±0.01	0.22±0.01
Nitrate (NO_3) mg/L	1.24±1.01	0.44±0.02	1.52±0.03
Ammonium (NH_4) mg/L	0.19±0.01	0.28±0.01	0.22±0.02
Phosphate (PO_4) mg/L	82.8±5.03	55.4±3.01	59.4±4.01
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.008±0.01
Sulphate (SO_4) mg/L	41.0±2.02	57.0±4.01	53.0±2.01

Table 4.14c (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Carica papayas* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.0±4.01	17.1±4.01	17.4±3.02
Phosphorus (P) mg/L	1.79±1.01	1.53±0.03	2.15±1.01
Potassium (K) mg/L	3.5±1.01	3.3±2.00	3.5±1.01
Calcium (Ca) mg/L	161.2±2.11	185.0±1.20	97.0±2.02
Magnesium (Mg) mg/L	24.0±3.01	24.0±3.01	33.0±2.02
Copper (Cu) mg/L	1.3±1.02	1.4±0.03	1.75±1.01
Zinc (Zn) mg/L	10.2±1.01	11.0±0.01	12.1±1.01
Iron (Fe) mg/L	3.0±1.01	2.6±1.01	3.11±1.01
Aluminium (Al) mg/L	0.17±0.01	0.19±0.01	0.24±0.02
Nitrate (NO_3) mg/L	1.39±1.01	1.43±0.01	1.51±0.02
Ammonium (NH_4) mg/L	0.5±0.02	0.18±0.01	0.27±5.01
Phosphate (PO_4) mg/L	64.5±2.01	68.7±2.01	56.2±0.00
Manganese (Mn) mg/L	0.007±0.01	0.007±0.01	0.010±0.01
Sulphate (SO_4) mg/L	47.0±1.01	49.0±1.04	59.0±3.01

Table 4.14c (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Carica papayas* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.7±2.01	17.6±4.01	18.0±1.01
Phosphorus (P) mg/L	1.77±1.01	1.51±1.01	2.15±1.01
Potassium (K) mg/L	3.3±1.02	3.1±0.02	3.4±2.01
Calcium (Ca) mg/L	165.0±4.00	182.0±5.02	99.2±3.02
Magnesium (Mg) mg/L	25.0±3.01	23.0±3.02	34.0±1.01
Copper (Cu) mg/L	1.3±1.01	1.4±1.01	1.65±0.02
Zinc (Zn) mg/L	12.5±3.01	11.0±1.01	12.8±2.01
Iron (Fe) mg/L	3.0±1.01	2.4±2.01	3.10±2.01
Aluminium (Al) mg/L	0.17±0.01	0.19±0.02	0.23±0.01
Nitrate (NO_3) mg/L	1.38±1.01	1.42±1.01	1.54±1.01
Ammonium (NH_4) mg/L	0.2±0.02	0.20±0.01	0.25±4.01
Phosphate (PO_4) mg/L	67.1±3.01	69.2±4.01	56.8±0.02
Manganese (Mn) mg/L	0.007±0.01	0.007±0.01	0.009±0.01
Sulphate (SO_4) mg/L	48.0±1.01	50.0±0.02	53.0±4.01

Table 4.14c (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Carica papayas* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.8±3.03	19.0±4.01	17.1±3.03
Phosphorus (P) mg/L	1.01±1.01	2.19±2.01	2.01±1.01
Potassium (K) mg/L	2.9±2.01	3.6±0.03	3.4±2.01
Calcium (Ca) mg/L	64.0±4.01	72.0±3.01	145.0±5.01
Magnesium (Mg) mg/L	16.0±2.01	36.0±3.01	30.0±3.01
Copper (Cu) mg/L	1.2±1.02	1.8±2.01	1.65±1.01
Zinc (Zn) mg/L	6.6±3.01	13.0±0.03	11.0±3.01
Iron (Fe) mg/L	1.9±1.01	3.0±1.01	3.0±2.02
Aluminium (Al) mg/L	0.11±0.03	0.27±0.03	0.21±1.01
Nitrate (NO_3) mg/L	1.12±0.01	1.46±0.02	1.4±1.02
Ammonium (NH_4) mg/L	0.29±0.01	0.27±0.01	0.25±0.03
Phosphate (PO_4) mg/L	60.4±8.06	72.3±4.01	38.6±3.02
Manganese (Mn) mg/L	0.004±0.01	0.001±0.01	0.009±0.01
Sulphate (SO_4) mg/L	38.0±2.01	53.0±3.03	50.0±4.01

Tables 4.14d (i-vi) show the results of the nutrient bioavailability and accessibility to maize plant with the application of *Telfairia occidentalis* biofertilizer. From the experiment, almost all the nutrients and mineral elements evaluated recorded their highest accumulation levels in the roots except calcium with the highest value of 204 mg/L in the 50 kg N/ha experiment in the leaves. The highest levels of nitrogen (19.6 mg/L recorded in the 30 kg N/ha experiment), phosphorus (2.41 mg/L recorded in the 10 kg N/ha experiment), potassium (3.6 mg/L recorded in the 30 kg N/ha experiment), magnesium (34 mg/L recorded in the 30 kg N/ha experiment), copper (1.98 mg/L recorded in the 40 kg N/ha experiment), zinc (14.4 mg/L recorded in the 20 kg N/ha experiment), iron (3.7 mg/L recorded in the 40 kg N/ha experiment), aluminium (0.34 mg/L recorded in the 10 kg n/ha experiment), nitrates (1.56 mg/L recorded in the 10 kg N/ha experiment), ammonium (0.32 mg/L recorded in the 10 kg N/ha experiment), phosphate (89.4 mg/L recorded in the 10 kg N/ha experiment), manganese (0.011 mg/L recorded in the 40 kg N/ha experiment) and sulphate (66 mg/L recorded in the 10 kg N/ha experiment) were all recorded in the roots of the tested plants. In all, the plant organ showing the highest levels of nutrient accumulation was the root and the experiment with the highest nutrient availability to plants was the 10 kg N/ha experimental set up.

Table 4.14d (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Telfairia occidentalis* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.3±3.03	17.1±3.01	15.4±2.01
Phosphorus (P) mg/L	1.56±0.02	1.96±1.01	2.41±1.01
Potassium (K) mg/L	3.0±0.01	3.4±1.01	3.5±1.01
Calcium (Ca) mg/L	150.0±8.01	110.0±7.02	80.0±6.01
Magnesium (Mg) mg/L	26.0±4.01	31.0±3.01	33.0±3.01
Copper (Cu) mg/L	1.3±1.01	1.7±1.01	1.95±1.01
Zinc (Zn) mg/L	10.0±2.02	12.5±3.03	14.5±2.02
Iron (Fe) mg/L	2.35±2.02	3.0±2.01	3.30±1.01
Aluminium (Al) mg/L	0.17±0.01	0.24±0.01	0.34±0.02
Nitrate (NO_3) mg/L	1.32±0.02	1.46±1.01	1.56±1.01
Ammonium (NH_4) mg/L	0.17±0.03	0.25±0.01	0.32±0.01
Phosphate (PO_4) mg/L	63.6±3.03	61.2±4.00	89.4±6.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.010±0.02
Sulphate (SO_4) mg/L	46.0±4.02	54.0±4.01	66.0±6.02

Table 4.14d (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Telfairia occidentalis* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.7±6.21	18.5±4.01	15.9±3.01
Phosphorus (P) mg/L	1.54±0.01	2.13±1.01	2.31±2.01
Potassium (K) mg/L	3.1±2.01	3.4±2.01	3.3±0.01
Calcium (Ca) mg/L	190.0±11.01	105.0±8.02	145.0±5.02
Magnesium (Mg) mg/L	24.0±3.03	30.0±3.01	29.0±2.01
Copper (Cu) mg/L	1.15±1.01	1.75±1.01	1.6±1.01
Zinc (Zn) mg/L	14.0±2.01	11.0±3.01	10.5±2.01
Iron (Fe) mg/L	2.8±2.01	3.2±2.01	3.1±1.01
Aluminium (Al) mg/L	0.17±0.11	0.23±0.01	0.26±0.01
Nitrate (NO_3) mg/L	1.2±0.02	1.3±1.01	1.36±1.01
Ammonium (NH_4) mg/L	0.2±0.01	0.22±0.01	0.31±2.01
Phosphate (PO_4) mg/L	57.9±4.01	60.3±5.02	64.0±3.11
Manganese (Mn) mg/L	0.009±0.01	0.008±0.01	0.008±0.01
Sulphate (SO_4) mg/L	47.0±3.03	52.0±4.03	54.0±4.01

Table 4.14d (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Telfairia occidentalis* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.4±4.01	14.5±3.03	19.6±0.03
Phosphorus (P) mg/L	1.64±1.01	2.15±2.01	1.89±2.01
Potassium (K) mg/L	3.2±1.01	3.4±1.01	3.6±0.03
Calcium (Ca) mg/L	190.0±7.01	180.0±4.02	82.0±5.01
Magnesium (Mg) mg/L	22.0±3.01	32.0±3.01	34.0±3.01
Copper (Cu) mg/L	1.45±1.01	1.95±1.01	1.85±0.04
Zinc (Zn) mg/L	8.8±2.02	12.0±2.01	12.0±1.01
Iron (Fe) mg/L	2.15±1.01	3.0±3.01	3.4±2.01
Aluminium (Al) mg/L	0.28±0.02	0.23±1.01	0.28±0.01
Nitrate (NO_3) mg/L	1.16±1.01	1.42±0.04	1.50±1.01
Ammonium (NH_4) mg/L	0.15±0.02	0.31±0.02	0.3±0.01
Phosphate (PO_4) mg/L	54.0±4.01	60.3±5.01	85.3±5.01
Manganese (Mn) mg/L	0.006±0.01	0.010±0.01	0.011±0.01
Sulphate (SO_4) mg/L	42.0±3.01	54.0±5.01	60.0±4.02

Table 4.14d (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Telfairia occidentalis* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.7±4.01	16.0±2.01	17.7±3.01
Phosphorus (P) mg/L	1.47±1.01	1.57±1.01	2.19±1.01
Potassium (K) mg/L	3.1±0.03	3.3±1.01	3.5±1.03
Calcium (Ca) mg/L	200.0±10.01	155.0±8.01	110.0±6.03
Magnesium (Mg) mg/L	22.0±3.01	27.0±3.01	31.0±3.01
Copper (Cu) mg/L	1.35±0.03	1.75±0.01	1.98±6.00
Zinc (Zn) mg/L	11.0±2.01	12.5±3.01	12.5±3.01
Iron (Fe) mg/L	2.7±2.01	3.2±3.01	3.7±1.01
Aluminium (Al) mg/L	0.18±0.02	0.28±0.01	0.25±0.01
Nitrate (NO_3) mg/L	1.28±1.01	1.46±1.01	1.28±0.04
Ammonium (NH_4) mg/L	0.17±0.01	0.24±0.02	0.28±0.02
Phosphate (PO_4) mg/L	52.7±4.01	67.5±6.01	69.8±2.01
Manganese (Mn) mg/L	0.007±0.01	0.008±0.01	0.011±0.01
Sulphate (SO_4) mg/L	46.0±3.01	54.0±3.01	55.0±3.02

Table 4.14d (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Telfairia occidentalis* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.2±3.02	17.7±2.01	16.2±3.30
Phosphorus (P) mg/L	1.92±1.01	1.59±2.01	1.97±1.01
Potassium (K) mg/L	3.0±1.01	3.2±1.01	3.4±1.01
Calcium (Ca) mg/L	204.0±0.01	105.0±4.01	135.0±5.01
Magnesium (Mg) mg/L	22.0±2.01	24.0±3.01	29.0±3.01
Copper (Cu) mg/L	1.4±1.01	1.5±1.01	1.7±1.01
Zinc (Zn) mg/L	8.0±1.01	11.5±3.01	13.0±2.01
Iron (Fe) mg/L	2.3±0.02	3.10±1.01	2.9±1.02
Aluminium (Al) mg/L	0.14±0.01	0.22±0.02	0.23±0.21
Nitrate (NO_3) mg/L	1.06±0.02	1.32±1.01	1.42±1.01
Ammonium (NH_4) mg/L	0.16±0.01	0.31±0.01	0.25±0.05
Phosphate (PO_4) mg/L	59.5±4.02	58.5±4.01	65.9±5.05
Manganese (Mn) mg/L	0.006±0.01	0.007±0.01	0.009±0.01
Sulphate (SO_4) mg/L	51.0±3.01	61.0±5.01	54.0±4.02

Table 4.14d (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Telfairia occidentalis* Fruit Peels Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	14.4±3.01	19.0±4.11	16.1±4.01
Phosphorus (P) mg/L	1.44±1.01	1.96±2.01	1.96±1.01
Potassium (K) mg/L	3.0±3.01	3.4±2.01	3.3±2.01
Calcium (Ca) mg/L	160.0±10.01	100.0±1.01	115.0±9.01
Magnesium (Mg) mg/L	23.0±2.01	28.0±5.01	28.0±5.01
Copper (Cu) mg/L	1.2±1.01	1.65±1.01	1.65±1.03
Zinc (Zn) mg/L	8.4±3.01	12.0±3.01	11.5±4.03
Iron (Fe) mg/L	2.25±2.02	3.2±2.01	2.85±1.01
Aluminium (Al) mg/L	0.13±1.01	0.24±0.02	0.21±0.02
Nitrate (NO_3) mg/L	1.18±1.01	1.48±1.01	1.36±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.31±0.01	0.22±0.01
Phosphate (PO_4) mg/L	39.8±5.02	68.4±4.11	61.0±4.01
Manganese (Mn) mg/L	0.005±0.01	0.008±0.01	0.008±0.01
Sulphate (SO_4) mg/L	40.0±4.02	52.0±2.05	52.0±4.01

Tables 4.14e (i-vi) show the results of the nutrient bioavailability and accessibility to maize plants with the application of *Arachis hypogaea* hull biofertilizer plot. From the experiment, the highest levels of nitrogen (19 mg/L recorded in the 30 kg N/ha experiment), phosphorus (2.41 mg/L recorded in the 10 kg N/ha experiment), potassium (3.6 mg/L recorded in the 30 kg N/ha experiment), magnesium (34 mg/L recorded in the 10 kg N/ha experiment), copper (1.98 mg/L recorded in the 40 kg N/ha experiment), zinc (14.5 mg/L recorded in the 10 kg N/ha experiment), iron (3.7 mg/L recorded in the 40 kg N/ha experiment), aluminium (0.35 mg/L recorded in the 10 kg n/ha experiment), nitrates (1.58 mg/L recorded in the 10 kg N/ha experiment), ammonium (0.34 mg/L recorded in the 60 kg N/ha experiment), phosphate (85.3 mg/L recorded in the 30 kg N/ha experiment), manganese (0.011 mg/L recorded in the 30 kg N/ha experiment) and sulphate (62 mg/L recorded in the 60 kg N/ha experiment) were all recorded in the roots of the tested plants. Only calcium recorded its highest value of 209 mg/l in the leaves and in the 40 kg N/ha experiment. In all, the plant organ showing the highest levels of nutrient accumulation was the root and the experiment with the highest nutrient availability to plants was the 10 kg N/ha experimental set up.

Table 4.14e (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Arachis hypogaea* Hull Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.3±3.03	17.1±3.01	15.4±2.01
Phosphorus (P) mg/L	1.56±0.02	1.96±1.01	2.41±1.01
Potassium (K) mg/L	3.0±0.01	3.4±1.01	3.5±1.01
Calcium (Ca) mg/L	150.0±8.01	110.0±7.02	80.0±6.01
Magnesium (Mg) mg/L	26.0±4.01	31.0±3.01	33.0±3.01
Copper (Cu) mg/L	1.3±1.01	1.7±1.01	1.95±1.01
Zinc (Zn) mg/L	10.0±2.02	12.5±3.03	14.5±2.02
Iron (Fe) mg/L	2.35±2.02	3.0±2.01	3.30±1.01
Aluminium (Al) mg/L	0.17±0.01	0.24±0.01	0.35±0.02
Nitrate (NO_3) mg/L	1.32±0.02	1.46±1.01	1.58±1.01
Ammonium (NH_4) mg/L	0.17±0.03	0.25±0.01	0.32±0.01
Phosphate (PO_4) mg/L	63.6±3.03	61.2±4.00	77.7±6.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.010±0.02
Sulphate (SO_4) mg/L	46.0±4.02	54.0±4.01	61.0±6.02

Table 4.14e (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Arachis hypogaea* Hull Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.7±6.21	18.5±4.01	15.9±3.01
Phosphorus (P) mg/L	1.54±0.01	2.13±1.01	2.31±2.01
Potassium (K) mg/L	3.1±2.01	3.4±2.01	3.3±0.01
Calcium (Ca) mg/L	190.0±11.01	105.0±8.02	145.0±5.02
Magnesium (Mg) mg/L	24.0±3.03	30.0±3.01	29.0±2.01
Copper (Cu) mg/L	1.15±1.01	1.75±1.01	1.6±1.01
Zinc (Zn) mg/L	14.0±2.01	11.0±3.01	10.5±2.01
Iron (Fe) mg/L	2.8±2.01	3.2±2.01	3.1±1.01
Aluminium (Al) mg/L	0.17±0.11	0.23±0.01	0.26±0.01
Nitrate (NO_3) mg/L	1.2±0.02	1.3±1.01	1.36±1.01
Ammonium (NH_4) mg/L	0.2±0.01	0.22±0.01	0.34±2.01
Phosphate (PO_4) mg/L	57.9±4.01	60.3±5.02	64.0±3.11
Manganese (Mn) mg/L	0.009±0.01	0.008±0.01	0.008±0.01
Sulphate (SO_4) mg/L	47.0±3.03	52.0±4.03	54.0±4.01

Table 4.14e (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Arachis hypogaea* Hull Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.4±4.01	14.5±3.03	19.0±0.03
Phosphorus (P) mg/L	1.64±1.01	2.15±2.01	1.89±2.01
Potassium (K) mg/L	3.2±1.01	3.4±1.01	3.6±0.03
Calcium (Ca) mg/L	190.0±7.01	180.0±4.02	82.0±5.01
Magnesium (Mg) mg/L	22.0±3.01	32.0±3.01	34.0±3.01
Copper (Cu) mg/L	1.45±1.01	1.91±1.01	1.85±0.04
Zinc (Zn) mg/L	8.8±2.02	12.0±2.01	12.0±1.01
Iron (Fe) mg/L	2.15±1.01	3.0±3.01	3.4±2.01
Aluminium (Al) mg/L	0.28±0.02	0.23±1.01	0.28±0.01
Nitrate (NO_3) mg/L	1.16±1.01	1.42±0.04	1.50±1.01
Ammonium (NH_4) mg/L	0.15±0.02	0.32±0.02	0.3±0.01
Phosphate (PO_4) mg/L	54.0±4.01	60.3±5.01	85.3±5.01
Manganese (Mn) mg/L	0.006±0.01	0.010±0.01	0.011±0.01
Sulphate (SO_4) mg/L	42.0±3.01	54.0±5.01	60.0±4.02

Table 4.14e (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Arachis hypogaea* Hull Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.7±4.01	16.0±2.01	17.7±3.01
Phosphorus (P) mg/L	1.47±1.01	1.57±1.01	2.19±1.01
Potassium (K) mg/L	3.1±0.03	3.3±1.01	3.5±1.03
Calcium (Ca) mg/L	209.0±10.01	155.0±8.01	110.0±6.03
Magnesium (Mg) mg/L	22.0±3.01	27.0±3.01	31.0±3.01
Copper (Cu) mg/L	1.35±0.03	1.75±0.01	1.98±6.00
Zinc (Zn) mg/L	11.0±2.01	12.5±3.01	12.5±3.01
Iron (Fe) mg/L	2.7±2.01	3.2±3.01	3.7±1.01
Aluminium (Al) mg/L	0.18±0.02	0.28±0.01	0.25±0.01
Nitrate (NO_3) mg/L	1.28±1.01	1.46±1.01	1.28±0.04
Ammonium (NH_4) mg/L	0.17±0.01	0.24±0.02	0.28±0.02
Phosphate (PO_4) mg/L	52.7±4.01	67.5±6.01	69.8±2.01
Manganese (Mn) mg/L	0.007±0.01	0.008±0.01	0.009±0.01
Sulphate (SO_4) mg/L	46.0±3.01	54.0±3.01	55.0±3.02

Table 4.14e (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Arachis hypogaea* Hull Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.2±3.02	17.7±2.01	16.2±3.30
Phosphorus (P) mg/L	1.92±1.01	1.59±2.01	1.97±1.01
Potassium (K) mg/L	3.0±1.01	3.2±1.01	3.4±1.01
Calcium (Ca) mg/L	200.0±0.01	105.0±4.01	135.0±5.01
Magnesium (Mg) mg/L	22.0±2.01	24.0±3.01	29.0±3.01
Copper (Cu) mg/L	1.4±1.01	1.5±1.01	1.7±1.01
Zinc (Zn) mg/L	8.0±1.01	11.5±3.01	13.0±2.01
Iron (Fe) mg/L	2.3±0.02	3.10±1.01	2.9±1.02
Aluminium (Al) mg/L	0.14±0.01	0.22±0.02	0.23±0.21
Nitrate (NO_3) mg/L	1.06±0.02	1.32±1.01	1.42±1.01
Ammonium (NH_4) mg/L	0.16±0.01	0.31±0.01	0.25±0.05
Phosphate (PO_4) mg/L	59.5±4.02	58.5±4.01	65.9±5.05
Manganese (Mn) mg/L	0.006±0.01	0.007±0.01	0.009±0.01
Sulphate (SO_4) mg/L	51.0±3.01	61.0±5.01	54.0±4.02

Table 4.14e (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Arachis hypogaea* Hull Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	14.4±3.01	17.0±4.11	16.1±4.01
Phosphorus (P) mg/L	1.44±1.01	1.96±2.01	1.96±1.01
Potassium (K) mg/L	3.0±3.01	3.4±2.01	3.3±2.01
Calcium (Ca) mg/L	160.0±10.01	100.0±1.01	115.0±9.01
Magnesium (Mg) mg/L	23.0±2.01	28.0±5.01	28.0±5.01
Copper (Cu) mg/L	1.2±1.01	1.65±1.01	1.65±1.03
Zinc (Zn) mg/L	8.4±3.01	12.0±3.01	11.5±4.03
Iron (Fe) mg/L	2.25±2.02	3.2±2.01	2.85±1.01
Aluminium (Al) mg/L	0.13±1.01	0.24±0.02	0.21±0.02
Nitrate (NO_3) mg/L	1.18±1.01	1.48±1.01	1.36±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.31±0.01	0.34±0.01
Phosphate (PO_4) mg/L	39.8±5.02	68.4±4.11	61.0±4.01
Manganese (Mn) mg/L	0.005±0.01	0.008±0.01	0.008±0.01
Sulphate (SO_4) mg/L	40.0±4.02	52.0±2.05	62.0±4.01

Tables 4.14f (i-vi) show the results of the nutrient bioavailability and accessibility to maize plant with the application of *Tithonia diversifolia* + poultry dropping biofertilizer. From the experiment, almost all the nutrients and mineral elements evaluated recorded their highest accumulation levels in the roots except calcium and zinc with the highest values of 210 mg/L (recorded in the 50 kg N/ha experiment) and 14 mg/L (recorded in the 20 kg N/ha experiment) respectively in the leaves. The highest levels of nitrogen (19 mg/L recorded in the 30 kg N/ha experiment), phosphorus (2.36 mg/L recorded in the 60 kg N/ha experiment), potassium (3.6 mg/L recorded in the 50 kg N/ha experiment), magnesium (34 mg/L recorded in the 30 kg N/ha experiment), copper (1.98 mg/L recorded in the 40 kg N/ha experiment), iron (3.6 mg/L recorded in the 40 kg N/ha experiment), aluminium (0.31 mg/L recorded in the 60 kg n/ha experiment), nitrates (1.56 mg/L recorded in the 60 kg N/ha experiment), ammonium (0.32 mg/L recorded in the 60 kg N/ha experiment), phosphate (85.3 mg/L recorded in the 30 kg N/ha experiment), manganese (0.011 mg/L recorded in the 40 kg N/ha experiment) and sulphate (65 mg/L recorded in the 40 kg N/ha experiment) were all recorded in the roots of the tested plants. In all, the plant organ showing the highest levels of nutrient accumulation was the root and the experiment with the highest nutrient availability to plants were the 40 and 60 kg N/ha experimental set ups.

Table 4.14f (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Tithonia diversifolia* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.3±3.03	17.1±3.01	15.4±2.01
Phosphorus (P) mg/L	1.56±0.02	1.96±1.01	2.32±1.01
Potassium (K) mg/L	3.0±0.01	3.4±1.01	3.5±1.01
Calcium (Ca) mg/L	150.0±8.01	110.0±7.02	86.0±6.01
Magnesium (Mg) mg/L	26.0±4.01	31.0±3.01	32.0±3.01
Copper (Cu) mg/L	1.3±1.01	1.7±1.01	1.97±1.01
Zinc (Zn) mg/L	10.0±2.02	12.5±3.03	13.5±2.02
Iron (Fe) mg/L	2.35±2.02	3.0±2.01	3.40±1.01
Aluminium (Al) mg/L	0.17±0.01	0.24±0.01	0.23±0.02
Nitrate (NO_3) mg/L	1.32±0.02	1.46±1.01	1.48±1.01
Ammonium (NH_4) mg/L	0.17±0.03	0.25±0.01	0.30±0.01
Phosphate (PO_4) mg/L	63.6±3.03	61.2±4.00	80.2±6.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.010±0.02
Sulphate (SO_4) mg/L	46.0±4.02	54.0±4.01	61.0±6.02

Table 4.14f (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Tithonia diversifolia* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.7±6.21	18.5±4.01	15.9±3.01
Phosphorus (P) mg/L	1.54±0.01	2.13±1.01	2.31±2.01
Potassium (K) mg/L	3.1±2.01	3.4±2.01	3.3±0.01
Calcium (Ca) mg/L	190.0±11.01	105.0±8.02	145.0±5.02
Magnesium (Mg) mg/L	24.0±3.03	30.0±3.01	29.0±2.01
Copper (Cu) mg/L	1.15±1.01	1.75±1.01	1.6±1.01
Zinc (Zn) mg/L	14.0±2.01	11.0±3.01	10.5±2.01
Iron (Fe) mg/L	2.8±2.01	3.2±2.01	3.1±1.01
Aluminium (Al) mg/L	0.17±0.11	0.23±0.01	0.26±0.01
Nitrate (NO_3) mg/L	1.2±0.02	1.3±1.01	1.36±1.01
Ammonium (NH_4) mg/L	0.2±0.01	0.22±0.01	0.31±2.01
Phosphate (PO_4) mg/L	57.9±4.01	60.3±5.02	64.0±3.11
Manganese (Mn) mg/L	0.009±0.01	0.008±0.01	0.008±0.01
Sulphate (SO_4) mg/L	47.0±3.03	52.0±4.03	54.0±4.01

Table 4.14f (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Tithonia diversifolia* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.4±4.01	14.5±3.03	19.0±0.03
Phosphorus (P) mg/L	1.64±1.01	2.15±2.01	1.89±2.01
Potassium (K) mg/L	3.2±1.01	3.4±1.01	3.5±0.03
Calcium (Ca) mg/L	190.0±7.01	180.0±4.02	82.0±5.01
Magnesium (Mg) mg/L	22.0±3.01	32.0±3.01	34.0±3.01
Copper (Cu) mg/L	1.45±1.01	1.95±1.01	1.85±0.04
Zinc (Zn) mg/L	8.8±2.02	12.0±2.01	12.0±1.01
Iron (Fe) mg/L	2.15±1.01	3.0±3.01	3.4±2.01
Aluminium (Al) mg/L	0.28±0.02	0.23±1.01	0.28±0.01
Nitrate (NO_3) mg/L	1.16±1.01	1.42±0.04	1.50±1.01
Ammonium (NH_4) mg/L	0.15±0.02	0.32±0.02	0.3±0.01
Phosphate (PO_4) mg/L	54.0±4.01	60.3±5.01	85.3±5.01
Manganese (Mn) mg/L	0.006±0.01	0.010±0.01	0.009±0.01
Sulphate (SO_4) mg/L	42.0±3.01	54.0±5.01	60.0±4.02

Table 4.14f (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Tithonia diversifolia* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.7±4.01	16.0±2.01	18.7±3.01
Phosphorus (P) mg/L	1.47±1.01	1.57±1.01	2.19±1.01
Potassium (K) mg/L	3.1±0.03	3.3±1.01	3.5±1.03
Calcium (Ca) mg/L	202.0±10.01	155.0±8.01	110.0±6.03
Magnesium (Mg) mg/L	22.0±3.01	27.0±3.01	31.0±3.01
Copper (Cu) mg/L	1.35±0.03	1.75±0.01	1.98±6.00
Zinc (Zn) mg/L	11.0±2.01	12.5±3.01	12.5±3.01
Iron (Fe) mg/L	2.7±2.01	3.2±3.01	3.6±1.01
Aluminium (Al) mg/L	0.18±0.02	0.28±0.01	0.25±0.01
Nitrate (NO_3) mg/L	1.28±1.01	1.46±1.01	1.28±0.04
Ammonium (NH_4) mg/L	0.17±0.01	0.24±0.02	0.28±0.02
Phosphate (PO_4) mg/L	52.7±4.01	67.5±6.01	69.8±2.01
Manganese (Mn) mg/L	0.007±0.01	0.008±0.01	0.011±0.01
Sulphate (SO_4) mg/L	46.0±3.01	54.0±3.01	65.0±3.02

Table 4.14f (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Tithonia diversifolia* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.2±3.02	17.7±2.01	16.2±3.30
Phosphorus (P) mg/L	1.92±1.01	1.59±2.01	1.97±1.01
Potassium (K) mg/L	3.0±1.01	3.2±1.01	3.6±1.01
Calcium (Ca) mg/L	210.0±0.01	105.0±4.01	135.0±5.01
Magnesium (Mg) mg/L	22.0±2.01	24.0±3.01	29.0±3.01
Copper (Cu) mg/L	1.4±1.01	1.5±1.01	1.7±1.01
Zinc (Zn) mg/L	8.0±1.01	11.5±3.01	13.0±2.01
Iron (Fe) mg/L	2.3±0.02	3.10±1.01	2.9±1.02
Aluminium (Al) mg/L	0.14±0.01	0.22±0.02	0.23±0.21
Nitrate (NO_3) mg/L	1.06±0.02	1.32±1.01	1.42±1.01
Ammonium (NH_4) mg/L	0.16±0.01	0.31±0.01	0.25±0.05
Phosphate (PO_4) mg/L	59.5±4.02	58.5±4.01	65.9±5.05
Manganese (Mn) mg/L	0.006±0.01	0.007±0.01	0.009±0.01
Sulphate (SO_4) mg/L	51.0±3.01	61.0±5.01	54.0±4.02

Table 4.14f (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Tithonia diversifolia* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	14.4±3.01	18.0±4.11	16.1±4.01
Phosphorus (P) mg/L	1.44±1.01	1.96±2.01	2.36±1.01
Potassium (K) mg/L	3.0±3.01	3.4±2.01	3.3±2.01
Calcium (Ca) mg/L	160.0±10.01	100.0±1.01	115.0±9.01
Magnesium (Mg) mg/L	23.0±2.01	28.0±5.01	28.0±5.01
Copper (Cu) mg/L	1.2±1.01	1.65±1.01	1.65±1.03
Zinc (Zn) mg/L	8.4±3.01	12.0±3.01	11.5±4.03
Iron (Fe) mg/L	2.25±2.02	3.2±2.01	2.85±1.01
Aluminium (Al) mg/L	0.13±1.01	0.24±0.02	0.31±0.02
Nitrate (NO_3) mg/L	1.18±1.01	1.48±1.01	1.56±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.31±0.01	0.32±0.01
Phosphate (PO_4) mg/L	39.8±5.02	68.4±4.11	61.0±4.01
Manganese (Mn) mg/L	0.005±0.01	0.008±0.01	0.008±0.01
Sulphate (SO_4) mg/L	40.0±4.02	52.0±2.05	52.0±4.01

Tables 4.14g (i-vi) show the results of nutrients bioavailability and accessibility to maize plants with the application of *Chromolaena odorata* + Poultry dropping biofertilizer. From the experiment, the highest accumulation of nitrogen, (20.8 mg/L recorded in the 60 kg N/ha experiment), potassium (4.3 mg/L recorded in the 60 kg N/ha experiment), calcium (162 mg/L recorded in the 60 kg N/ha experiment) And nitrates (1.7 mg/L recorded in the 60 kg N/ha experiment) were all recorded in the plants leaves. The highest values of phosphorus (2.68 mg/L recorded in the 60 kg N/ha experiment), magnesium (48 mg/L recorded in the 60 kg N/ha experiment), copper (1.97 mg/L recorded in the 60 kg N/ha experiment), zinc (13.3 mg/L recorded in the 10 kg N/ha experiment), iron (3.7 mg/L recorded in the 60 kg N/ha experiment), ammonium (0.32 mg/L recorded in the 60 kg N/ha experiment), phosphate (82.1 mg/L recorded in the 60 kg N/ha experiment) and sulphate (67.5 mg/L recorded in the 60 kg N/ha experiment) were all recorded in the roots. Only aluminium (0.28 mg/L recorded in the 60 kg N/ha experiment) and manganese (0.011 mg/L recorded in the 60 kg N/ha experiment) had their highest levels recorded in the stem of the plants. In all, the plant organ that showed the highest accumulation ability in this plot was the roots and the experimental set up that showed the highest level of nutrient availability to the tested plants was the 60 kg N/ha experiment.

Table 4.14g (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Chromolaena odorata* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.5±1.02	16.9±2.01	17.6±1.03
Phosphorus (P) mg/L	1.69±0.02	1.61±0.03	2.27±0.01
Potassium (K) mg/L	3.3±0.01	3.1±0.01	3.5±0.02
Calcium (Ca) mg/L	140.4±0.01	95±0.01	82±2.01
Magnesium (Mg) mg/L	27.3±0.02	33.3±0.03	39.2±0.02
Copper (Cu) mg/L	1.45±0.11	1.8±0.02	1.95±0.03
Zinc (Zn) mg/L	8.8±1.02	10.5±1.01	13.3±0.02
Iron (Fe) mg/L	2.9±0.21	2.7±0.03	3.3±0.01
Aluminium (Al) mg/L	0.21±0.00	0.28±0.02	0.26±0.01
Nitrate (NO_3) mg/L	1.5±0.01	1.32±0.01	1.5±0.01
Ammonium (NH_4) mg/L	0.22±0.03	0.27±0.01	0.31±0.03
Phosphate (PO_4) mg/L	58.5±2.01	64.3±0.04	78.8±4.01
Manganese (Mn) mg/L	0.009±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	49.5±0.04	58.6±3.01	60±3.01

Table 4.14g (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Chromolaena odorata* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.7±1.12	16.8±1.01	17.8±1.00
Phosphorus (P) mg/L	1.72±0.01	1.66±0.02	2.29±0.01
Potassium (K) mg/L	3.5±0.01	3.3±0.01	3.7±0.01
Calcium (Ca) mg/L	146.1±0.03	97±0.01	85±2.01
Magnesium (Mg) mg/L	26.1±0.02	33.3±0.03	39.2±0.02
Copper (Cu) mg/L	1.45±0.11	1.8±0.02	1.95±0.03
Zinc (Zn) mg/L	8.7±1.02	11.1±1.01	13.1±0.02
Iron (Fe) mg/L	2.8±0.21	2.8±0.03	3.4±0.01
Aluminium (Al) mg/L	0.21±0.00	0.27±0.02	0.26±0.01
Nitrate (NO_3) mg/L	1.5±0.01	1.32±0.01	1.5±0.01
Ammonium (NH_4) mg/L	0.23±0.03	0.27±0.01	0.31±0.03
Phosphate (PO_4) mg/L	60.1±2.01	65.1±0.04	79.1±4.01
Manganese (Mn) mg/L	0.009±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	51.6±0.04	58.1±3.01	63±1.01

Table 4.14g (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Chromolaena odorata* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.9±0.12	17.2±1.01	17.6±1.00
Phosphorus (P) mg/L	1.75±0.01	1.71±0.02	2.31±0.01
Potassium (K) mg/L	3.7±0.01	3.6±0.01	3.8±0.01
Calcium (Ca) mg/L	154.5±0.02	102±0.01	95±1.02
Magnesium (Mg) mg/L	25.1±0.01	34.5±0.03	41.2±0.02
Copper (Cu) mg/L	1.45±0.11	1.8±0.02	1.95±0.03
Zinc (Zn) mg/L	8.8±1.01	10.1±0.01	12.5±0.03
Iron (Fe) mg/L	2.9±0.11	2.8±0.03	3.6±0.01
Aluminium (Al) mg/L	0.21±0.00	0.27±0.02	0.26±0.01
Nitrate (NO_3) mg/L	1.5±0.01	1.32±0.01	1.5±0.01
Ammonium (NH_4) mg/L	0.23±0.03	0.26±0.01	0.30±0.03
Phosphate (PO_4) mg/L	65.1±2.01	67.1±0.02	81.1±2.01
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	54.2±0.02	58.5±3.01	65±1.01

Table 4.14g (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Chromolaena odorata* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	19.8±0.15	17.4±1.01	17.5±1.10
Phosphorus (P) mg/L	1.76±0.01	1.70±0.02	2.41±0.01
Potassium (K) mg/L	3.6±0.01	3.4±0.01	3.9±0.01
Calcium (Ca) mg/L	156.1±0.03	100±0.01	97±1.02
Magnesium (Mg) mg/L	25.1±0.01	35.5±0.03	44.2±0.02
Copper (Cu) mg/L	1.45±0.11	1.8±0.02	1.95±0.03
Zinc (Zn) mg/L	8.8±1.01	10.1±0.01	11.9±0.01
Iron (Fe) mg/L	2.9±0.11	2.8±0.01	3.6±0.01
Aluminium (Al) mg/L	0.21±0.00	0.27±0.02	0.26±0.01
Nitrate (NO_3) mg/L	1.6±0.01	1.32±0.01	1.5±0.01
Ammonium (NH_4) mg/L	0.23±0.05	0.27±0.03	0.29±0.03
Phosphate (PO_4) mg/L	66.2±2.01	67.4±0.02	80.5±2.01
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	55.5±0.02	57.8±2.01	66±1.05

Table 4.14g (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Chromolaena odorata* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	20.6±2.00	17.9±2.01	18.2±1.10
Phosphorus (P) mg/L	1.79±0.01	1.80±0.02	2.61±0.01
Potassium (K) mg/L	4.2±0.02	3.6±0.01	4.0±0.01
Calcium (Ca) mg/L	160.2±0.05	110±0.01	104±2.02
Magnesium (Mg) mg/L	25.4±0.01	37.4±0.05	47±0.02
Copper (Cu) mg/L	1.46±0.11	1.8±0.02	1.96±0.03
Zinc (Zn) mg/L	9.1±1.01	11.1±0.01	12.1±0.01
Iron (Fe) mg/L	3.1±0.11	2.9±0.01	3.6±0.01
Aluminium (Al) mg/L	0.21±0.00	0.27±0.02	0.26±0.01
Nitrate (NO_3) mg/L	1.6±0.01	1.32±0.01	1.5±0.02
Ammonium (NH_4) mg/L	0.23±0.05	0.26±0.03	0.30±0.03
Phosphate (PO_4) mg/L	68.5±1.03	67.9±0.02	81.1±1.02
Manganese (Mn) mg/L	0.009±0.01	0.010±0.01	0.010±0.01
Sulphate (SO_4) mg/L	59.1±0.02	60.2±2.01	64.5±1.02

Table 4.14g (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Chromolaena odorata* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	20.8±1.02	17.7±3.01	18.9±1.10
Phosphorus (P) mg/L	1.81±0.01	1.78±0.02	2.68±0.01
Potassium (K) mg/L	4.3±0.02	3.8±0.01	4.1±0.01
Calcium (Ca) mg/L	162.1±0.06	114±0.01	120±1.02
Magnesium (Mg) mg/L	26.1±0.01	35.8±0.03	48±0.02
Copper (Cu) mg/L	1.46±0.11	1.8±0.02	1.97±0.03
Zinc (Zn) mg/L	9.1±1.01	11.1±0.01	10.8±0.01
Iron (Fe) mg/L	3.3±0.11	2.9±0.01	3.7±0.01
Aluminium (Al) mg/L	0.23±0.00	0.28±0.02	0.26±0.01
Nitrate (NO_3) mg/L	1.7±0.01	1.32±0.01	1.5±0.02
Ammonium (NH_4) mg/L	0.23±0.02	0.27±0.01	0.32±0.02
Phosphate (PO_4) mg/L	68.5±1.01	67.9±0.02	82.1±1.02
Manganese (Mn) mg/L	0.009±0.01	0.011±0.01	0.010±0.01
Sulphate (SO_4) mg/L	59.1±0.02	60.2±2.01	67.5±1.02

Tables 4.14h (i-vi) show the results of the nutrients bioavailability and accessibility to maize plants with the application of *Carica papaya* + poultry dropping biofertilizer. From the experiment, almost all the nutrients and mineral elements evaluated recorded their highest accumulation levels in the roots except nitrogen, potassium and calcium with the highest values of 18.9 mg/L (recorded in the 60 kg N/ha experiment), 3.5 mg/L (recorded in the 60 kg N/ha experiment) and 150 mg/L (recorded in the 30 kg N/ha experiment) respectively in the leaves. Also, aluminium had its highest accumulation level of 0.29 mg/L (recorded in the 60 kg N/ha experiment) in the stem. The highest levels of phosphorus (2.35 mg/L recorded in the 30 kg N/ha experiment), magnesium (39 mg/L recorded in the 40 kg N/ha experiment), copper (1.96 mg/L recorded in the 60 kg N/ha experiment), zinc (16 mg/L recorded in the 30 kg N/ha experiment), iron (3.4 mg/L recorded in the 50 kg N/ha experiment), nitrates (1.52 mg/L recorded in the 30 kg N/ha experiment), ammonium (0.42 mg/L recorded in the 30 kg N/ha experiment), phosphate (81.1 mg/L recorded in the 60 kg N/ha experiment), manganese (0.010 mg/L recorded in the 60 kg N/ha experiment) and sulphate (63 mg/L recorded in the 30 kg N/ha experiment) were all recorded in the roots of the tested plants. In all, the plant organ showing the highest levels of nutrient accumulation was the root and the experiments with the highest nutrient availability to plants were the 30 and 60 kg N/ha experimental set ups.

Table 4.14h (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Carica papayas* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.3±1.00	14.3±3.00	15.1±2.01
Phosphorus (P) mg/L	1.37±0.01	2.19±0.01	2.31±0.02
Potassium (K) mg/L	2.6±0.02	2.6±1.02	2.4±0.01
Calcium (Ca) mg/L	110.0±2.02	95.0±4.04	111.0±4.02
Magnesium (Mg) mg/L	20.0±0.02	24.0±2.02	26.0±0.02
Copper (Cu) mg/L	1.2±0.01	1.6±0.03	1.5±0.01
Zinc (Zn) mg/L	7.5±1.01	10.5±2.02	10.5±0.03
Iron (Fe) mg/L	2.41±0.03	2.55±1.05	3.0±0.04
Aluminium (Al) mg/L	0.12±0.02	0.27±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.40±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.22±0.01	0.28±0.02
Phosphate (PO_4) mg/L	72.8±2.02	66.2±2.00	77.1±2.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.008±0.01
Sulphate (SO_4) mg/L	42.0±1.02	46.0±0.05	45.0±0.02

Table 4.14h (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Carica papayas* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.5±1.00	14.8±1.00	15.4±1.01
Phosphorus (P) mg/L	1.38±0.01	2.19±0.01	2.33±0.02
Potassium (K) mg/L	2.8±0.02	2.8±1.02	2.6±0.01
Calcium (Ca) mg/L	117.0±1.02	99.0±4.04	119.0±1.03
Magnesium (Mg) mg/L	23.0±0.02	24.0±2.02	26.2±0.01
Copper (Cu) mg/L	1.2±0.01	1.6±0.03	1.5±0.01
Zinc (Zn) mg/L	7.8±1.01	10.1±2.02	11.1±0.03
Iron (Fe) mg/L	2.41±0.03	2.58±1.05	3.2±0.04
Aluminium (Al) mg/L	0.12±0.02	0.26±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.25±0.01	1.46±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.25±0.01	0.29±0.01
Phosphate (PO_4) mg/L	74.2±2.02	68.2±2.00	77.1±2.02
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	46.0±1.04	47.0±0.03	49.0±0.03

Table 4.14h (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Carica papayas* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.0±3.02	16.4±3.05	16.8±3.02
Phosphorus (P) mg/L	1.7±0.03	1.97±0.01	2.35±1.01
Potassium (K) mg/L	3.2±1.01	3.4±1.01	3.4±1.01
Calcium (Ca) mg/L	150.0±5.01	120.0±4.01	85.0±4.01
Magnesium (Mg) mg/L	25.0±3.01	30.0±3.01	30.0±2.03
Copper (Cu) mg/L	1.5±0.03	1.8±0.03	1.8±1.01
Zinc (Zn) mg/L	10.0±3.01	11.0±2.01	16.0±2.01
Iron (Fe) mg/L	2.55±1.01	2.9±0.04	3.1±0.01
Aluminium (Al) mg/L	0.19±0.01	0.24±0.04	0.23±0.01
Nitrate (NO_3) mg/L	1.36±0.03	1.44±0.02	1.52±0.02
Ammonium (NH_4) mg/L	0.22±0.02	0.22±0.02	0.42±0.03
Phosphate (PO_4) mg/L	50.9±3.01	58.7±4.05	62.4±3.01
Manganese (Mn) mg/L	0.007±0.01	0.008±0.01	0.009±0.01
Sulphate (SO_4) mg/L	48.0±3.01	53.0±3.01	63.0±4.01

Table 4.14h (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Carica papayas* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.1±2.03	16.1±1.02	16.8±2.02
Phosphorus (P) mg/L	1.64±0.03	1.51±0.01	1.71±0.02
Potassium (K) mg/L	2.9±0.01	2.7±0.01	3.3±0.01
Calcium (Ca) mg/L	138.0±0.02	102.0±0.03	122.0±5.02
Magnesium (Mg) mg/L	27.0±2.03	33.0±0.02	39.0±1.01
Copper (Cu) mg/L	1.45±0.03	1.8±0.01	1.95±0.02
Zinc (Zn) mg/L	8.8±1.01	10.5±1.02	13.0±1.02
Iron (Fe) mg/L	2.9±0.01	2.7±0.03	3.3±0.01
Aluminium (Al) mg/L	0.21±0.02	0.28±0.01	0.26±0.01
Nitrate (NO_3) mg/L	1.5±0.01	1.32±0.02	1.5±0.11
Ammonium (NH_4) mg/L	0.22±0.03	0.27±0.01	0.31±0.01
Phosphate (PO_4) mg/L	58.5±3.05	64.3±3.02	78.8±4.01
Manganese (Mn) mg/L	0.009±0.02	0.009±0.01	0.008±0.01
Sulphate (SO_4) mg/L	49.0±2.02	58.0±2.03	60.0±3.02

Table 4.14h (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Carica papayas* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.5±2.01	16.5±1.01	17.4±2.01
Phosphorus (P) mg/L	1.65±0.02	1.61±0.01	2.22±0.01
Potassium (K) mg/L	3.2±0.01	2.7±0.01	3.2±0.05
Calcium (Ca) mg/L	140.0±1.04	95.0±2.02	82.0±1.05
Magnesium (Mg) mg/L	28.0±1.03	23.0±1.01	35.0±2.02
Copper (Cu) mg/L	1.45±0.03	1.8±0.01	1.95±0.01
Zinc (Zn) mg/L	8.8±1.01	10.5±2.00	13.0±2.02
Iron (Fe) mg/L	2.9±1.02	2.7±0.02	3.4±0.01
Aluminium (Al) mg/L	0.21±0.04	0.28±0.04	0.26±0.01
Nitrate (NO_3) mg/L	1.5±0.02	1.32±0.03	1.5±0.01
Ammonium (NH_4) mg/L	0.22±0.01	0.27±0.02	0.31±0.02
Phosphate (PO_4) mg/L	58.5±3.01	64.3±2.05	78.8±1.02
Manganese (Mn) mg/L	0.009±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	51.0±1.01	59.0±0.01	56.0±2.00

Table 4.14h (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Carica papayas* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.9±3.00	17.3±3.00	17.9±3.01
Phosphorus (P) mg/L	1.47±0.01	2.19±0.01	2.31±0.02
Potassium (K) mg/L	3.5±0.02	3.1±1.02	3.4±0.01
Calcium (Ca) mg/L	145.0±2.02	95.0±4.04	125.0±2.02
Magnesium (Mg) mg/L	22.0±0.02	34.0±2.02	29.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.8±0.03	1.96±0.01
Zinc (Zn) mg/L	9.8±1.02	13.5±2.02	12.5±0.03
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.29±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.42±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.24±0.01	0.28±0.02
Phosphate (PO_4) mg/L	76.8±4.02	71.7±4.00	81.1±5.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	44.0±3.05	56.0±0.05	55.0±0.05

Tables 4.14i (i-vi) show the results of nutrients bioavailability and accessibility to maize plants with the application of *Telfairia occidentalis* + poultry dropping biofertilizer. From the experiment, almost all the nutrients and mineral elements evaluated recorded their highest accumulation levels in the roots except nitrogen, potassium and calcium with the highest values of 18.2 mg/L (recorded in the 50 kg N/ha experiment), 3.7 mg/L (recorded in the 50 kg N/ha experiment) and 143 mg/L (recorded in the 50 kg N/ha experiment) respectively in the leaves. Also, magnesium, zinc and aluminium had their highest accumulation levels of 35 mg/L (recorded in the 50 kg N/ha experiment), 13.5 mg/L (recorded in the 60 kg N/ha) and 0.32 mg/L (recorded in the 60 kg N/ha) respectively in the stem. The highest levels of phosphorus (2.43 mg/L recorded in the 60 kg N/ha experiment), copper (1.9 mg/L recorded in the 50 kg N/ha experiment), iron (3.3 mg/L recorded in the 60 kg N/ha experiment), nitrates (1.43 mg/L recorded in the 60 kg N/ha experiment), ammonium (0.34 mg/L recorded in the 60 kg N/ha experiment), phosphate (85.5 mg/L recorded in the 60 kg N/ha experiment), manganese (0.012 mg/L recorded in the 60 kg N/ha experiment) and sulphate (61 mg/L recorded in the 60 kg N/ha experiment) were all recorded in the roots of the tested plants. In all, the plant organ showing the highest levels of nutrient accumulation was the root and the experiment with the highest nutrient availability to plants was the 60 kg N/ha experimental set up.

Table 4.14i (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Telfairia occidentalis* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.2±1.00	15.3±3.00	15.9±2.01
Phosphorus (P) mg/L	1.47±0.01	1.44±0.01	1.45±0.02
Potassium (K) mg/L	3.0±0.02	3.4±1.02	3.4±0.01
Calcium (Ca) mg/L	120.0±5.02	93.0±4.04	115.0±4.02
Magnesium (Mg) mg/L	22.0±0.02	34.0±2.02	29.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.8±0.03	1.7±0.01
Zinc (Zn) mg/L	7.8±1.02	11.5±2.02	12.5±0.03
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.29±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.42±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.27±0.01	0.31±0.02
Phosphate (PO_4) mg/L	72.3±2.02	70.7±4.00	78.6±5.02
Manganese (Mn) mg/L	0.007±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	46.0±2.02	52.0±0.05	55.0±0.05

Table 4.14i (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Telfairia occidentalis* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.6±1.00	15.1±3.00	15.4±2.01
Phosphorus (P) mg/L	1.47±0.01	1.44±0.01	1.45±0.02
Potassium (K) mg/L	3.3±0.02	3.1±1.02	3.2±0.01
Calcium (Ca) mg/L	122.0±2.02	96.0±2.04	121.0±1.02
Magnesium (Mg) mg/L	22.0±0.02	34.0±2.02	29.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.8±0.03	1.7±0.01
Zinc (Zn) mg/L	7.8±1.02	11.2±2.02	11.5±0.03
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.29±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.21±0.01	1.40±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.25±0.01	0.29±0.02
Phosphate (PO_4) mg/L	75.3±2.02	7.7±4.00	84.0±5.02
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.010±0.01
Sulphate (SO_4) mg/L	48.0±1.02	54.0±0.02	56.0±0.02

Table 4.14i (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Telfairia occidentalis* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.4±1.00	16.3±2.00	16.5±3.01
Phosphorus (P) mg/L	1.49±0.01	1.46±0.01	1.46±0.02
Potassium (K) mg/L	3.4±0.03	3.2±1.02	3.2±0.01
Calcium (Ca) mg/L	135.0±2.02	99.0±2.04	129.0±1.02
Magnesium (Mg) mg/L	21.0±0.02	34.0±2.02	29.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.5±0.03	1.7±0.01
Zinc (Zn) mg/L	7.8±1.02	11.4±2.02	12.5±0.03
Iron (Fe) mg/L	2.55±0.02	2.76±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.30±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.22±0.01	1.41±1.02	1.33±0.02
Ammonium (NH_4) mg/L	0.18±0.01	0.23±0.01	0.30±0.02
Phosphate (PO_4) mg/L	73.3±2.02	71.7±4.00	86.1±5.02
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.008±0.01
Sulphate (SO_4) mg/L	47.0±1.02	53.0±0.02	57.0±0.01

Table 4.14i (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Telfairia occidentalis* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.6±1.00	16.7±3.00	16.8±2.02
Phosphorus (P) mg/L	1.53±0.01	1.46±0.01	1.46±2.02
Potassium (K) mg/L	3.6±0.05	3.2±1.02	3.2±0.01
Calcium (Ca) mg/L	137.0±2.02	102.0±1.04	135.0±1.02
Magnesium (Mg) mg/L	23.0±0.02	34.0±2.02	29.2±0.02
Copper (Cu) mg/L	1.3±0.01	1.5±0.03	1.7±0.01
Zinc (Zn) mg/L	7.8±1.02	11.4±2.02	12.5±0.03
Iron (Fe) mg/L	2.56±0.02	2.76±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.30±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.41±1.02	1.35±0.02
Ammonium (NH_4) mg/L	0.18±0.01	0.23±0.01	0.29±0.02
Phosphate (PO_4) mg/L	73.5±2.02	73.7±4.00	86.6±5.02
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	49.0±1.02	54.0±0.02	59.0±2.01

Table 4.14i (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Telfairia occidentalis* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	18.2±1.00	16.7±3.00	16.8±2.02
Phosphorus (P) mg/L	1.54±0.01	1.47±0.01	1.49±2.02
Potassium (K) mg/L	3.7±0.02	3.4±1.02	3.5±0.01
Calcium (Ca) mg/L	143.0±2.02	112.0±1.04	138.0±1.02
Magnesium (Mg) mg/L	22.0±0.02	35.0±2.02	28.4±0.02
Copper (Cu) mg/L	1.3±0.01	1.5±0.03	1.9±0.01
Zinc (Zn) mg/L	7.8±1.02	11.4±2.02	12.5±0.03
Iron (Fe) mg/L	2.56±0.02	2.76±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.30±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.40±1.02	1.36±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.25±0.01	0.29±0.02
Phosphate (PO_4) mg/L	73.5±2.02	73.7±4.00	88.1±5.02
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.011±0.01
Sulphate (SO_4) mg/L	47.0±1.02	56.0±0.02	61.0±2.01

Table 4.14i (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Telfairia occidentalis* + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	17.9±3.00	17.1±3.00	17.6±4.01
Phosphorus (P) mg/L	2.41±0.01	2.41±0.01	2.43±0.02
Potassium (K) mg/L	3.5±0.02	3.2±1.02	3.6±0.01
Calcium (Ca) mg/L	134.0±2.02	105.0±2.04	125.0±4.02
Magnesium (Mg) mg/L	22.0±0.02	34.0±2.02	27.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.8±0.03	1.7±0.01
Zinc (Zn) mg/L	9.8±1.02	13.5±2.02	12.5±0.03
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	3.3±0.04
Aluminium (Al) mg/L	0.17±0.02	0.32±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.42±1.02	1.43±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.25±0.01	0.34±0.02
Phosphate (PO_4) mg/L	76.8±4.02	71.7±4.00	85.5±5.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.012±0.01
Sulphate (SO_4) mg/L	47.0±1.03	52.0±0.03	53.0±0.02

Tables 4.14j (i-vi) shows the results of nutrients bioavailability and accessibility to maize plants with the application of *Arachis hypogaea* hull + poultry dropping biofertilizer. From the experiment, almost all the nutrients and mineral elements evaluated recorded their highest accumulation levels in the roots except nitrogen, potassium and calcium with the highest values of 16.9 mg/L (recorded in the 60 kg N/ha experiment), 3.8 mg/L (recorded in the 50 kg N/ha experiment) and 129 mg/L (recorded in the 50 kg N/ha experiment) respectively in the leaves. Also, magnesium, zinc, aluminium and nitrates had their highest accumulation levels of 34 mg/L (recorded in the 60 kg N/ha experiment), 13.5 mg/L (recorded in the 60 kg N/ha experiment), 0.31 mg/L (recorded in the 50 kg N/ha experiment) and 1.42 mg/L (recorded in the 60 kg N/ha experiment) respectively in the stem. The highest levels of phosphorus (2.34 mg/L recorded in the 20 kg N/ha experiment), copper (1.9 mg/L recorded in the 50 kg N/ha experiment), iron (3.0 mg/L recorded in the 60 kg N/ha experiment), ammonium (0.29 mg/L recorded in the 60 kg N/ha experiment), phosphate (83.6 mg/L recorded in the 60 kg N/ha experiment), manganese (0.010 mg/L recorded in the 60 kg N/ha experiment) and sulphate (58.5 mg/L recorded in the 60 kg N/ha experiment) were all recorded in the roots of the tested plants. In all, the plant organ showing the highest levels of nutrient accumulation was the root and the experiment with the highest nutrient availability to plants was the 60 kg N/ha experimental set up.

Table 4.14j (i): Nutrient Bioavailability and Accessibility to Plant Organs with 10 kg N/ha *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.2±1.00	14.6±3.00	14.3±1.01
Phosphorus (P) mg/L	1.47±0.01	2.19±0.01	2.31±0.02
Potassium (K) mg/L	2.3±0.02	3.6±1.02	3.4±0.01
Calcium (Ca) mg/L	110.0±5.02	95.0±4.04	115.0±4.02
Magnesium (Mg) mg/L	22.0±0.02	31.0±2.02	27.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.6±0.03	1.5±0.01
Zinc (Zn) mg/L	7.8±1.02	8.5±2.02	12.5±0.03
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	2.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.29±0.01	0.23±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.42±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.22±0.01	0.26±0.02
Phosphate (PO_4) mg/L	72.8±2.02	70.7±4.00	78.1±5.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.007±0.01
Sulphate (SO_4) mg/L	42.0±1.02	50.0±0.04	53.0±0.05

Table 4.14j (ii): Nutrient Bioavailability and Accessibility to Plant Organs with 20 kg N/ha *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.6±1.00	14.8±2.00	14.4±1.01
Phosphorus (P) mg/L	1.47±0.01	2.19±0.01	2.34±0.02
Potassium (K) mg/L	2.3±0.02	3.6±1.02	3.4±0.01
Calcium (Ca) mg/L	117.0±3.02	98.0±2.04	112.0±4.02
Magnesium (Mg) mg/L	23.0±0.02	32.0±2.02	29.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.6±0.03	1.7±0.01
Zinc (Zn) mg/L	7.6±1.02	8.2±2.02	10.2±0.03
Iron (Fe) mg/L	2.4±0.03	2.8±1.05	2.4±0.04
Aluminium (Al) mg/L	0.17±0.02	0.27±0.01	0.22±0.05
Nitrate (NO_3) mg/L	1.24±0.01	1.40±1.02	1.36±0.02
Ammonium (NH_4) mg/L	0.16±0.01	0.22±0.01	0.27±0.02
Phosphate (PO_4) mg/L	70.8±2.02	70.7±4.00	76.1±2.02
Manganese (Mn) mg/L	0.006±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	43.0±1.02	51.0±0.04	54.0±0.02

Table 4.14j (iii): Nutrient Bioavailability and Accessibility to Plant Organs with 30 kg N/ha *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.8±1.00	14.7±1.00	15.5±1.01
Phosphorus (P) mg/L	1.49±0.01	2.02±0.01	2.11±0.01
Potassium (K) mg/L	2.3±0.02	3.6±1.02	3.4±0.01
Calcium (Ca) mg/L	129.0±2.02	100.0±1.03	122.0±3.02
Magnesium (Mg) mg/L	21.0±0.02	33.0±2.02	28.0±0.02
Copper (Cu) mg/L	1.4±0.01	1.8±0.03	1.8±0.01
Zinc (Zn) mg/L	7.6±1.02	8.2±2.02	10.2±0.01
Iron (Fe) mg/L	2.45±0.03	2.65±1.05	2.1±0.04
Aluminium (Al) mg/L	0.15±0.02	0.28±0.01	0.23±0.02
Nitrate (NO_3) mg/L	1.22±0.01	1.41±1.02	1.35±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.24±0.01	0.28±0.01
Phosphate (PO_4) mg/L	72.2±2.02	70.7±4.00	76.6±2.01
Manganese (Mn) mg/L	0.008±0.01	0.009±0.01	0.009±0.01
Sulphate (SO_4) mg/L	47.0±2.02	55.0±0.04	56.0±0.01

Table 4.14j (iv): Nutrient Bioavailability and Accessibility to Plant Organs with 40 kg N/ha *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	15.6±1.00	14.4±2.00	15.1±2.02
Phosphorus (P) mg/L	1.46±0.01	2.02±0.01	2.11±0.01
Potassium (K) mg/L	2.5±0.02	3.6±1.02	3.4±0.01
Calcium (Ca) mg/L	123.0±2.02	103.0±1.05	125.0±2.02
Magnesium (Mg) mg/L	20.0±0.02	32.0±2.02	27.0±0.02
Copper (Cu) mg/L	1.4±0.01	1.7±0.03	1.7±0.01
Zinc (Zn) mg/L	7.8±1.02	8.2±2.02	10.5±0.01
Iron (Fe) mg/L	2.49±0.03	2.85±1.05	2.5±0.04
Aluminium (Al) mg/L	0.17±0.02	0.27±0.01	0.25±0.02
Nitrate (NO_3) mg/L	1.23±0.01	1.40±1.02	1.38±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.20±0.01	0.28±0.01
Phosphate (PO_4) mg/L	74.2±2.02	70.7±4.00	76.6±2.01
Manganese (Mn) mg/L	0.008±0.01	0.007±0.01	0.008±0.01
Sulphate (SO_4) mg/L	49.0±2.02	50.0±0.04	56.0±0.01

Table 4.14j (v): Nutrient Bioavailability and Accessibility to Plant Organs with 50 kg N/ha *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.6±3.00	15.8±3.00	16.4±1.02
Phosphorus (P) mg/L	2.26±0.01	2.02±0.01	2.11±0.01
Potassium (K) mg/L	3.8±0.01	3.5±1.02	3.4±0.01
Calcium (Ca) mg/L	129.0±5.02	108.0±1.04	125.0±2.02
Magnesium (Mg) mg/L	22.0±0.02	32.0±2.02	30.0±0.02
Copper (Cu) mg/L	1.2±0.01	1.8±0.03	1.9±0.01
Zinc (Zn) mg/L	7.6±1.02	8.6±2.02	11.2±0.01
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	2.0±0.04
Aluminium (Al) mg/L	0.19±0.02	0.31±0.01	0.26±0.02
Nitrate (NO_3) mg/L	1.20±0.01	1.42±1.02	1.37±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.22±0.01	0.28±0.01
Phosphate (PO_4) mg/L	73.2±2.02	74.7±4.00	76.9±2.01
Manganese (Mn) mg/L	0.009±0.01	0.007±0.01	0.008±0.01
Sulphate (SO_4) mg/L	54.3±2.02	55.5±0.04	58.5±0.01

Table 4.14j (vi): Nutrient Bioavailability and Accessibility to Plant Organs with 60 kg N/ha *Arachis hypogaea* Hull + Poultry Droppings Biofertilizer Application Using Maize (*Zea mays*) as Test Plant

Parameter	Leaves	Stem	Roots
Nitrogen (N) mg/L	16.9±3.00	17.3±3.00	17.9±3.01
Phosphorus (P) mg/L	2.27±0.01	2.19±0.01	2.31±0.02
Potassium (K) mg/L	3.1±0.02	3.6±1.02	3.4±0.01
Calcium (Ca) mg/L	120.0±5.02	95.0±4.04	115.0±4.02
Magnesium (Mg) mg/L	22.0±0.02	34.0±2.02	29.0±0.02
Copper (Cu) mg/L	1.3±0.01	1.8±0.03	1.8±0.01
Zinc (Zn) mg/L	9.8±1.02	13.5±2.02	12.5±0.03
Iron (Fe) mg/L	2.45±0.03	2.75±1.05	3.0±0.04
Aluminium (Al) mg/L	0.17±0.02	0.29±0.01	0.22±0.05
Nitrate (NO_3) mg/L	1.20±0.01	1.40±1.02	1.39±0.02
Ammonium (NH_4) mg/L	0.17±0.01	0.26±0.01	0.29±0.02
Phosphate (PO_4) mg/L	76.8±4.02	71.7±4.00	83.6±5.02
Manganese (Mn) mg/L	0.006±0.01	0.008±0.01	0.010±0.01
Sulphate (SO_4) mg/L	44.0±3.05	56.0±0.05	55.0±0.05

4.15. Soil Fertility Improvement Assessment

Table 4.15 shows the result of the chemical properties and microbial composition of the soil used in the phyto-assessment. From the table, all the elements recorded low values suggesting that the soil is low or depleted in nutrient. Also, the microbial composition revealed lesser diversity and population of organisms unlike richer soils. The bacterial TPC of the soil was 4.1×10^5 cfu/ml while that of fungi was 3.0×10^3 cfu/ml.

Table 4.15: Physicochemical Properties and Microbial Composition of Soil Used for the Phyto-Assessment

Chemical Properties		Microbial Composition			
Parameter	**Soil (mg/L)**	**Bacteria**		**Fungi**	
		Organism	**TBPC (cfu/ml)**	**Organism**	**TFC (cfu/ml)**
Nitrogen (N)	9.2	*Bacillus* sp.	4.1×10^5	*Aspergillus niger*	3.0×10^3
Phosphorus (P)	1.4			*Mucor* sp.	
Potassium (K)	2.6	*Clostridium* sp.			
Calcium (Ca)	43.3				
Magnesium (Mg)	21.6				
Copper (Cu)	1.25				
Zinc (Zn)	10				
Iron (Fe)	2.1				
Aluminium (Al)	0.05				
Nitrate (NO_3)	1.09				
Ammonium (NH_4)	0.21				
Phosphate (PO_4)	44.4				
Manganese (Mn)	0.008				
Sulphate (SO_4)	41.5				

TBPC = Total bacterial plate count; TFC = Total fungal count

Tables 4.16 (a-e) shows the comparative soil fertility improvement achieved in the experimental soils after harvesting of the tested plants. In the *Tithonia diversifolia* biofertilizer plots, soil nutrients increase was consistent with increase in the quantity of applied fertilizer. The lowest fertile soil was the negative control which had no fertilizer application while the most fertile soil was the 50 kg N/ha experiment. The highest levels of nitrogen (18.8 mg/L), phosphorus (3.6 mg/L), iron (4.4 mg/L), nitrates (2.2 mg/L), ammonium (0.39 mg/L) and manganese (0.022 mg/L) were found in the 60 kg N/ha experimental soil while those of potassium (4.6 mg/L), calcium (85.6 mg/L), magnesium (44.5 mg/L), copper (2.8 mg/L), zinc (17.2 mg/L), aluminium (0.38 mg/L), phosphate (88.5 mg/L) and sulphate (68 mg/L) were all recorded in the 50 kg N/ha experimental soil after harvesting.

In the *Chromolaena odorata* biofertilizer plot, soil nutrient improvement also followed an increasing pattern with the quantity of applied fertilizer. Here, the lowest fertile soil soil after plant harvesting was the negative control with no initial fertilizer application while the most fertile soil was the 60 kg N/kg experimental soil. The highest levels of nitrogen (19.1 mg/L), phosphorus (3.7 mg/L), potassium (4.9 mg/L), calcium (85.5 mg/L), copper (2.9 mg/L), zinc (17.9 mg/L), iron (4.6 mg/L), aluminium (0.41 mg/L), nitrates (2.4 mg/L), phosphate (89.5 mg/L) and manganese (0.022 mg/L) were all recorded in the 60 kg N/ha experimental soils while those of magnesium (46.5 mg/L), ammonium (0.44 mg/L) and sulphate (68 mg/L) were recorded in the 50 kg N/ha experimental soil.

In the *Carica papaya* biofertilizer plot, the improvement in the fertility of soils after plant harvesting was lowest in the negative control and highest in the 60 kg N/ha experimental soil. The highest values of nitrogen (18.2 mg/L), phosphorus (3.3 mg/L), potassium (4.3 mg/L), calcium (76.2 mg/L), magnesium (43 mg/L), copper (2.7 mg/L), zinc (17 mg/L), iron (4.2 mg/L), aluminium (0.38 mg/L), nitrates (2.0 mg/L), ammonium (0.39 mg/L), phosphate (84.5 mg/L), manganese (0.018 mg/L) and sulphate (67 mg/L) were all recorded in the 60 kg N/ha experimental soils.

In the *Telfairia occidentalis* biofertilizer plot, soil nutrient improvement also followed an increasing pattern with the quantity of applied fertilizer. Here also, the lowest fertile soil

soil after plant harvesting was the negative control with no initial fertilizer application while the most fertile soil was the 60 kg N/kg experimental soils. The highest levels of nitrogen (18.3 mg/L), phosphorus (3.2 mg/L), potassium (4.2 mg/L), calcium (71.6 mg/L), magnesium (42 mg/L), copper (2.4 mg/L), zinc (16.7 mg/L), iron (3.7 mg/L), aluminium (0.33 mg/L), nitrates (2.0 mg/L), ammonium (0.37), phosphate (81.1 mg/L) and manganese (0.015 mg/L) were all recorded in the 60 kg N/ha experimental soils while that of sulphate (64.2 mg/L) was recorded in the 50 kg N/ha experimental soil.

In the *Arachis hypogaea* hull biofertilizer plot, improvement of nutrients in the tested soils also followed an increasing pattern with the quantity of applied fertilizer. In this plot, the lowest fertile soil soil after plant harvesting was the negative control with no initial fertilizer application while the most fertile soil was the 60 kg N/kg experimental soils. The highest levels of nitrogen (16.9 mg/L), phosphorus (2.7 mg/L), potassium (4.0 mg/L), calcium (68.1 mg/L), magnesium (39 mg/L), copper (2.6 mg/L), zinc (16.6 mg/L), iron (3.7 mg/L), nitrates (1.6 mg/L), ammonium (0.36 mg/L), phosphate (76.2 mg/L), manganese (0.013 mg/L) and sulphate (63.6 mg/L) were all recorded in the 60 kg N/ha experimental soils while that of aluminium (0.34 mg/L) was recorded in the 50 kg N/ha experimental soils.

Table 4.16a: Soil Improvement with *Tithonia diversifolia* Shoot Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.2±0.01	16.1±0.01	17.3±0.12	17.4±0.02	18.2±1.02	18.8±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.4±0.02	2.6±0.02	2.8±0.02	2.8±0.02	3.5±0.02	3.6±0.02
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.6±0.02	3.8±0.02	3.9±0.02	4.1±0.05	4.6±0.02	4.4±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	55±2.01	66.5±0.01	69.5±0.05	70±0.04	85.6±0.03	79.4±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	36±0.03	37.5±0.03	39.5±0.03	42±0.03	44.5±0.04	44±0.04
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.9±0.02	2.1±0.02	2.1±0.02	2.4±0.02	2.8±0.02	2.6±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	16±0.02	15.6±0.01	16.5±0.01	16.8±0.02	17.2±1.02	17.1±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.4±0.02	3.4±0.02	3.7±0.02	4.0±0.02	4.4±0.02	4.4±0.02
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.34±0.02	0.35±0.02	0.36±0.02	0.38±0.02	0.38±0.02	0.37±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.5±0.01	1.7±0.01	1.9±0.00	2.0±0.01	2.2±0.02	2.2±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.35±0.02	0.36±0.02	0.38±0.02	0.39±0.02	0.39±0.02	0.39±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	77.2±2.02	78±3.02	79.1±1.02	81±2.03	88.5±2.01	87.9±2.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.015±0.01	0.014±0.01	0.017±0.01	0.021±0.01	0.021±0.01	0.022±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	61.5±2.03	61.3±2.02	63.2±2.03	63.5±2.02	68±2.02	66±2.05

Table 4.16b: Soil Improvement with *Chromolaena odorata* Shoot Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.3±0.01	16.3±0.01	17.2±0.12	17.8±0.02	18.9±1.02	19.1±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.6±0.02	2.8±0.02	2.9±0.02	3.4±0.02	3.5±0.02	3.7±0.02
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.6±0.02	3.8±0.02	4.1±0.02	4.2±0.05	4.7±0.02	4.9±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	56±2.01	64.5±0.01	69.5±0.05	74±0.04	79.6±0.03	85.5±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	35±0.03	34.5±0.03	37.5±0.03	42±0.03	46.5±0.04	44.5±0.04
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	2.0±0.02	2.2±0.02	2.1±0.02	2.5±0.02	2.7±0.02	2.9±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	15±0.02	15.2±0.01	16.3±0.01	16.8±0.02	17.2±1.02	17.9±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.3±0.02	3.3±0.02	3.6±0.02	4.1±0.02	4.4±0.02	4.6±0.02
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.34±0.02	0.36±0.02	0.37±0.02	0.38±0.02	0.39±0.02	0.41±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.6±0.01	1.6±0.05	1.7±0.00	1.9±0.01	2.1±0.02	2.4±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.35±0.02	0.36±0.02	0.39±0.02	0.39±0.02	0.44±0.02	0.41±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	76.2±2.02	78±3.02	78.1±1.02	82±2.03	85.5±2.01	89.5±2.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.013±0.01	0.013±0.01	0.015±0.01	0.017±0.01	0.019±0.01	0.022±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	62.5.±2.03	63±1.02	63.5±2.03	64.5±2.02	68±2.02	67.5±2.05

Table 4.16c: Soil Improvement with *Carica papayas* Shoot Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10.0±0.02	17.6±2.02	16.2±0.01	16.2±0.01	16.7±0.12	16.9±0.02	17.6±1.03	18.2±0.02
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.5±0.01	2.6±0.02	2.8±0.02	2.9±0.02	3.2±0.02	3.3±0.02
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.5±0.02	3.6±0.02	3.7±0.02	3.9±0.02	4.1±0.02	4.3±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	56±2.01	67.5±0.02	69.5±0.05	68±0.02	73.6±0.03	76.2±0.02
Magnesium (Mg) mg/L	21±1.01	36±1.02	35±0.03	36.5±0.03	36.6±0.01	38±0.02	41.5±0.02	43±0.03
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.95±0.02	1.8±0.02	2.1±0.02	2.1±0.03	2.5±0.02	2.7±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	14.5±0.02	15±0.01	16.5±0.01	16.7±0.01	17±1.02	17±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.2±0.02	3.1±0.02	3.5±0.02	3.7±0.02	3.9±0.02	4.2±0.02
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.31±0.02	0.32±0.02	0.34±0.02	0.36±0.02	0.35±0.02	0.38±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.4±0.01	1.45±0.01	1.55±0.00	1.52±0.01	1.7±0.02	2.0±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.35±0.02	0.35±0.02	0.36±0.01	0.37±0.12	0.35±0.05	0.39±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	75.4±1.02	76±1.02	77.5±1.02	78±2.01	83±2.01	84.5±1.00
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.012±0.01	0.012±0.01	0.013±0.01	0.014±0.01	0.014±0.01	0.018±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	61±2.03	61±2.02	62.5±2.01	62.5±0.02	64±1.02	67±2.03

Table 4.16d: Soil Improvement with *Telfairia occidentalis* Fruit Peels Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	13.6±0.01	14.6±0.05	16.6±0.10	16.4±0.02	17.8±1.02	18.3±0.03
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.2±0.02	2.4±0.01	2.6±0.02	2.7±0.05	2.7±0.02	3.2±0.00
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.4±0.02	3.6±0.02	3.7±0.04	3.9±0.02	4.1±0.02	4.2±0.02
Calcium (Ca) mg/L	36±2.02	54±2.02	57±0.01	62.5±0.02	64±0.05	66±0.03	68.2±0.01	71.6±0.03
Magnesium (Mg) mg/L	21±1.01	36±1.02	31±0.03	34.9±0.01	36.5±0.03	39±0.03	41.5±0.04	42±0.04
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.7±0.02	1.8±0.02	2.0±0.03	2.1±0.02	2.2±0.02	2.4±0.01
Zinc (Zn) mg/L	6.9±1.02	16±1.02	14±0.02	14.1±0.03	14.9±0.04	15.8±0.02	16.2±1.02	16.7±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.1±0.02	3.2±0.05	3.2±0.02	3.3±0.02	3.4±0.02	3.7±0.04
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.25±0.02	0.23±0.05	0.26±0.02	0.28±0.00	0.31±0.02	0.33±0.03
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.4±0.03	1.3±0.02	1.4±0.00	1.6±0.01	1.6±0.05	2.0±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.31±0.02	0.32±0.01	0.33±0.02	0.35±0.01	0.36±0.02	0.37±0.03
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	71.2±0.02	73±0.02	73.1±1.02	75±2.03	78.5±2.01	81.1±1.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.011±0.01	0.011±0.01	0.013±0.01	0.015±0.01	0.015±0.01	0.015±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	61.5±0.03	61.3±0.02	62.5±1.03	63.2±1.05	64.2±2.00	64±0.05

Table 4.16e: Soil Improvement with *Arachis hypogaea* Hull Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	13.5±0.02	13.6±0.01	14.2±0.10	14.8±0.05	16.4±0.02	16.9±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.3±0.02	2.4±0.05	2.6±0.02	2.4±0.02	2.6±0.02	2.7±0.02
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.2±0.01	3.3±0.02	3.5±0.01	3.6±0.05	3.8±0.02	4.0±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	56±1.01	61.1±0.01	62.5±0.01	63±0.04	65.1±0.03	68.1±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	32±0.01	35.5±0.02	36.5±0.03	37±0.03	37.5±0.02	39±0.01
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.9±0.02	1.9±0.01	2.0±0.02	2.2±0.01	2.4±0.02	2.6±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	15±0.02	16.1±0.01	16.2±0.01	16.3±0.01	16.6±1.02	16.6±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.1±0.02	3.2±0.00	3.2±0.02	3.3±0.02	3.4±0.05	3.7±0.03
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.29±0.02	0.31±0.01	0.33±0.02	0.33±0.03	0.34±0.02	0.33±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.0±0.01	1.1±0.02	1.3±0.02	1.5±0.01	1.6±0.02	1.6±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.33±0.01	0.34±0.02	0.34±0.00	0.35±0.02	0.36±0.02	0.36±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	69.2±0.02	71±1.02	71.4±1.02	73±0.03	74.4±2.00	76.2±2.00
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.011±0.01	0.010±0.01	0.011±0.01	0.011±0.01	0.012±0.01	0.013±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	57.3.1±1.03	60.1±2.02	60.2±0.03	61.2±0.02	63±2.00	63.6±0.05

Tables 4.16 (f-j) shows the comparative soil fertility improvement achieved in the experimental soils with application of biofertilizers from co-digestion experiments. In the *Tithonia diversifolia* shoot + poultry dropping biofertilizer plot, the improvement in the fertility of tested soils after harvesting was lowest in the negative control and highest in the 60 kg N/ha experimental soil. In all, the highest values of nitrogen (19.7 mg/L), phosphorus (3.8 mg/L), potassium (4.8 mg/L), calcium (83 mg/L), magnesium (46 mg/L), copper (2.9 mg/L), zinc (17.7 mg/L), iron (4.6 mg/L), aluminium (0.40 mg/L), nitrates (2.5 mg/L), ammonium (0.43 mg/L), phosphate (89.9 mg/L), manganese (0.020 mg/L) and sulphate (70.5 mg/L) were all recorded in the 60 kg N/ha experimental soils.

In the *Chromolaena odorata* shoot + poultry dropping biofertilizer plot, soil nutrient improvement also followed an increasing pattern with the quantity of applied fertilizer. Here, the lowest fertile soil soil after plant harvesting was the negative control with no initial fertilizer application while the most fertile soil was the 60 kg N/kg experimental soil. The highest levels of phosphorus (3.8 mg/L), potassium (4.9 mg/L), calcium (84 mg/L), magnesium (47 mg/L), copper (2.8 mg/L), zinc (17.8 mg/L), iron (4.7 mg/L), aluminium (0.38 mg/L), nitrates (2.4 mg/L), ammonium (0.45 mg/L), phosphate (89 mg/L) and sulphate (73 mg/L) were all recorded in the 60 kg N/ha experimental soils while those of nitrogen (20.6 mg/L) and manganese (0.021 mg/L) were recorded in the 50 kg N/ha experimental soil.

In the *Carica papaya* peels + poultry dropping biofertilizer plot, soil fertility improvement was highly enhanced with the addition of the biofertilizer. after harvesting of plants, the soils with the lowest soil fertility was the control while the highest fertility was found in the 60 kg N/ha experimental soils like other plots. The highest levels of nitrogen (19 mg/L), phosphorus (3.4 mg/L), potassium (4.4 mg/L), calcium (76.4 mg/L), iron (4.4 mg/L), nitrates (2.0 mg/L), manganese (0.016 mg/L) and sulphate (65.5 mg/L) were all recorded in the 60 kg N/ha experimental soils. The highest values of magnesium (44.5 mg/L), copper (2.8 mg/L), zinc (17.2 mg/L) aluminium (0.38 mg/L) and phosphate (88.5 mg/L) were recorded in the 50 kg N/ha experimental soils while that of ammonium (0.39 mg/L) was found in the 40 kg N/ha experimental soils.

In the *Telfairia occidentalis* fruit peels + poultry dropping biofertilizer plot, soil fertility improvement was observed after harvesting of plants. The soils with the lowest soil fertility was the control while the highest fertility was found in the 60 kg N/ha experimental soils like other plots. The highest levels of nitrogen (18.6 mg/L), phosphorus (2.9 mg/L), potassium (4.2 mg/L), calcium (74.2 mg/L), zinc (16.9 mg/L), iron (4.2 mg/L), nitrates (2.3 mg/L), phosphate (85.9 mg/L), manganese (0.016 mg/L) and sulphate (65 mg/L) were all recorded in the 60 kg N/ha experimental soils. The highest values of magnesium (44.5 mg/L), copper (2.8 mg/L), aluminium (0.38 mg/L) and ammonium (0.38 mg/L) were recorded in the 50 kg N/ha experimental soils.

In the *Arachis hypogaea* hull + poultry dropping biofertilizer plot, the improvement in the fertility of soils after plant harvesting was lowest in the negative control and highest in the 60 kg N/ha experimental soil as observed in some other plots. The highest values of nitrogen (18.4 mg/L), phosphorus (3.5 mg/L), potassium (4.5 mg/L), calcium (76.5 mg/L), magnesium (43 mg/L), copper (2.7 mg/L), zinc (17.8 mg/L), iron (4.2 mg/L), aluminium (0.38 mg/L), nitrates (2.0 mg/L), ammonium (0.39 mg/L), phosphate (84.5 mg/L), manganese (0.018 mg/L) and sulphate (68 mg/L) were all recorded in the 60 kg N/ha experimental soils.

Overall, the soils in all the ten biofertilizer plots were observed to be richer than both the negative control and the NPK 15-15-15 experimental plots in the composition of all the nutrients evaluated. The experimental plot with the highest levels of soil nutrient improvement was the *Chromolaena odorata* shoot + poultry dropping biofertilizer plot where the highest values of all important soil nutrients and elements were found after plant harvest. Also, the 60 kg N/ha experimental runs recorded the highest levels of nutrient improvement in all the biofertilizer experimental plots.

Table 4.16f: Soil Improvement with *Tithonia diversifolia* Shoot + Poultry Dropping Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.4±0.02	16.8±0.01	17.3±0.12	18.7±0.02	19.6±1.02	19.7±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.6±0.02	2.6±0.02	2.8±0.02	2.9±0.02	3.6±0.02	3.8±0.05
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.5±0.02	3.6±0.12	3.7±0.02	3.8±0.01	4.6±0.02	4.8±0.02
Calcium (Ca) mg/L	36±2.02	54±2.02	57±2.01	60.5±0.01	64.5±0.05	75±0.04	78.6±0.03	83±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	35±0.01	37.5±0.03	39.5±0.03	44±0.03	45±0.02	46±0.02
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.95±0.02	2.0±0.02	2.3±0.01	2.2±0.01	2.9±0.02	2.9±0.01
Zinc (Zn) mg/L	6.9±1.02	16±1.02	14±0.02	13.9±0.01	16.5±0.01	16.8±0.02	17.5±0.02	17.7±0.05
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.0±0.02	3.5±0.02	3.3±0.02	3.7±0.01	4.5±0.02	4.6±0.01
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.32±0.02	0.33±0.02	0.36±0.02	0.37±0.02	0.38±0.02	0.40±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.3±0.01	1.5±0.01	1.6±1.00	2.2±0.01	2.4±0.02	2.5±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.34±0.02	0.38±0.02	0.38±0.01	0.40±0.02	0.41±0.02	0.43±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	74.2±2.02	76±3.02	79±1.02	85±2.03	89±2.01	89.9±0.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.015±0.01	0.012±0.01	0.017±0.01	0.019±0.01	0.018±0.01	0.020±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	65±2.03	63±0.02	63.3±0.03	65±0.02	67±2.02	70.5±2.00

Table 4.16g: Soil Improvement with *Chromolaena odorata* Shoot + Poultry Dropping Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.4±0.02	17.1±0.01	17.3±0.12	18.7±0.02	20.6±1.02	19.8±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.6±0.02	2.6±0.02	2.8±0.02	2.9±0.02	3.6±0.02	3.8±0.05
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.5±0.02	3.6±0.12	3.7±0.02	3.8±0.01	4.6±0.02	4.9±0.02
Calcium (Ca) mg/L	36±2.02	54±2.02	57±2.01	60.5±0.01	64.5±0.05	75±0.04	77.6±0.03	84±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	35±0.01	37.5±0.03	39.5±0.03	44±0.03	45±0.02	47±0.02
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.9±0.02	2.1±0.02	2.1±0.02	2.6±0.01	2.8±0.02	2.8±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	16±0.02	15.6±0.01	16.5±0.01	16.8±0.02	17.3±1.00	17.8±0.05
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.4±0.02	3.4±0.00	3.7±0.02	4.0±0.02	4.4±0.02	4.7±0.04
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.34±0.02	0.35±0.02	0.36±0.02	0.35±0.02	0.36±0.02	0.38±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.5±0.01	1.7±0.01	1.9±0.00	2.1±0.01	2.2±0.02	2.4±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.35±0.02	0.36±0.02	0.38±0.02	0.39±0.02	0.42±0.02	0.45±0.01
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	74.2±2.02	78±3.02	79.1±1.02	81±2.03	86±1.05	89±0.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.015±0.01	0.014±0.01	0.017±0.01	0.020±0.01	0.021±0.01	0.020±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	65±0.03	61.3±0.02	66±2.03	65±2.00	71±2.02	73±0.05

Table 4.16h: Soil Improvement with *Carica papaya* Peels + Poultry Dropping Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.2±0.01	16.5±0.02	17.4±0.12	18.4±0.02	18.8±1.02	19±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.4±0.02	2.6±0.02	2.6±0.02	2.8±0.02	3.3±0.02	3.4±0.02
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.6±0.02	3.8±0.02	3.9±0.02	4.1±0.05	4.3±0.02	4.4±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	59±2.01	66.5±0.01	65.8±0.05	70±0.04	72±0.03	76.4±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	36±0.03	37.5±0.03	39.5±0.03	42±0.03	44.5±0.04	44±0.04
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.9±0.02	2.1±0.02	2.1±0.02	2.4±0.02	2.8±0.02	2.6±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	16±0.02	15.6±0.01	16.5±0.01	16.8±0.02	17.2±1.02	17.1±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.4±0.02	3.4±0.02	3.7±0.02	4.0±0.02	4.4±0.02	4.4±0.02
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.34±0.02	0.35±0.02	0.36±0.02	0.38±0.02	0.38±0.02	0.37±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.5±0.01	1.7±0.01	1.7±0.00	2.0±0.01	2.0±0.02	2.0±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.35±0.02	0.35±0.01	0.36±0.02	0.39±0.02	0.37±0.02	0.38±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	77.2±2.02	78±3.02	79.1±1.02	81±2.03	88.5±2.01	87.9±2.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.015±0.01	0.014±0.01	0.015±0.01	0.014±0.01	0.013±0.01	0.016±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	61.5±1.03	61.3±2.02	63.2±2.03	63.5±2.02	64.3±2.02	65.5±2.00

Table 4.16i: Soil Improvement with *Telfairia occidentalis* Peels + Poultry Dropping Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.2±0.01	16.5±0.02	16.4±0.12	17.4±0.02	18.3±0.02	18.6±0.01
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.4±0.02	2.6±0.02	2.6±0.02	2.65±0.00	2.53±0.02	2.9±0.01
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.5±0.02	3.6±0.02	3.7±0.02	3.8±0.05	4.0±0.02	4.2±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	56±0.01	64.5±0.01	65.8±0.05	70±0.04	70±0.03	74.2±0.05
Magnesium (Mg) mg/L	21±1.01	36±1.02	36±0.03	37.5±0.03	39.5±0.03	42±0.03	44.5±0.04	44±0.04
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.9±0.02	2.1±0.02	2.1±0.02	2.4±0.02	2.8±0.02	2.6±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	16±0.02	15.6±0.01	15.8±0.01	16.8±0.02	16.8±1.02	16.9±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.4±0.02	3.4±0.02	3.5±0.02	4.0±0.02	4.0±0.02	4.2±0.02
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.34±0.02	0.35±0.02	0.36±0.02	0.38±0.02	0.38±0.02	0.37±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.5±0.01	1.7±0.01	1.7±0.00	2.0±0.01	2.0±0.02	2.3±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.33±0.02	0.35±0.01	0.36±0.02	0.37±0.02	0.38±0.02	0.36±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	73.2±2.02	71±3.02	74.1±0.02	81±2.03	84.5±2.01	85.9±1.05
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.015±0.01	0.014±0.01	0.015±0.01	0.014±0.01	0.014±0.01	0.016±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	61.5±1.13	61.3±2.02	63.2±2.03	63.5±2.02	64.5±0.02	65±2.01

Table 4.16j: Soil Improvement with *Arachis hypogaea* Hull + Poultry Dropping Biofertilizer

Parameters	Control	NPK	10 kg N/ha	20 kg N/ha	30 kg N/ha	40 kg N/ha	50 kg N/ha	60 kg N/ha
Nitrogen (N) mg/L	10±0.02	17.6±2.02	16.5±0.01	16.5±0.01	16.8±0.12	17.4±0.02	17.9±1.03	18.4±0.05
Phosphorus (P) mg/L	1.1±0.01	2.8±0.02	2.5±0.01	2.6±0.02	2.8±0.02	2.9±0.02	3.4±0.02	3.5±0.02
Potassium (K) mg/L	1.6±0.01	3.7±0.01	3.5±0.02	3.6±0.01	3.8±0.02	3.9±0.02	4.1±0.02	4.5±0.03
Calcium (Ca) mg/L	36±2.02	54±2.02	56±2.01	67.5±0.02	69.5±0.05	68±0.05	73.6±0.03	76.5±0.02
Magnesium (Mg) mg/L	21±1.01	36±1.02	35±0.03	36.5±0.03	36.6±0.01	38±0.02	41.5±0.02	43±0.03
Copper (Cu) mg/L	1.01±0.02	2.0±0.02	1.95±0.02	1.8±0.02	2.1±0.02	2.1±0.03	2.5±0.02	2.7±0.02
Zinc (Zn) mg/L	6.9±1.02	16±1.02	14.5±0.02	15±0.01	16.5±0.01	16.7±0.01	17.5±1.02	17.8±0.02
Iron (Fe) mg/L	1.6±0.01	3.3±0.02	3.2±0.02	3.1±0.02	3.5±0.02	3.7±0.02	3.9±0.02	4.2±0.02
Aluminium (Al) mg/L	0.11±0.02	0.33±0.02	0.31±0.01	0.32±0.02	0.34±0.02	0.36±0.02	0.35±0.02	0.38±0.02
Nitrate (NO_3) mg/L	0.4±0.02	1.6±0.02	1.4±0.01	1.45±0.01	1.55±0.00	1.52±0.01	1.7±0.02	2.0±0.02
Ammonium (NH_4) mg/L	0.11±0.01	0.36±0.02	0.35±0.02	0.35±0.02	0.36±0.01	0.37±0.12	0.35±0.05	0.39±0.02
Phosphate (PO_4) mg/L	43.2±1.02	75.6±4.02	75.4±1.02	76.7±1.02	77.5±1.02	78±2.01	83±2.01	84.5±1.00
Manganese (Mn) mg/L	0.006±0.01	0.013±0.02	0.012±0.01	0.012±0.01	0.013±0.01	0.013±0.01	0.014±0.01	0.018±0.01
Sulphate (SO_4) mg/L	34±1.02	62.5±2.02	61±2.03	61±2.02	65±2.01	62.5±0.02	64±1.02	68±2.03

CHAPTER FIVE

5.0 DISCUSSION

5.1. Effects of Thermo-Alkaline Pretreatment on Biomass

The application of thermo-alkaline pretreatment in this study brought about the solubilization of important components of the lignocellulosic biomass used in both mono and co-digestion as evident in the quantities of biogas generated and the methane contents.A major factor that brought about the difference in this study is the use of heating for the substrates pretreatment. Heating in this context ensured adequate breakdown and solubilization of the lignin, cutin, suberin and other cellulosic components of the materials. Again, the range of the chosen temperature (80^{o} C) helped to counterbalance the negative effects earlier reported with the use of higher temperature ranges (>100^{o} C) (Rafique *et al*., 2012). Prior to this research, a similar result with the application of alkaline pretreatment at 100^{o} C using 1 g/100 g VS NaOH concentration on grass silage has been reported (Xie *et al*., 2011). Other studies have reported similar trends with the application of thermo-alkaline pretreatment to corn stover and other lignocellulosic substrates (Zhu *et al*., 2010). Alkaline pretreatment has also been reported as adequate for ensiled sorghum as an increase of 25% biogas yield was obtained and further use of pretreated biomass as appropriate feedstock for biomethane generation was suggested (Sambusiti *et al*., 2012).

5.2. Physical Parameters of Biomass

The pH values recorded for all the digestion regimes in this study are within the experimental design range and agrees with previous works that the suitable pH for the efficient proliferation and activities of anaerobic microorganisms especially members of the archaea is between 6.5 and 8 (Marrugo *et al*., 2016). A pH range of less than 6.5 or higher than 8 has been reported to hinder the success of anaerobiosis in biogas generation (Zonta *et al*., 2013; Fierro *et al*., 2016; Yap *et al*., 2016). The relative abundance of anaerobic microbial species has been reported to increase at alkaline pH (Pang *et al*., 2008). This was reported in this study as the population of facultative anaerobes and methanogens were at their peak between the 4^{th} and 6^{th} weeks of digestion when the pH of the medium was alkaline. Thus, maintenance of suitable pH in anaerobic digesters is therefore fundamental to ensuring adequate substrate bioconversion, high biogas yield and

subsequent digester stability (Zheng *et al*., 2014; Zahedi *et al*., 2016). Temperature is another important factor in anaerobiosis because the various arrays of bacteria and archaea responsible for the bioconversion of substrates are known to be efficient at specific temperatures (Jain *et al*., 2015; Mckennedy and Sherlock, 2015). The mesophilic temperature such as employed in this study ensured better digester stability and provides the needed condition for bacteria proliferation and efficiency (Kwietniewska and Tys, 2014; Mao *et al*., 2015).

The retention time for all the digestion regimes in this study remained between 20 and 30 days according to experimental design. This also agrees with the work of Mao *et al.,* (2015) who reported that for the efficiency of anaerobic microorganisms and to ensure high digestion rate of lignocellulosic biomass, a retention day of between 20 and 30 days should be adopted. The same trend was observed for total solids and volatile solids whose values were between 4 and 12 g/kg throughout the experiment according to experimental design. The values obtained in this study, therefore, agree with the report of Kougias *et al.,* (2013) and Kim *et al.,* (2015).

5.3. Chemical Parameters of Biomass

The analysis shows that all the five substrates and their mixtures with poultry droppings were rich in nutrients and basic mineral elements required for microbial growth and subsequent substrate degradation in a fermentation medium. The bulkiness of the poultry manure could be traced to the less efficient digestion systems in poultry which would usually leave much of the components of their diets undigested thus ensuring bulkiness of the droppings. Another factor is the increased moisture content of the mixture of the weed and poultry manure due to dilution prior to digestion. Again, the rumen content had undergone more prior digestion in the stomach compartment as against the poultry dropping that went through little or no digestion as a result of less efficiency in the bird's alimentary canal (Alfa *et al*., 2014a). The characteristics of most of these materials are comparable with those of *Cymbopogon citratus* earlier utilized in anaerobic digestion processes (Alfa *et al*., 2014b). The high nutrients and elemental content of these substrates could be due to the high ability of some of them, especially the succulent plants for absorption and storage of elemental nutrients in their tissues. The C/N ratio of the substrates in all experiments was similar to the value (17/1) previously obtained by

Degueurce *et al*. (2016) from digesting spent cow bedding but lower than the 20-28 reported for the digestion of different animal beddings (Riggio *et al*., 2017). The types and concentration of intermediate acids (VFAs) reported in this study corresponds to those reported earlier (Zhang *et al*., 2016; Riggio *et al*., 2017).

5.4. Biogas Generation

The quantity of gas generated from the substrates could be as a result of high carbon contents and the combination of different (Mechanical and thermo-alkaline) pretreatments prior to digestion as an improvement over previous studies carried out without pretreatment or those that employed only one pretreatment method. Generally, the use of appropriate pretreatment procedures has been recommended for enhancement of biogas generation from lignocellulosic biomasses (Jain *et al*., 2015; Kim *et al*., 2015; Li *et al*., 2015).

5.5. Microbial Diversity and Succession

Diverse microorganisms belonging to different acidogenic and methanogenic genera were isolated during the anaerobic digestion process for all studied biomass. Most of the organisms have been earlier encountered in anaerobic digestion processes (Jain *et al*., 2015). Their source could be traced to the inoculum and poultry dropping used as co-substrate in the co-digestion regimes. The diverse and high population of bacterial species especially members of the genera *Clostridia* (usually found abundant in poultry wastes) may have contributed to the pronounced acetogenesis/methanogenesis stage. These bacteria are amino-acid-utilizing and are capable of degrading amino-acids thereby producing acetate, propionate, and ammonia as end-products (Zhang *et al*., 2016).

The succession pattern of the microorganisms revealed changes in population at different points of the digestion. These population changes are due to fluctuations in environmental condition of digestion i.e temperature and pH. Anaerobic organisms (Anaerobes and methanogens) are highly sensitive to extremities of environmental factors such as pH and temperature (Zonta *et al*., 2013; Jain *et al*., 2015; Mao *et al*., 2015; Mckennedy and Sherlock, 2015). Aerobes and fungi had their highest populations during the first week of the experiment and this is due to the slightly acidic state of the fermenting material which supported fermenting aerobes and fungal proliferation. Facultative anaerobes and methanogens thrived most during the latter part of the experiments due to the very alkaline

nature of the medium which is the best pH range for their growth (Zonta *et al*., 2013; Mao *et al*., 2015). The microbial optimization studies carried out in this work revealed lower biogas yield (highest value was 3474.50 10^{-3} m^3/ kg VS) than in the use of microbial consortia (highest value was 4178.81 10^{-3} m^3/ kg VS). This could be due to factors such as less synergy between the organisms in terms of substrate co-metabolism, reduced population and diversity.

5.6. Composition of Anaerobic Digestates

The anaerobic digestates obtained in this study had elevated nutrient compositions as against their lower values prior to digestion. This trend was recorded for elements such as nitrogen, phosphorus, potassium, magnesium, manganese, iron, zinc, aluminium and copper. Therefore, these digestates are rich in nutrient and have great potentials to increase both the microbial and nutrient status of soil when applied through the rhizosphere as fertilizers especially in nutrient-depleted soils. Plant growth and general well-being could also be enhanced via the use of such digestate especially in the Sub-Saharan Africa and beyond where issues of soil nutrient depletion, toxicity to soil microflora and pollution is rampant (Alfa *et al.,* 2014b). Several studies have reported the potentials of anaerobic digestates as suitable replacements for inorganic chemical fertilizers which over the decades have impacted adversely on the ecosystem (Alfa *et al*., 2013a, b; Pivato *et al*., 2015; Sun *et al*., 2015; Westphal *et al*., 2016). The reduction observed in the values of total carbon and calcium in the digestate could be due to the uptake/usage of these components for metabolism and as precursors for microbial cell wall synthesis. The moderate nitrogen levels (25.12 to 37.31 g/kg TS) of the substrates in this study prevented the nitrogen inhibition usually encountered in anaerobic digestions and similar results have been obtained in the co-digestion of food wastes and spent animal beddings (Zhang *et al*., 2016; Riggio *et al*., 2017). The anaerobic digestion in this study was very efficient in COD removal. The values recorded (up to 64%) are higher than the 41-47% reduction obtained in some previous anaerobic digestion studies (Qiao *et al*., 2011; Alfa *et al*., 2014b).

5.7. OptimizationStudy

The F-values of all the models in this study with low p-values obtained revealed the significance of the regression models and this agrees with the report of Montingelli *et al*. (2016). In the use of the"coefficient of determination" (R^2)in checking the goodness of fit

of all the models, it has been severally reported that R^2 should be at least 0.80 for the good fit of a model (Guan and Yao, 2008; Niladevi *et al*., 2009; Reungsang *et al*., 2012; Pei *et al*., 2014). The R^2 values obtained for the optimization of all the five mon-digestions were 0.8802, 0.8680, 0.9239, 0.8996 and 0.8676 respectively. Those obtained for the co-digestions were 0.8674, 0.9009, 0.9181, 0.8827 and 0.9045 respectively. These values implied that the sample variations of 88.02, 86.80, 92.39, 89.96, 86.76, 86.74, 90.09, 91.81, 88.27 and 90.45 % for the biogas yield are a function of the interaction between the five independent variables used in the modeling and optimization studies. The adequate precision of a model is a measure of the accuracy and a ratio greater than 4 is usually acceptable for the good fitting of a model. The values of 10.596, 7.607, 13.883, 8.009, 10.764, 9.270, 11.950, 10.461, 12.438 and 11.627 obtained for the ten different digestions are good indications that the models are suitable for the design. All the low p-values i.e. > 0.05 (Appendix 2 a-j) is a further proof that the models are adequate for describing the relationships among the variables in the study. The lack-of-fit terms of 0.1755, 0.9727, 0.5770, 0.1735, 0.0275, 0.0949, 0.0590, 0.6235, 0.7359 and 0.2255 for the ten digestions were not significant except for the mono-digestion of *Arachis hypogaea* hull (0.0275) and this implied that the models are effective in the theoretically predicting biogas generation. All negative and positive values in equations 4.1 to 4.10 suggests that the variables have both negative and positive effects on the biogas yield.

All the RSM' 3-dimensional response surface plots (Figures 4.1 and Appendix 8) for the regression equation showed moderate relationships between the five variables while those of ANNs showed pronounced relationships/interactions. Such kind of interactions had been observed in earlier studies involving the use of RSM and ANNs for prediction in bioprocessing operations (Yusof *et al*., 2014; Betiku *et al*., 2015; Emeko *et al*., 2015).

In all the experiments, though RSM predicted higher gas yield than ANN, the latter gave higher accuracy. This was premised on the fact that all the RSM models recorded higher error values than ANNs while their R^2 were equally lower than those of ANNs. Similar results have been obtained in the use and comparison of RSM and ANNs for prediction of biogas and biodiesel productions from different materials (Yusof *et al*., 2014; Betiku *et al*., 2015; Emeko *et al*., 2015).

5.8. Physicochemical Composition of Biofertilizers

In the physicochemical assessments of the newly produced biofertilizers, the *Telfairia occidentalis* + poultry dropping biofertilizer was the richest in terms of some major and minor elements like copper, iron, magnesium, manganese, phosphate, sulphate and phosphorus. This could be related to the richness of the raw materials (*Telfairia occidentalis* and poultry dropping) used in the production of the biofertilizer. Both materials are veritable sources of nutrients and essential elements needed for crop plant's growth and general wellness (Table 4.1a). The *Tithonia diversifolia* + poultry dropping biofertilizer was equally found to be the richest in the composition of potassium, zinc, and aluminium; Calcium was highest in the *Chromolaena odorata* biofertilizer while the richest biofertilizer in terms of nitrogen composition was the *Chromolaena odorata* + poultry dropping biofertilizer. As already stated above, all these compositions are functions of the type of materials used in producing the different biofertilizers (Westphal *et al*., 2016). It has earlier been reported that the quality of a digestate is determined by the nature of raw materials used in the production (Alfa *et al*., 2014a, b; Martí-Herrero *et al*., 2015). All the biofertilizers produced from the anaerobic co-digestion were richer in nutrients and elemental compositions than the ones without poultry dropping and this could be due to the richness of poultry waste as a good source of nutrients especially nitrogen and ammonium compounds (Borowski *et al*., 2014). It has been opined that for the generation of richer digestate, the co-digestion of poultry waste with other high energy-yielding substrates such as grasses, silage, and other green biomasses be embraced (Dalkilic and Ugurlu, 2015; Dareioti and Kornaros 2015; Khoufi *et al*., 2015; Riggio *et al*., 2015).

5.9. Microbial Composition of Biofertilizers

Analysis of all the anaerobic digestates and the resulting biofertilizers revealed higher microbial load in the digestates than in the dewatered biofertilizers. This could be due to the absence or reduction of water/moisture in the biofertilizers since water aids microbial proliferation and its absence could cause desiccation and death of microbial cells. However, all the biofertilizers are rich in microorganisms which are often used as microbial inoculant for soil fertilization and nutrient improvement. Suitable inoculants like *Bacillus, Clostridium* and *Aspergillus* were present in all the biofertilizers and these are

known to quicken the microbial processes in the soil by increasing the availability of nutrients that can be assimilated by plants (Tamil Nadu Agricultural University, 2008). *Clostridium species* are free living nitrogen fixers while *Bacillus species* are phosphate solubilizers (Alfa *et al*., 2014a). Earlier researches reported that biofertilizers contain more readily available nutrients than undigested materials and it is thus better for crops fertilization (Lansing *et al*., 2010; Garfi*et al*., 2011; Goberna *et al*., 2011). Unlike chemical fertilizers, they are also important in the provision of many ecological benefits including food quality improvement and environmental quality enhancement (Grigatti *et al*., 2011; Johansen *et al*., 2013).

5.10. Phyto-Assessments

As seen in all experiments in the phyto assessments, comparison between values obtained from the phyto experiments involving all the ten produced biofertilizers, the NPK 15-15-15 inorganic fertilizer and the negative control (No fertilizer application) showed that all the ten biofertilizers produced better results in the performance of the maize plants especially at higher biofertilizer application (30 to 60 kg N/ha) rates. All the phyto-parameters evaluated recorded higher values than the NPK inorganic fertilizer and the negative control experiments except for root length where the value recorded for NPK was higher than that of *Chromolaena odorata, Tithonia diversifolia, Telfairia occidentalis* and *Arachis hypogaea* biofertilizers respectively. This is an indication that the biofertilizers contained and subsequently released more nutrients and required elements to the plants which in turn enhanced their performance than those with NPK 15-15-15 application. This corroborates the earlier report that anaerobic digestate contains a relatively high proportion of mineral nutrients, which gives digestate enormous fertilizing potentials to replace inorganic fertilizers, especially because of the nutrients originally present in the raw materials before digestion remains after digestion (Alburquerque *et al*., 2012). This also agrees with the report of Babalola, (2010) and that of Suarez *et al*. (2015) that biofertilizers application stimulates plant growth by different mechanisms such as atmospheric nitrogen fixation, phosphorus solubilization, and mobilization, sequestration of iron by siderophores, phytohormones production etc.

The over reliance on inorganic fertilizers especially in the tropics has resulted in soil quality reduction and environmental degradation, risks to biodiversity, eutrophication, and

heavy metals pollution are becoming increasingly serious (Zhu *et al*., 2010). Organic fertilizers such as produced and used in this study have the potential for balanced and sustainable nutrient supply which is an advantage over the inorganic fertilizers (Dittmar *et al*., 2000; Chen, 2006; Sun *et al.,* 2015).

5.11. Soil Quality Improvement

Organic fertilizers are also known to modify soil physical conditions via the improvementof soil aggregation, increased soil hydraulic conductivity and reduction of mechanical resistance (Hati *et al*., 2006; Bhattacharyya *et al*., 2007; Sun *et al.,* 2015; Zhao *et al*., 2016). One of the most attractive ways to manage anaerobic digestate is its application as biofertilizer to the soil, since this allows the nutrients (mostly nitrogen and phosphorus) to be recovered and also to control the loss of organic matter by soils under agricultural exploitation (Pivato *et al*., 2015; Riva *et al*., 2016). In the soil fertility improvement studies carried out, the soils in all the ten biofertilizer plots were observed to be richer than both the negative control and the NPK 15-15-15: experimental plots in the composition of all the nutrients evaluated. The experimental plot with the highest levels of soil nutrient improvement was the *Chromolaena odorata* shoot + poultry dropping biofertilizer plot where the highest values of all important soil nutrients and elements were found after plant harvest. This is due to the fact that the *Chromolaena odorata* shoot + poultry dropping biofertilizer was the richest in nitrogen and also contained very high quantities of other essential mineral elements. Biofertilizer application/addition is a popularpractice geared towards the management of soil via the enhancement of soil fertility and agricultural productivity (Shen *et al*., 2010). To this extent, the use of digestates from anaerobic digestion has increased fertilizer utilization and reduced chemical consumption in many cropping systems globally (Sun *et al*., 2015).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The mono digestion of the five biomass and their co-digestions with poultry droppings were found to yield biogas/methane as seen in this study as well as achieving a high reduction in the initial COD values of all the fermenting materials. The present study utilized *Tithonia diversifolia* and *Chromolaena odorata* shoot for energy generation since green plants are natural sinks for enormous energy as a result of photosynthesis. Their abundance in several locations around the world is an indication that a veritable and environmental-friendly usage needs to be sought for the weeds. Based on available literature, this appears to be the first study that elucidated the impacts of pretreatment combinations on biomass degradation for biogas production enhancement as well as the process parameters optimization of biogas generation from the succulent plants as improvement over previous studies. Since no permanent solution has been documented for these weed's invasion across the world and the challenges they pose to agriculture, this research has proposed solution to this barrier.

Carica papaya, Telfairia occidentalis, and *Arachis hypogaea* are crops well adapted to several geographical locations especially in the tropics and have been put to numerous uses. However, the fruit peels of these crops still remain grossly unutilized despite their abundant biomass potentials via production of very big/numerous fruits. Their biogas-producing potentials with pretreatment combinations have also been reported in this study. On the other hand, poultry waste/dropping is a common resource in most environments. The richness of all the biomass used in this study and those of the poultry droppings in terms of minerals and elemental composition suggest that they are all suitable candidates for the biotechnological biofuel and biofertilizer productions. Both RSM and ANNs models are efficient in the biogas production prediction from all the substrates in mono and co-digestions with poultry droppings with ANNs being the desirable model.Access to cheap and basic energy is one of the major challenges in Nigeria and Sub-Saharan Africa despite the presence of huge biomass that could be exploited in the generation of environmental-friendly, sustainable and renewable energy. It is therefore proposed that profound and further use of the biomass used in this study be carried out due to their

abundance in several localities in Nigeria as this could contribute to solving our energy and environment crisis.

Analyses showed that all the anaerobic digestates produced after the digestions in this study were very rich in mineral elemental compositions. Their subsequent applications on soils planted with maize revealed their crop growth enhancement and soil fertility improvement abilities. All the produced biofertilizers performed better than the NPK 15-15-15 used as a control. The over dependence on inorganic chemical fertilizers in Nigeria and other nations in the tropics has resulted in soil quality reduction, environmental degradation, risks to biodiversity (soil micro and macro fauna), eutrophication, as well as heavy metals pollution. The adoption of organic agriculture via the usage of anaerobic digestates as biofertilizers and soil conditioners is a veritable way of overcoming this challenge.

6.2. Recommendations for Further Studies

1. Further use of the five biomass used in this study is solicited especially in co-digestion with other available high energy-yielding biomasses in order to provide better nutrient balance and microbial diversity
2. The use of molecular biology techniques for the characterization of the microorganisms isolated in this study is recommended so as to fully exploit their biogas-producing potentials
3. Further experiments should be carried out on the biofertilizers produced in this study using different soil types so as to establish a baseline for their effective usage.

CONTRIBUTIONS TO KNOWLEDGE

The following contributions were added to existing knowledge in this project:

1. The biogas producing abilities of five locally available biomass in both mono and co-digestion with poultry dropping with the application of suitable pretreatment procedures documented which revealed higher yield in the co-digestions over the mono-digestions.
2. The optimal conditions for the most efficient biogas generation from the five biomass were established using the Response Surface Methodology and the Artificial Neural Networks with the latter more accurate and precise than the former.
3. The possibility of microbial combination for optimized biogas production from the biomass was documented from which the combination of species of *Clostridium, Fusobacterium* and *Methanosarcinales* gave the highest biogas yield from the digestion of *Chromolaena ododrata* shoot and poultry droppings.
4. The microorganisms responsible for the bioconversion of the five biomass and their succession patterns were documented.
5. The physical, chemical and biological compositions of the anaerobic digestates and biofertilizers from the mono and co-digestions of the biomass were established with the biofertilizers from the co-digestions richer than those from the mono-digestions.
6. The nutrient value and soil improvement abilities of the produced biofertilizers were established and this showed that *Chromolaena odorata* shoot and poultry dropping was the best substrate for biofertilizer production.

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