EVALUATION OF BIOGAS YIELD AND MICROBIAL SPECIES FROM MULTI-BIOMASS FEEDSTOCKS

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CERTIFICATION

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DEDICATION

This dissertation is dedicated to the one and only God that is higher than the highest, wider than the widest, the all supreme God in whose Mercy and Grace, I have been able to complete this course.

I also want to dedicate this dissertation to my parents, Mr. and Mrs. J. N. Oseji for their wonderful support. May God grant you long life to reap the fruit of your labour, Amen!

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ABSTRACT

Virtually all countries, especially the developing nations, are being confronted with the twin problems of waste management and energy deficit. This development has led to the search for renewable energy sources. Although pig dung, water hyacinth and maize cob have been identified as good feedstocks for biogas production, studies utilising their mixtures have not been fully explored. This study was therefore designed to evaluate the biogas yield and microbial species from mixtures of biomass feedstocks.

The feedstocks utilised for this study comprised Pig Dung (PD), Water Hyacinth (WH), and Maize Cob (MC). The PD and WH were sourced from University of Ibadan Teaching and Research Farm while MC was sourced from refuse bins in Oje Market. Six feedstock groups were selected namely PD, WH, MC, PD:MC (PM), PD:WH (PW), and PD:MC:WH (PMW). Each mixture was made in equal proportion on dry weight basis. A simple biogas digester was fabricated from a 10-litre plastic keg for feedstock biodegradation. Each feedstock sample to be digested was prepared by mixing 0.75 kg dry feedstock with 8.25 litres of water in the ratio of 1:11 (w/v) to form slurry. The slurry was fed into the corresponding digester, and kept for 35 days for anaerobic digestion while samples of the effluent were taken at seven days interval for five weeks for laboratory analyses. Parameters including temperature, pH, carbon, nitrogen, carbon to nitrogen (C-N) ratio, potassium, phosphorus, and microbial identification characteristics were determined using standard methods. Gas generated was estimated based on Archimedes' Principle. Data were analyzed using descriptive statistics and ANOVA at $p < 0.05$.

Temperature and pH of all slurries ranged from 25.75±0.4^{o}C to 28.75±0.4^{o}C and 5.80±0.0 to 7.85±0.1 respectively. There was a significant difference in percentage nitrogen, phosphorus and potassium of the various slurries. Mean C-N ratio of the various slurries decreased from day 0 to day 35 as follows: 20.05±2.1 to 16.27±1.1, 23.28±0.1 to 12.95±1.2, 97.54±3.3 to 47.70±1.3, 57.27±0.2 to 28.34±2.1, 28.52±4.2 to 24.19±2.0 and 49.86±2.9 to 37.24±2.4 for PD, WH, MC, PM, PW, and PWM respectively. Predominant organisms identified at day 35 were: *Methanobacterium, Enterobacter* and *Aspergillus spp.* The anaerobic, coliform and fungal counts ranged from 6.80×10^{2} to 1.0×10^{5}cfu/g, 4.3×10^{4} to 6.2×10^{6}cfu/g, and 9.1×10^{3} to 6.3×10^{6}cfu/g respectively throughout the duration of the study. The highest anaerobic count

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($1.0\times10^5\pm0.03\times10^5$cfu/g) was recorded in PW on day 28. Peak biogas production was observed on day 23 for PD (987.50±3.5mL); day 24 for PW (1095.00±7.1mL), and PM (732.50±17.7mL); day 25 for MC (560.00±7.1mL), day 26 for WH (635.00±7.1mL) and PMW (662.50±10.6mL). Group PW had the highest biogas yield of 6067.00±38.2mL for the entire duration of the study. There was a significant difference between the mean biogas yields of the various feedstock groups.

Co-digestion of pig dung with water hyacinth had the highest number of anaerobes and biogas yield as compared to single feedstocks. Therefore, the use of multi-biomass feedstocks for biogas production as a source of alternative energy production should be fully optimised.

Keywords: Biogas yield, Renewable energy, Multi-feedstock, Anaerobic digestion.

Word count: 488

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GLOSSARY OF TECHNICAL TERMS AND ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DM	Dry Matter
DW	Dry Weight
HS	High Solid Systems
IITA	International Institute of Tropical Agriculture
IMRAT	Institute of Medical Research and Advanced Training
LS	Low solid systems
MS	Medium solid Systems
mg/L	Miligram per litre
TAC	Total Aerobic Count
TCC	Total Coliform Count
TFC	Total Fungal Count
TOC	Total Organic Carbon
TS	Total Solid
UCH	University College Hospital
UI	University of Ibadan
UITRF	University of Ibadan Teaching and Research Farm
UASB	Upflow Anaerobic Sludge Blanket Reactor
WW	Wet Weight

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CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Currently, 85% of the world's energy demand is met by combustion of fossil fuels which are depletable. The global energy demand is expected to grow by about 50% by 2025, the major part of this increase coming from rapidly developing countries. Given the growing world population, increasing energy demand per capital and global warming, the need for a long term alternative energy supply is clear (Marchaim and Criden, 1981; Sandia National Laboratories, 2010). Biofuels offer the advantage of coming from large, mainly under-utilised biomass resources that are sustainable and renewable in a closed carbon cycle that reduces environmental input (Sandia National Laboratories, 2010). Biofuels apply to solid, liquid or gaseous fuel produced from biological materials (biomass) which can be used for the generation of power, heat or fuel for motive power (Ugochukwu, 2010; Agba *et al.*, 2010; Bamikole *et al.*, 2008; Leo *et al.*, 2007). Biomass constitutes the feedstock utilized for conversion into biofuels.

The use of biomass as a source of energy has two main advantages: First is its nearly unlimited availability and second is the fact that it can be used without essential damage to the environment. In addition, biomass resources are considered renewable as they are naturally occurring and by comparison with other renewable energy resources such as solar and wind, biomass is a storable resource, inexpensive and has favourable energetic efficiency. Biomass resources available in Nigeria include: Agricultural crops, agricultural crop residues, fuel wood and forestry residues, waste paper, sawdust and wood shavings, residues from food industries, energy crops, animal dung/poultry droppings, industrial effluent/municipal solid waste. (Ugochukwu, 2010 ; Sambo, 2009; Ajueyitsi, 2009; Tayo, 2008).

In Africa, water pollution and access to energy resources present challenges to human health, environmental health, and economic development. In 21 sub-Saharan African countries, less than 10% of the populations have access to electricity (Parawira, 2004). The need for alternative renew-able energy sources from locally available resources cannot be over emphasized, biogas being a viable option. Biogas technology, being an appropriate and economically feasible

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technology that combines solid waste, wastewater treatment and energy production can simultaneously protect the surrounding water resources and enhance energy availability. Biogas technology in which biogas is derived through anaerobic digestion of biomass, such as agricultural wastes, municipal and Industrial waste (water), is one such appropriate technology Africa should adopt to easy its energy and environmental problems. Anaerobic digestion consists of several interdependent, complex sequential and parallel biological reactions in the absence of oxygen, during which the products from one group of microorganisms serve as the substrates for the next, resulting in transformation of organic matter (biomass) mainly into a mixture of methane and carbon dioxide (Parawira, 2004).

1.2 Problem statement

Solid waste disposal is of enormous concern in developing countries, as poverty and urbanization combined with underfunded government prevent efficient management of wastes generated from domestic and industrial activities (UNEP, 2002). Nigeria as a developing nation exemplifies chronic solid waste management problems in conjunction with population growth. It is the most populous country in Africa, with over 162.5 million people in 2011 from 45.2 million in 1960, changing by 251 percent during the last 50 years (National Population Commission). It has 2.29 percent of the world's population (National Population Commission), and over the past 50 years, has had the third largest urban growth rate in the world at 5.51% annually (UNWUP, 1999). In developing countries like Nigeria, there is a much higher proportion of organics, and considerably less plastics. The large amount of organic material makes the waste more dense, with greater moisture and smaller particle size (Cointreau, 1982).

Consequently, the environmental and health impacts of solid wastes are enormous, in the absence of proper management and consist of a large number of components. Putrescible organics have a tendency to generate leachates that need careful management (Haug 1993). Leachates can be acidic, especially when they are generated under anaerobic conditions. They can cause the dissolution of metals and metallic compounds that may be present in organics. Under aerobic conditions, alkaline leachates can be formed from organics with low carbon/high nitrogen ratios, such as food and animal organics. Leachates from composting and related

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organics-processing facilities have the potential to pollute groundwater and surface water bodies (such as rivers, creeks and dams). They can be high in nutrients; this makes them favourable host media for bacteria and other micro-organisms and gives them a high biological oxygen demand (BOD) (Tchobanoglous et al. 1993).

The enormous growth in the world populations, during the last few decades has led to a difficult situation in the field of energy supply and demand. At present, the world is confronted with the twin crises of fossil fuel depletion and environmental degradation. Indiscriminate extraction and consumption of fossil fuels have led to a reduction in the underground carbon sources. The global reserves of primary energy and raw materials are obviously limited. According to an estimate, the reserves will last for 218 years for coal, 41 years for oil, and 63 years for natural gas under a business-as-usual scenario coupled with their inherent environmental impact (Agarwal, 2005). This has made the search for alternative and renewable sources of energy inevitable.

Studies have showed that indiscriminate use of fossil fuels and poor waste disposal methods coupled with natural degradation have led to a significant increase in green house gases over the past decades (Agarwal, 2005). The emission of methane to the atmosphere is reported as the principal greenhouse impact of concern for composting and related organics-processing facilities, because methane has more than 20 times the greenhouse warming potential of carbon dioxide (World Bank, 2006).

1.3 Rationale for the study

The Nation Nigeria, in an attempt to address environmental problems came up with Vision 2010. The report proposed goals to be accomplished by the year 2010 that would lead to sustainable development. As regards to solid waste management; the report says the goal is to “achieve not less than 80% effective management of the volume of municipal solid waste generated at all levels and ensure environmentally sound management” (Vision 2010). Therefore, this research is

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a welcome development, revealing the biogas potential of organic waste; thereby contributing to the realization of the aforementioned goal.

Kyoto's protocol is one of the environmental agreements signed by the firstline countries to reduce green house gas production. This commitment established that every country has to reduce from 40% to 25% green house gas emission by 2020 in relation to its 1990 rate. According to this commitment, the production of biofuels has become a global agenda; hence it becomes imperative for Nigeria as part of the global environment and also as a signatory to the Kyoto's Protocol on Global Warming & Climate Change commitment to participate in this 'bioeconomy revolution'. So most countries are evolving new technologies of producing and using biofuels; biofuels have two main goals: to substitute fossil fuels and to reduce green house gases which are the main culprit in climate change.

Biogas technology constitutes a widely propagated branch of technology with a history of over 30 years ISAT/GTZ (1999). The technology is efficient, well demonstrated and provides a cost-effective method of disposing organic wastes and producing fuel and fertilizers without releasing greenhouse gases (UNDP, 1994). Anaerobic digesters have the ability to destroy pathogenic organisms in wastewater, to produce energy in the form of methane gas, to run water pump engines, electric generators, agricultural machinery, and to produce fertiliser for use in agriculture (LEISA, 2005; Kangmin and Ho, 2006). Integrated systems for the recovery of waste resources and improvements in sanitation should have, at their centre, a biogas reactor (Van Buuren, 1996; Doelle et al, 1998). Biogas is an excellent source of energy and can be used to produce electricity as well as cooking and lighting gas (Doelle *et al*, 1998).

The long term benefits of this research will be to introduce a sustainable solid waste management strategy for a number of livestock manure and other lignocellulosic waste materials; contribute towards the mitigation of greenhouse gases emissions through sustained carbon and nutrient recycling; reduce the potential for water, air, and soil contamination associated with land disposal of organic waste materials; and to broaden the feedstock source of raw materials for the production of biogas.

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1.4 OBJECTIVES OF THE STUDY

1.41 Broad objective

The broad objective of this research is to evaluate the biogas generated and the corresponding microbial species from mixtures of pig dung, maize cob and water hyacinth biomass feedstocks.

1.42 Specific objectives

The specific objectives were to:

1. Assess the availability of the substrates for biogas production.
2. Measured the physic-chemical characteristics of the substrates.
3. Determine the biogas yield from the various digesters.
4. Assess the microbial content of the slurry.
5. Determine the relationship between the biogas yield and the microbial content of slurries across the pig dung, maize cob and water hyacinth digesters.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of wastes

From the first generations, human beings have been facing and adapting to the conditions the planet has imposed on them. But nowadays man is changing life conditions creating a risk to lose control. Waste generation is increasing rapidly with urbanization and industrialization. The quantum of waste generated varies from place to place depending upon the population density and demand from society. Compared to other places, waste generation is more where the population density is maximum. This is because waste is related directly or indirectly, to the growth and development of human society. This is the reason why the developing countries face more problems due to waste in their day-to-day activities. Waste is generated from various sources such as domestic and industrial ones, which cover mainly municipal solid and liquid waste; chemical, pharmaceutical, and agro industrial waste; plastic waste; waste water effluent; and so on (Lal and Reddy, 2005).

Waste, being the most dangerous and hazardous in nature, poses many harmful effects on the environment and society, for example it increases prevalence of public health diseases, it also has adverse effect on non humans such as including those in phylogenetic kingdoms, that is, monera, protista, fungi, plantae, and animalia. This spells out the need for proper waste management in order to mitigate its detrimental impact. Management of waste is a key element in the protection of public health and failure to manage and dispose waste properly may lead to severe consequences (Lucas and Gilles, 2003). Although nature has already created the process of converting one type of waste to another form by its natural biological cycle, however with growing demands of humans, these natural efforts have become more or less adequate in maintaining the supply. On the contrary, sometimes, the natural biological phenomenon also generates some form of waste that has a direct impact on the environment.

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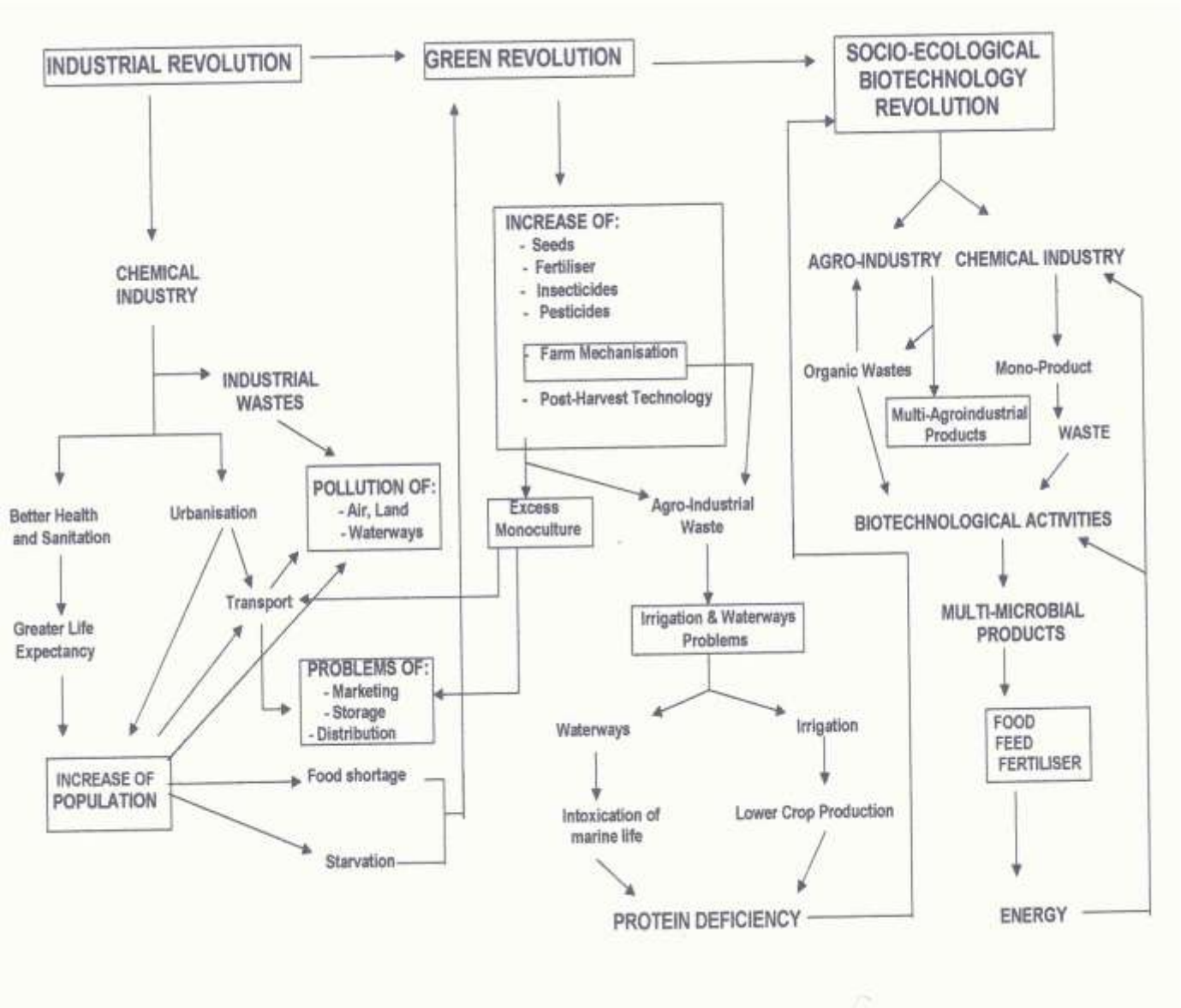

Source: Doelle *et al*, 1998

Fig 2.1 Development of societies

Annually almost 250 million tones of methane gas is generated by anaerobic digestion by methanogenic bacteria, world over: methane traps 30 times more heat than carbon dioxide and contributes to 18% of the global warming (Lal and Reddy, 2005). Also the polluting gases

produced by human beings especially by fossils fuels (carbon dioxide, methane, nitrous oxide, hydrofluoro carbon, perfluoro carbon, hexafluoro azide) have been degrading our ozone layer provoking green house effects.

This has led to the fabrication of some alternatives for disposing off the hazardous wastes and making the environment free from the detrimental impacts of the said wastes. Various scientists and industrialists have made efforts to solve the challenging problem faced by the environment due to hazardous wastes. Presently, various methods of treatment of solid and liquid waste have been successfully developed and implemented globally. However, the waste management practice is not sufficient to make the environment completely free from the detrimental impact of the wastes (Lal and Reddy, 2005). But, with continuous improvement in the fields of science and technology, nowadays, scientist have developed new technologies by which human beings will not only restrict their knowledge for disposing off the hazardous wastes but also harness some alternative products generated from the same waste, which can be highly useful to society. Today, examples of such efforts are seen in the production of BIOGAS from wastes of organic origin, often known as biomass.

2.2 Biomass

Biomass, being defined as all organic matter such as wood and wood waste, agricultural residues and farming manure (Boyle, 2004; Demirbas, 2007) is one of the most wide-spread energy resources worldwide. Its high availability and dispersed location enable it to be used for decentralised power generation. By being renewable, a long-term energy supply on the basis of biomass can emerge. While its low energy density could be seen as a potential barrier for implementation, when using biomass in small- and micro-scale applications these shortfalls can be overcome and it can even substitute grid connection for remotely located customers with sufficient amounts of feedstock on site (Lin, 2007).

Biomass in general is divided into wet and dry feedstock, the first with a moisture content of significantly less than 50%, and the latter with up to more than 90% for animal manures (McKendry, 2002). Wet biomass is normally treated biochemically, whereas dry biomass is

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processed thermochemically. In both ways, an intermediate fuel is produced to be used for electricity generation purposes.

In Nigeria, the primary biomass fuels are wood fuels and various waste products. Biofuels include alcohols, synfuels and biodiesel, a fuel made from grain and animal fats. Waste consists of municipal solid waste, landfill gas, agricultural byproducts and other material. Most biomass energy used in the U.S. – 65 percent – comes from wood (U.S. Department of Energy, 2006). Another 23 percent of biomass energy used comes from biofuels while the remaining 12 percent comes from waste energy. While cattle manure has the most potential for power use, other forms of agricultural waste have significant possibilities, too. These include poultry litter, rice straw, peanut shells, cotton gin trash and corn stover. In fact, a recent report from the Houston Advanced Research Center estimated that Texas agricultural wastes have the potential to produce 418.9 megawatts of electricity, or enough to power over 250,000 homes, based on average Texas electric use in 2006 (Houston, 2008).

2.3 Components of biomass

The term "lignocellulosic biomass" is used when referring to higher plants, softwood or hardwood. The main components of the lignocellulosic materials are cellulose, hemicellulose and lignin. Cellulose is a major structural component of cell walls, and it provides mechanical strength and chemical stability to plants. Solar energy is absorbed through the process of photosynthesis and stored in the form of cellulose. (Raven *et al.*,1992) Hemicellulose is a copolymer of different C5 and C6 sugars that also exist in the plant cell wall. Lignin is polymer of aromatic compounds produced through a biosynthetic process and forms a protective layer for the plant walls. In nature, the above substances grow and decay during the year. It has been estimated that around $7.5x10^{10}$ tonnes of cellulose are consumed and regenerated every year (Kirk-Otmer, 2001). It is thereby the most abundant organic compound in the world. Apart from the three basic chemical compounds that lignocellulose consists of, water is also present in the complex. Furthermore, minor amounts of proteins, minerals and other components can be found in the lignocellulose composition as well. The composition of lignocellulose highly depends on its source. There is a significant variation of the lignin and (hemi)cellulose content of

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lignocellulose depending on whether it is derived from hard-wood, softwood, or grasses. Table 2.1 summarizes the composition of lignocellulose encountered in the most common sources of biomass.

Table 2.1 Composition of lignocellulose in several sources on dry basis

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA	24–29
Swine waste	6.0	28	NA
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switchgrass	45	31.4	12.0

Source: Sun and Cheng, 2002

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2.4 Potential Sources of Agro-based Waste Residue

The term agricultural residue is used to describe all the organic materials which are produced as by-products from harvesting and processing of agricultural crops. These residues can be further categorized into primary residues and secondary residues (Dhingra *et al*., 1996). Agricultural residues, which are generated in the field at the time of harvest, are defined as primary or field based residues whereas those co-produced during processing are called secondary or processing based residues.

- Primary residues – paddy straw, sugarcane top, maize stalks, coconut empty bunches and frond, palm oil frond and bunches;
- Secondary residues – paddy husk, bagasse, maize cob, coconut shell, coconut husk, coir dust, saw dust, palm oil shell, fiber and empty bunches, wastewater, black liquor.

Agricultural residues (Inyer *et al.*, 2002) are highly important sources of biomass fuels for both the domestic and industrial sectors. Availability of primary residues for energy application is usually low since collection is difficult and they have other uses as fertilizer, animal feed etc. However secondary residues are usually available in relatively large quantities at the processing site and may be used as captive energy source for the same processing plant involving minimal transportation and handling cost.

Rice produces both straw and rice husks at the processing plant which can be conveniently and easily converted into energy. Significant quantities of biomass remain in the fields in the form of cob when maize is harvested which can be converted into energy. Sugar cane harvesting leads to harvest residues in the fields while processing produces fibrous bagasse, both of which are good sources of energy. Harvesting and processing of coconuts produces quantities of shell and fibre that can be utilised while peanuts leave shells. All these materials can be converted into useful energy by a wide range of technologies. Thus, for known amounts of crop production, it may be possible to estimate the amounts of agricultural residues produced using the residue to crop ratio (Vimal and Tyagi 1984).

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2.5 Bioenergy of biomass

Bioenergy is energy contained in living or recently living biological organisms, a definition which specifically excludes fossil fuels. Plants get bioenergy through photosynthesis, and animals get it by consuming plants. Organic material containing bioenergy is known as biomass. Humans can use this biomass in many different ways, through something as simple as burning wood for heat, or as complex as genetically modifying bacteria to create cellulosic ethanol. Since almost all bioenergy can be traced back to energy from sunlight, bioenergy has the major advantage of being a renewable energy source (Rosillo and Cortez, 1998). However, it is important that bioenergy be harnessed in a sustainable fashion.

Three compelling arguments for investigating bioenergy opportunities are:

- every unit of energy sourced from biomass replaces one that would otherwise be derived from coal-fired power (producing harmful greenhouse gas emissions);
- capturing waste methane prevents it from escaping to the atmosphere where it adds to the heat-trapping gases and the Greenhouse Effect;
- the market in Australia is reaching the point where bioenergy can mean opportunities for extra income from a waste, particularly in rural areas (ATSR, 2000).

All managed sources of bioenergy will result in a net reduction in carbon dioxide emissions, if they replace coal-fired generation. In the case of plantation timbers or crops (like sugar cane), the cycle of growing, harvesting and energy production does not produce or absorb any additional carbon. Carbon stored in the crop is released at harvest, then reabsorbed by the next crop, similar to the natural carbon cycle (Agarwal, 2005).

2.6 Types of biofuels

2.6.1 Liquid biofuels

Liquid biofuels have attracted much attention and investment because they can be used to replace or supplement traditional petroleum-based transportation fuels and can be used in existing vehicles with little or no modification to engines and fueling systems. They can also be used for heating and electricity production. Large quantities of liquid biofuels are presently used in many

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countries, and the potential exists to greatly expand their use in the future (Lynd *et al*, 2003). The two most common kinds of liquid biofuels are ethanol and biodiesel, but a range of other liquid fuels exist or are being developed.

2.6.1.1 Bioethanol

Ethanol is currently produced in large quantities by fermenting the sugar or starch portions of agricultural raw materials. The feedstocks used for ethanol production vary by region, including sugar cane in Brazil, grain and corn (maize) in North America, grain and sugar beets in France, etc. The top three ethanol producers are Brazil, the US and China. Because ethanol from sugar and starch directly competes with food production, people are working to commercialize technologies to produce ethanol from cellulose, which makes up the bulk of all plants and trees and is inedible (Nomuro *et al*, 2002). Cellulosic ethanol is often referred to as a second-generation biofuel.

2.6.1.2 Biodiesel

Biodiesel is typically composed of methyl (or ethyl) esters of long chain fatty acids derived from plant oils. It is produced by chemically upgrading oils obtained from the pressing of oil plants, both edible like rapeseed, soybean and the fruits of oil palms and non-edible, like jatropha and karanj. Waste cooking oil can also be converted to biodiesel Agarwal, 2005.

2.6.1.3 Other Liquid Biofuels

(i) Biobutanol (butanol, butyl alcohol)

Butanol (called "biobutanol" if derived from biomass) is an alcohol similar to ethanol but with a higher energy density. Despite this and other advantages butanol is currently more expensive to produce than ethanol.

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(ii) Pure Plant Oil (PPO)

PPO (also known as SVO, Straight Vegetable Oil) is a diesel type fuel. It occurs natural in plant oils such as rapeseed, jatropha and many others. Waste cooking oil can also be converted to PPO. PPO can be used in its pure form or mixed with diesel/biodiesel (Agarwal, 2005).

(iii) Biokerosene

Kerosene is widely used to power jet engines. At present biokerosene is not produced for large-scale aviation because aviation fuels need to meet special requirements such as a very low freezing point and a high energy content by volume. There are, however, a variety of possible alternatives to petroleum-derived kerosene. The most promising is the synthetic biokerosene produced from Fischer-Tropsch processes using biomass feedstocks.

The most common kind of gaseous biofuel is biogas or biomethane, which is composed mostly of methane and carbon dioxide and is produced from the anaerobic digestion or fermentation of biomass including manure, sewage sludge, municipal solid waste, biodegradable waste or any other feedstock. Biogas can either be burned to produce heat and electricity or purified to be used as a vehicle fuel, sometimes mixed with natural gas.

(iv) Synthetic natural gas (SNG)

SNG is generated by gasification or fermentation of biomass and additional methanation and cleaning.

2.6.2: Solid biofuel

Solid biofuels include wood, manure or charcoal burned as fuel as well as more recent innovations like high-density clean burning pellets. Solid biomass can be burned for heat or to produce electricity either by itself or as part of a co-firing power plant.

- **Wood:**

  Wood can be utilized for bioenergy in the following forms: Firewood, Wood charcoal (charcoal), Wood-fired biomass boilers, Wood gasification (wood gas) - especially waste wood, Wood

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pellets , Wood residues (waste wood). Wood and other forms of biomass can be pressed into pellets. Due to their low moisture content, regular shape and high density, pellets can be burned very efficiently and are relatively easy to transport. They are often used for heating or electricity generation (Andrae and Merlet, 2000).

2.7 Biogas

Biogas typically refers to the gas that has been produced during the breakdown of organic materials without presence of oxygen, which consist of mainly methane and carbon dioxide.
This process is known as anaerobic digestion and is performed by microorganisms present in the anaerobic digester. This phenomenon occurs also naturally in anaerobic environments, like in ponds and marshes Microbially-controlled production of biogas is an important part of the global carbon cycle. Every year, natural biodegradation of organic matter under anaerobic conditions is estimated to release 590–800 million tons of methane into the atmosphere (ISAT/GTZ, 1999).

Biogas is a mixture of methane (45-75%) and carbon dioxide (25-55%), the actual proportion depending on the feedstock (substrate) used and the processes employed. For biogas to be flammable the methane content must be ≥ 40%. Apart from methane and carbon dioxide, biogas may also contain small amounts (≤ 3%) of impurities such as hydrogen sulphide, ammonia, carbon monoxide, and other gases (Monnet 2003).

The calorific value of biogas is not fixed and varies from between 500-700 BTU per cubic feet. The amount of air needed for combustion depends on the methane content of the biogas. The flame speed factor of biogas is 11.1, which is low and therefore, the flame will 'lift off' burners which are not properly designed, ie become unstable because of its distance from the burner. The critical pressure of methane is the main factor which acts as a hurdle in bottling of biogas (Lapp *et al*, 1975).

The methane content and hence the calorific value is higher the longer the digestion process. The methane content falls as low as 50% if retention time is short. If the methane content is considerably below 50%, biogas is no longer combustible. The gas formed in the first three to

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five days must therefore be discharged untapped. The methane content depends on the digestion temperature. Low digestion temperatures give high methane content, but then less gas is produced.

The methane content depends on the feed material. Some typical values are as follows:

Table 2.2: Methane content of different feedstock for biogas

Feedstock	**Methane content (%)**
Poultry manure	60
Pig manure	67
Farmyard manure	55
Straw	59
Grass	70
Leaves	56
Kitchen waste	50
Algae	63
Water hyacinth	52

FAO, 1997

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2.7.1 History of Biogas Production

The history of biogas production from organic waste goes back to 3000 years ago (He, 2010). There are a lot of evidences that have been found, which show that biogas has been used both in Assyria in 10th century BC and Persia in 16th century AD (He, 2010). Nevertheless, it was not until 1808 that the organic waste was recognized as a source of energy by Davy, who documented that methane, was produced during the breakdown of cattle manure (Tietjen 1975). However, it was not until the end of 19th century that methanogenesis was associated to microbial activity (McCarty et al. 1982). Jan Baptista and Van Helmont first determined in 17th century that flammable gases could evolve from decaying organic matter. Count Alessandro Volta concluded in 1776 that there was a direct correlation between the amount of decaying organic matter and the amount of flammable gas produced (Tietjen, 1975). In 1808, Sir Humphrey Davy determined that methane was present in the gases produced during the anaerobic decay of cattle manure (Tietjen 1975). Methane was first recognised as having practical and commercial value in England, where a specially designed septic was used to generate gas for the purpose of lighting in the 1890s (Cheremisinoff *et al.* 1980). There are also reports of successful methane production units in several parts of the world, and many farmers wonder if such small scale methane production units can be installed at their farms to convert waste into something more valuable (Lewis 1983).

2.7.2 Biogas technology in Nigeria

Nigeria is an energy rich resource country in terms of both fossil fuels such as crude oil, natural gas, coal, and renewable energy resources like solar, wind and biomass. The urban poor and the rural households however, still depend on biomass for their energy needs.

In Nigeria, identified feedstock substrate for an economically feasible biogas production includes water lettuce, water hyacinth, grass, dung, cassava leaves and processing waste, algae, urban refuse, solid (including industrial) waste, agricultural residues and sewage (Akinbami *et al*., 1996, 2001; Okagbue, 1988; Ubalua, 2008). It has been estimated that Nigeria produces about 227,500 tons of fresh animal waste daily. Since 1 kg of fresh animal waste produces about 0.03m^3 biogas, then Nigeria can potentially produce about 6.8 million m^3 of biogas every day

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from animal waste only. In addition, 20 kg of municipal solid waste (MSW) per capita has been estimated to be generated in the country annually (Mathew, 1982). By the 1991 census figure of 88.5 million inhabitants, the total generated MSW will be at least 1.77 million tones every year. With increasing urbanisation and industrialization, the annual MSW generated will continue to increase (Akinbami *et al.,* 1996). Biogas production may therefore be a profitable means of reducing or even eliminating the menace and nuisance of urban wastes in many cities in Nigeria (Akinbami *et al.*, 2001).

By the 2006 census figure of 88.5 million inhabitants, the total generated MSW will be at least 1.77 million tones every year. With increasing urbanisation and industrialisation, the annual MSW generated will continue to increase (Akinbami *et al.,* 1996). Biogas production may therefore be a profitable means of reducing or even eliminating the menace and nuisance of urban wastes in many cities in Nigeria (Akinbami *et al.,* 2001). Although biogas technology is not common in Nigeria, various research works on the science, technology and policy aspects of biogas production has been carried by various scientists in the country. Some significant research has been done on reactor design by some Nigerian scientists that would lead to process optimization in the development of anaerobic digesters. For instance, the Usman Danfodiyo University, Sokoto, designed a simple biogas plant (with additional gas storage system) that could produce 425 L of biogas per day which could be sufficient to cook meals for one person (Dangogo and Fernado, 1986). Similarly, an engineering design and economic evaluation of a familysized plant was carried out at the Technology Planning and Development Unit, Obafemi Awolowo University, Ile-Ife (Adeoti, 1998). Igoni *et al.* (2008) provided a synthesis of the key issues and analyses concerning the design of a high-performance anaerobic digester. Ezekoye and Okeke, (2006) designed and constructed a plastic biodigester and used it to produce biogas from spent grains and rice husk mixed together. The digestion of the slurries was undertaken in a batch operation and good biogas production was reported.

Many other raw materials available in Nigeria have been critically assessed for their possible use in biogas production by (Odeyemi, 1983). They include refuse and sewage generated in urban areas, agricultural residues and manure. It was concluded that poultry manure generated in

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Nigerian homes and in commercial poultry farms could be economically feasible substrates for biogas production. The potential to utilise poultry, cow and kitchen wastes for biogas production was demonstrated by other workers including Akinluyi and Odeyemi (1986), Abubakar (1990), Lawal *et al.* (1995), Matthew (1982), *Ojolo et al.* (2007) and Zuru *et al.* (1998). Atuanya and Aigbirior, (2002) reported the feasibility of biogas production using a UASB reactor of 3.50 L capacity. Ilori *et al.* (2007) investigated production of biogas from co-digestion of banana and plantain peels using a 10 L laboratory scale anaerobic digester. The highest volume of biogas was obtained when the banana and plantain peels were in equal proportions as feedstock. Seeding of co-digested pig waste and cassava with wood ash was reported to result into significant increase in biogas production compared with unseeded mixture of pig waste and cassava peels (Adeyanju, 2008). Fariku and Kidah (2008) reported good biogas production from anaerobic digestion of waste shells of *Lophira lanceolata* fruit. The potential use of local algal biomass for biogas production in Nigeria was recognised by Weerasinghe and Naqvi (1983). Odeyemi (1981) compared four other substrates, namely *Eupatorium odoratum*, water lettuce, water hyacinth and cow dung as potential substrates for biogas production. *Eupatorium odoratum* gave the highest yield of biogas and cowdung was the poorest substrate. He concluded that *E. odoratum* was a cheap source of biogas in Nigeria because of its luxuriant and ubiquitous growth. These laboratory studies demonstrated the potential of biogas production from agricultural waste, industrial and urban waste and animal waste in Nigeria. It appears that some groundwork for biogas research and development have been initiated in Nigeria.

Although biogas technology is not common in Nigeria, various research works on the science, technology and policy aspects of biogas production has been carried by various scientists in the country. Some significant research has been done on reactor design by some Nigerian scientists that would lead to process optimization in the development of anaerobic digesters. For instance, the Usman Danfodiyo University, Sokoto, designed a simple biogas plant (with additional gas storage system) that could produce 425 L of biogas per day which could be sufficient to cook meals for one person (Dangogo and Fernado, 1986). Similarly, an engineering design and economic evaluation of a family sized plant was carried out at the Technology Planning and

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Seeding of co-digested pig waste and cassava with wood ash was reported to result into significant increase in biogas production compared with unseeded mixture of pig waste and cassava peels (Adeyanju, 2008). Fariku and Kidah (2008) reported good biogas production from anaerobic digestion of waste shells of *Lophira lanceolata* fruit. The potential use of local algal biomass for biogas production in Nigeria was recognized by Weerasinghe and Naqvi (1983). Odeyemi (1981) compared four other substrates, namely *Eupatorium odoratum*, water lettuce, water hyacinth and cow dung as potential substrates for biogas production. *Eupatorium odoratum* gave the highest yield of biogas and cowdung was the poorest substrate. He concluded that *E. odoratum* was a cheap source of biogas in Nigeria because of its luxuriant and ubiquitous growth. These laboratory studies demonstrated the potential of biogas production from agricultural waste, industrial and urban waste and animal waste in Nigeria. It appears as some groundwork for biogas research

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2.7.3 Sustainability of Biogas Technology

Biogas technology is a manure management tool that promotes the recovery and use of biogas as energy by adapting manure management practices to collect biogas. The biogas can be used as a fuel source to generate electricity for on farm use or for sale to the electrical grid, or for heating or cooling needs. The biologically stabilized byproducts of anaerobic digestion can be used in a number of ways, depending on local needs and resources. Successful byproduct applications include use as a crop fertilizer, bedding, and as aquaculture supplements.

Adaramola and Oyewola, opined that Nigeria is endowed with huge resources of conventional energy resources (crude oil, tar sands, natural gas and coal) as well as reasonable amount of renewable energy resources (e.g. hydro, solar, wind and biomass). Most of the developing nations are facing serious shortage of fuels, the most commonly used fuel being wood fuel. According to Nepal DHS (2001) population census, percent of million Nepalese households are using fuel wood for cooking purposes. As a result, million tons of fuel wood is being burnt annually. The case in Nigeria is not different. A biogas plant or latrine when successful is an appropriate and sustainable method to deal with human or animal waste. This system produces two extremely useful products from the waste: biogas and slurry. Using biogas for cooking and lighting reduces the strain on the environment by decreasing the use of biomass and the production of greenhouse gases (as methane that is produced normally from manure is now captured and used). The biogas system also provides a barrier protecting ground water from contamination from untreated waste. To save the environment from further deterioration and also supplement the energy needs of the rural populace, a strategy incorporating local resources and new technology as biogas technology can be effectively utilized. Biofuels will be increasingly used to replace some of fossil fuel for our sustainable future. Anaerobic digestion with the addition of co-substrates, i.e. co-digestion, has been considered an effective, low-cost, and commercially flexible approach to reduce process limitations and improve methane yields.

In Nigeria, research into biogas technology and its practical application is on-going, though, has not really received the deserved attention. Lack of adequate funding from government and sponsorship by individuals or corporate bodies has hindered the development of this technology

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in Nigeria. The identification of feedstock substrate for an economically feasible biogas production in Nigeria, to include water lettuce, water hyacinth, dung, cassava leaves and processing waste, urban refuse, solid (including industrial) waste, agricultural residues and sewage have been made. Many other raw materials available in Nigeria have been critically assessed for their possible use in biogas production. They include refuse and sewage generated in urban areas, agricultural residues and manure. It was concluded that poultry manure generated in Nigerian homes and in commercial poultry farms could be economically feasible substrates for biogas production.

The potential to utilize poultry, cow and kitchen wastes for biogas production was demonstrated by other workers including. Atuanya and Aigbirior (2002) reported the feasibility of biogas production using an upflow anaerobic sludge blanket reactor (UASB) of 3.50 L capacity. Seeding of co-digested pig waste and cassava with wood ash was reported to result into significant increase in biogas production compared with unseeded mixture of pig waste and cassava peels. Fariku and Kidah (2008) reported good biogas production from anaerobic digestion of waste shells of *Lophira lanceolata* fruit. Zuru *et al*., (1998) recognized the potential use of local algal biomass for biogas production in Nigeria.

Odeyemi (1983) compared four other substrates, namely *Eupatorium odoratum*, water lettuce, water hyacinth and cow dung as potential substrates for biogas production. *Eupatorium odoratum* gave the highest yield of biogas and cow dung was the poorest substrate. He concluded that *E. odoratum* was a cheap source of biogas in Nigeria because of its luxuriant and ubiquitous growth. These laboratory studies demonstrated the potential of biogas production from agricultural waste, industrial and urban waste and animal waste in Nigeria. Numerous health problems have been reported to be associated with spread of human and animal waste. Human waste can leach into ground water from a functioning pit toilet, contamination of groundwater and reservoirs by running storm water and flash floods can result in significant sporadic pollution events, and the type of contamination includes enterobacteria, enteroviruses and a range of fungal spores (Pritchard *et al.*, 2009). Cattle slurry is known to introduce a range of pathogens

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including *Clostridium chavoie* (black leg disease), *Ascaris ova*, *E. coli* and *Salmonella spp*. as reported in cow dung slurries in Bauchi state, Nigeria and in poultry wastes in Cameroon (Yongabi *et al.,* 2003).

Pathogen prevalence in the environment is affected by local climate, soil type, animal host prevalence, topography, land cover and management, organic waste applications and hydrology (e.g. Gagliardi and Karns, 2000; Jamieson *et al.,* 2002; Hutchison *et al.,* 2004; Tyrrel and Quinton, 2003; Tate *et al.,* 2006). Installation of biogas digesters has potential to reduce the risks of encountering these pathogens if operated properly. The objective of this project therefore is to create a sustainable solid waste management system that supports greenhouse gas (GHG) emission reduction by the co-digestion of food waste and human excreta for biogas generation. The choice of these substrates was due to the fact that they are the most commonly generated wastes in every home in the country and also because the previous biogas researches in Nigeria, focused mostly on animal wastes (cow dung, 5 piggery wastes and chicken droppings) without any emphasis on human excreta or its co-digestion with other substrates. This is the first documented pilot scale attempt to use human excreta for biogas in Nigeria.

2.8 Biogas Process

A typical biogas system consists of the following components:

a. Manure collection

b. Anaerobic digester

c. Effluent storage

d. Gas handling

e. Gas use.

Each of these components is discussed briefly.

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2.8.1 Manure Collection

Livestock facilities use manure management systems to collect and store manure because of sanitary, environmental, and farm operational considerations. Manure is collected and stored either as liquids, slurries, semi-solids, or solids.

Raw Manure. Manure is excreted with a solids content of 8 to 25 percent, depending upon animal type. It can be diluted by various process waters or thickened by air drying or by adding bedding materials.

Liquid Manure. Manure handled as a liquid has been diluted to a solids content of less than 5 percent. This manure is typically "flushed" from where it is excreted, using fresh or recycled water. The manure and flush water can be pumped to treatment and storage tanks, ponds, lagoons, or other suitable structures before land application. Liquid manure systems may be adapted for biogas production and energy recovery in "warm" climates. In colder climates, biogas recovery can be used, but is usually limited to gas flaring for odor control.

Slurry Manure. Manure handled as slurry has been diluted to a solids content of about 5 to 10 percent. Slurry manure is usually collected by a mechanical "scraper" system. This manure can be pumped, and is often treated or stored in tanks, ponds, or lagoons prior to land application. Some amount of water is generally mixed with the manure to create slurry. For example, spilled drinking water mixes with pig manure to create slurry. Manure managed in this manner may be used for biogas recovery and energy production, depending on climate and dilution factors.

Semi-Solid Manure. Manure handled as a semi-solid has solid content of 10 to 20 percent. This manure is typically scraped. Water is not added to the manure, and the manure is typically stored until it is spread on local fields. Fresh scraped manure (less than one week old) can be used for biogas and energy production in all climates, because it can be heated to promote bacterial growth.

Solid Manure: Manure with a solids content of greater than 20 percent is handled as a solid by a scoop loader. Aged solid manure or manure that is left "unmanaged" (i.e., is left in the pasture where it is deposited by the animals) or allowed to dry is not suitable for biogas recovery.

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2.8.2 Digester Types

The digester is the component of the manure management system that optimizes naturally occurring anaerobic bacteria to decompose and treat the manure while producing biogas. Digesters are covered with an air-tight impermeable cover to trap the biogas for on-farm energy use. The choice of which digester to use is driven by the existing (or planned) manure handling system at the facility. The digester must be designed to operate as part of the facility's operations. One of three basic options will generally be suitable for most conditions:

(i) **Covered Lagoon Digester.** Covered lagoons are used to treat and produce biogas from liquid manure with less than 3 percent solids. Generally, large lagoon volumes are required, preferably with depths greater than 12 feet. The typical volume of the required lagoon can be roughly estimated by multiplying the daily manure flush volume by 40 to 60 days. Covered lagoons for energy recovery are compatible with flush manure systems in warm climates. Covered lagoons may be used in cold climates for seasonal biogas recovery and odor control (gas flaring). There are two types of covers, bank-to-bank and modular. A bank-to-bank cover is used in moderate to heavy rainfall regions. A modular cover is used for arid regions. Exhibit 1-2 illustrates a modular floating cover for lagoon applications. Typically, multiple modules cover the lagoon surface and can be fabricated from various materials.

(ii) **Complete Mix Digester:** Complete mix digesters are engineered tanks, above or below ground, that treats slurry manure with a solids concentration in the range of 3 to 10 percent. These structures require less land than lagoons and are heated. Complete mix digesters are compatible with combinations of scraped and flushed manure.

(iii) **Plug Flow Digester:** Plug flow digesters are engineered, heated, rectangular tanks that treat scraped *dairy* manure with a range of 11 to 13 percent total solids. Swine manure cannot be treated with a plug flow digester due to its lack of fiber.

(iv) **Fixed Film Digester:** Fixed-film digesters consist of a tank filled with plastic media. The media supports a thin layer of anaerobic bacteria called biofilm (hence the term "fixed-film"). As the waste manure passes through the media, biogas is produced. Like covered lagoon digesters fixed-film digesters are best suited for dilute waste streams typically associated with flush

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manure handling or pit recharge manure collection. Fixed-film digesters can be used for both dairy and swine wastes. However, separation of dairy manure is required to remove slowly degradable solids (FAO, 1997).

2.8.3 Effluent Storage

The products of the anaerobic digestion of manure in digesters are biogas and effluent. The effluent is a stabilized organic solution that has value as a fertilizer and other potential uses. Waste storage facilities are required to store treated effluent because the nutrients in the effluent cannot be applied to land and crops year round.

The size of the storage facility and storage period must be adequate to meet farm requirements during the non-growing season. Facilities with longer storage periods allow flexibility in managing the waste to accommodate weather changes, equipment availability and breakdown, and overall operation management.

2.8.4 Gas Handling

A gas handling system removes biogas from the digester and transports it to the end-use, such as an engine or flange. Gas handling includes: piping; gas pump or blower; gas meter; pressure regulator; and condensate drain(s).

Biogas produced in the digester is trapped under an airtight cover placed over the digester. The biogas is removed by pulling a slight vacuum on the collection pipe (e.g., by connecting a gas pump/blower to the end of the pipe), which draws the collected gas from under the cover. A gas meter is used to monitor the gas flow rate. Sometimes a gas scrubber is needed to clean or "scrub" the biogas of corrosive compounds contained in the biogas (e.g., hydrogen sulfide). Warm biogas cools as it travels through the piping and water vapor in the gas condenses. A condensate drain(s) removes the condensate produced.

2.8.5 Gas Use

Recovered biogas can be utilized in a variety of ways. The recovered gas is 60 - 80 percent methane, with a heating value of approximately 600 -800 Btu/ft^3. Gas of this quality can be used

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to generate electricity; it may be used as fuel for a boiler, space heater, or refrigeration equipment; or it may be directly combusted as a cooking and lighting fuel.

Electricity can be generated for on-farm use or for sale to the local electric power grid. The most common technology for generating electricity is an internal combustion engine with a generator. The predicted gas flow rate and the operating plan are used to size the electricity generation equipment.

Engine-generator sets are available in many sizes. Some brands have a long history of reliable operation when fueled by biogas. Electricity generated in this manner can replace energy purchased from the local utility, or can be sold directly to the local electricity supply system. In addition, waste heat from these engines can provide heating or hot water for farm use.

Biogas can also be used directly on-site as a fuel for facility operations. Equipment that normally uses propane or natural gas can be modified to use biogas. Such equipment includes boilers, heaters, and chillers. (Minnesota Project, 2010).

(i) **Boilers and Space Heaters.** Boilers and space heaters fired with biogas produce heat for use in the facility operations. Although this may not be the most efficient use of the gas, in some situations it may be a farm's best option.

(ii) **Chilling/Refrigeration.** Dairy farms use considerable amounts of energy for refrigeration. Approximately 15 to 30 percent of a dairy's electricity load is used to cool milk. Gas-fired chillers are commercially available and can be used for this purpose. For some dairies, this may be the most cost effective option for biogas utilization.

Other energy use options may exist. For example, a nearby greenhouse could be heated with the biogas, and carbon dioxide from the heater exhaust could be used to enhance plant growth. These options need to be evaluated on a case-by-case basis.

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2.9 Benefits of Biogas Technology

Most confined livestock operations handle manure as liquids, slurries, semi-solids, or solids that are stored in lagoons, concrete basins, tanks, and other containment structures. These structures are typically designed to comply with local and state environmental regulations and are a necessary cost of production.

Biogas technology can be a cost-effective, environment and neighborhood friendly addition to existing manure management strategies. Biogas technologies anaerobically digest manure, resulting in biogas and a liquefied, low-odor effluent. By managing the anaerobic digestion of manure, biogas technologies significantly reduce Biochemical Oxygen Demand (BOD), and pathogen levels; remove most noxious odors; and convert most of the organic nitrogen to plant available inorganic nitrogen.

The principal reasons a farmer or producer would consider installing a biogas system are:

(i) **On-Site Farm Energy.** By recovering biogas and producing on-farm energy, livestock producers can reduce monthly energy purchases from electric and gas suppliers.

(ii) **Reduced Odors.** Biogas systems reduce offensive odors from overloaded or improperly managed manure storage facilities. These odors impair air quality and may be a nuisance to nearby communities. These offensive odors are reduced due to the utilization of volatile organic acids which are the odor causing compounds by biogas producing bacteria.

(iii) **High Quality Fertilizer.** In the process of anaerobic digestion, the organic nitrogen in the manure is largely converted to ammonium. Ammonium is the primary constituent of commercial fertilizer, which is readily available and utilized by plants.

(iv) **Reduced Surface and Groundwater Contamination.** Digester effluent is a more uniform and predictable product than untreated manure. The higher ammonium content allows better crop utilization and the physical properties allow easier land application. Properly applied, digester effluent reduces the likelihood of surface or groundwater pollution.

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(v) **Pathogen Reduction.** Heated digesters reduce pathogen populations dramatically in a few days. Lagoon digesters isolate pathogens and allow pathogen kill and die-off prior to entering storage for land application.

Biogas recovery can improve profitability while improving environmental quality. Maximizing farm resources in such a manner may prove essential to remain competitive and environmentally sustainable in today's livestock industry. In addition, more widespread use of biogas technology will create jobs related to the design, operation, and manufacture of energy recovery systems and lead to the advancement of U.S. agribusiness. (Minnesota Project, 2010).

2.10 Comparison of anaerobic and aerobic digestion

In an anaerobic system there is an absence of gaseous oxygen as gaseous oxygen is prevented from entering the system through physical containment in sealed tanks. Anaerobes access oxygen from sources other than the surrounding air. The oxygen source for these microorganisms can be the organic material itself or alternatively may be supplied by inorganic oxides from within the input material. When the oxygen source in an anaerobic system is derived from the organic material itself, then the 'intermediate' end products are primarily alcohols, aldehydes, and organic acids plus carbon dioxide. In the presence of specialised methanogens, the intermediates are converted to the 'final' end products of methane, carbon dioxide with trace levels of hydrogen sulfide. In an anaerobic system the majority of the chemical energy contained within the starting material is released by methanogenic bacteria as methane (Fergusen & Mah, 2006 and Sharon *et al*, 2004).

In an aerobic system, such as composting, the microorganisms access free, gaseous oxygen directly from the surrounding atmosphere. The end products of an aerobic process are primarily carbon dioxide and water which are the stable, oxidised forms of carbon and hydrogen. If the biodegradable starting material contains nitrogen, phosphorus and sulfur, then the end products may also include their oxidised forms- nitrate, phosphate and sulfate. In an aerobic system the majority of the energy in the starting material is released as heat by their oxidization into carbon dioxide and water (Fergusen & Mah, 2006 and Sharon *et al*, 2004).

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Composting systems typically include organisms such as fungi that are able to break down lignin and celluloses to a greater extent than anaerobic bacteria. Due to this fact it is possible, following anaerobic digestion, to compost the anaerobic digestate allowing further volume reduction and stabilization (Sharon *et al*, 2004).

2.11 Biochemistry of anaerobic digestion

Biogas is produced in three main steps; hydrolysis, acidification and methane production. The active microorganisms consist of a large mixture of differently acting species that live under symbiotic relationship. The first step of biogas production called hydrolysis is known as the polymer breakdown stage (Ostrem, 2004). The second step is the process of acidification where the acid producing bacteria convert the monomers produced in the first step to different fermentation products, mainly acids. In the second stage of this process, called acetogenesis, the different fermentation products will be converted to acetic acid, which serve as one of the substrate for the methane production (Bilitewski *et al*., 1997; Mata-Alvarez, 2003. In the third and the last step, also known as methanogenesis, methane-producing bacteria utilize either acetate, or carbon dioxide and hydrogen to form methane and carbon dioxide (Verma, 2002). Below is a simple sketch of the above process.

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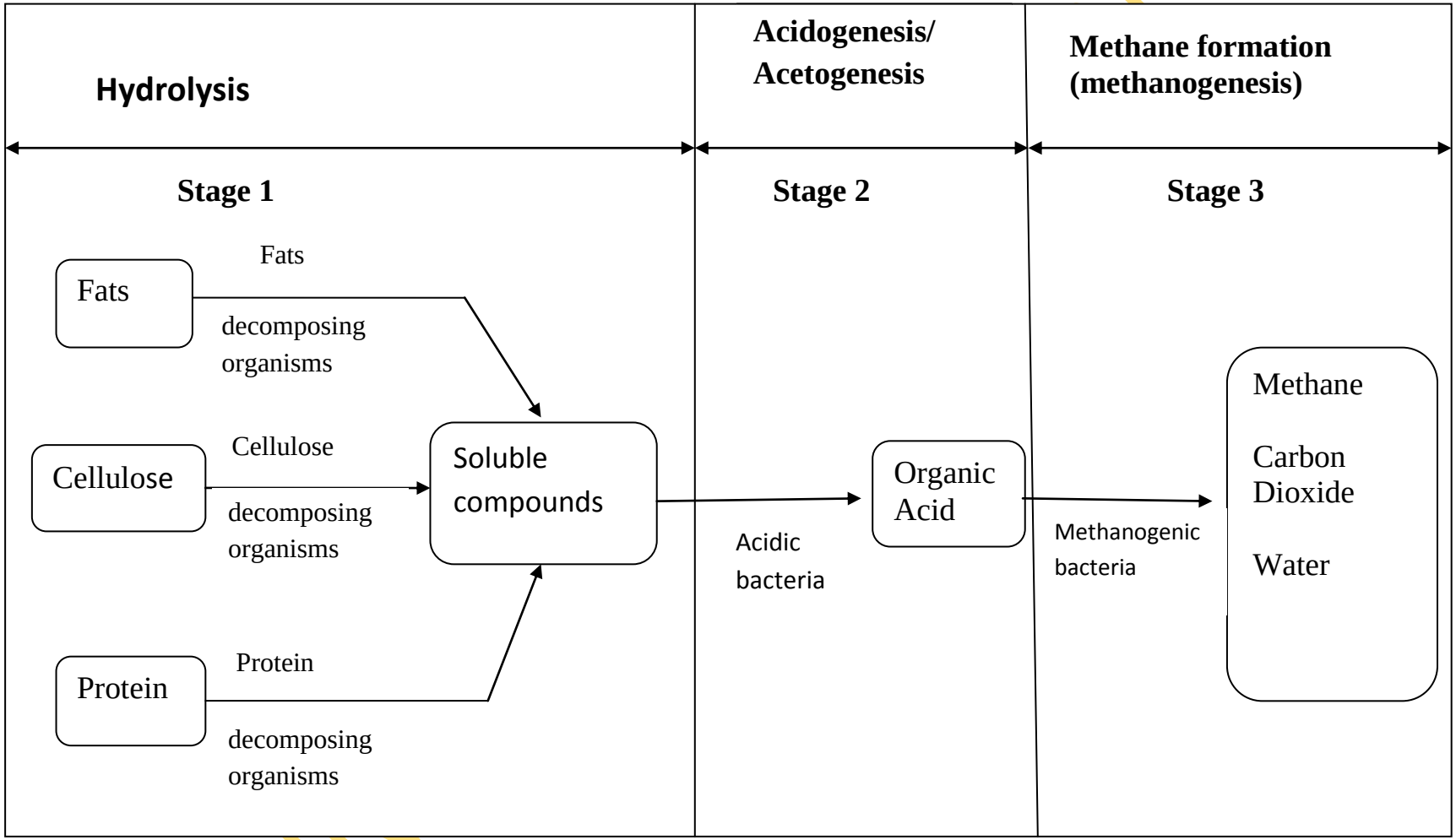

Fig 2.1 Biochemistry of anaerobic digestion:

Source: Parawira, 2004

Anaerobic digestion, which takes place in three stages inside an airtight container, produces biogas. Different kinds of micro-organisms are responsible.The chemical equation for the three stages above are as shown below:

1. Hydrolysis: $(C_6H_{10}O_5)\ n + nH_2O \longrightarrow n\ (C_6H_{12}O_6)$
2. Acidogenesis/Acetogenesis: $n\ (C_6H_{12}O_6) \longrightarrow nCH_3COOH$
3. Methane formation (methanogenesis): $3nCH_3COOH \longrightarrow nCH_4 + CO_2$

A variety of factors affect the rate of digestion and biogas production. The most important is temperature. Anaerobic bacteria communities can endure temperatures ranging from below freezing to above 135°F (57.2°C), but they thrive best at temperatures of about 98°F (36.7°C) (mesophilic) and 130°F (54.4°C) (thermophilic). Bacteria activity, and thus biogas production, falls off significantly between 103° and 125°F (39.4° and 51.7°C) and gradually from 95° to 32°F (35° to 0°C).

In the thermophilic range, decomposition and biogas production occur more rapidly than in the mesophilic range. However, the process is highly sensitive to disturbances, such as changes in feed materials or temperature. While all anaerobic digesters reduce the viability of weed seeds and disease-producing (pathogenic) organisms, the higher temperatures of thermophilic digestion result in more complete destruction. Although digesters operated in the mesophilic range must be larger (to accommodate a longer period of decomposition within the tank [residence time]), the process is less sensitive to upset or change in operating regimen.

To optimize the digestion process, the biodigester must be kept at a consistent temperature, as rapid changes will upset bacterial activity. In most areas of the United States, digestion vessels require some level of insulation and/or heating. Some installations circulate the coolant from their biogas-powered engines in or around the digester to keep it warm, while others burn part of the biogas to heat the digester. In a properly designed system, heating generally results in an increase in biogas production during colder periods. The trade-offs in maintaining optimum digester temperatures to maximize gas production while minimizing expenses are somewhat complex. Studies on digesters in the north-central areas of the country indicate that maximum net

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biogas production can occur in digesters maintained at temperatures as low as 72°F (22.2°C).

Other factors affect the rate and amount of biogas output. These include pH, water/solids ratio, carbon/nitrogen ratio, mixing of the digesting material, the particle size of the material being digested, and retention time. Pre-sizing and mixing of the feed material for a uniform consistency allows the bacteria to work more quickly. The pH is self-regulating in most cases. Bicarbonate of soda can be added to maintain a consistent pH; for example, when too much "green" or material high in nitrogen content is added. It may be necessary to add water to the feed material if it is too dry or if the nitrogen content is very high. A carbon/nitrogen ratio of 20/1 to 30/1 is best. Occasional mixing or agitation of the digesting material can aid the digestion process. Antibiotics in livestock feed have been known to kill the anaerobic bacteria in digesters. Complete digestion, and retention times, depends on all of the above factors.
There are many factors that play a significant role in this process, like pH value, temperature, organic loading rate, retention time, C/N ratio, the amount of available nutrients and toxicity. (Dela-Rubia *et al*., 2002; Bouallagui et al., 2009b; Riau et al., 2010).

2.12 Prospect and Potential of Microbes in Biogas Technology

2.12.1 Anaerobes.

Anaerobic bacteria do not grow on solid media in room air (10% carbon dioxide and 18% oxygen); facultative anaerobic bacteria can grow in the presence as well as in the absence of air. Microaerophilic bacteria do not grow at all aerobically or grow poorly, but grow better under 10% carbon dioxide or anaerobically. Anaerobic bacteria can be divided into strict anaerobes that cannot grow in the presence of more than 0.5% oxygen and moderate anaerobic bacteria that are able of growing between 2 to 8% oxygen (Jousimies-Somer HR et al, 2002)

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2.12.2 Methanogens

These are diverse group of strict anaerobes which are widely distributed in nature and can be found in variety of permanently flooded soils, sediments, sewage-sludge digestors or the digestive tract of certain animals. The identified methanogens are grouped under archae and are extremely sensitive to oxygen. The most distinct feature of methanogens is the reduction of C-1 compounds (e.g., CO_2, methanol, formate, or Nmethyl groups) to methane (CH_4). Among the enzyme and cofactors involved in this metabolicpathway, some are very unique and found only in methanogens. The coenzyme F420 involved in methanogenesis causes an intense autofluorescence of cells under excitation by shortwave UV light. This phenomenon is a diagnostic feature and can be used to check cultures of methanogens for contaminants by epifluorescence microscopy.

Methanogens are important members of microbiological consortia in natural environments, subterranean formations including petroleum reservoirs and also in marine and land animals, insects and human gut, peat bogs, waste streams, etc. However, there is no standard method of detecting methanogens. One method of methanogen detection is to culture them. Cultivating methanogen anaerobically in a laboratory is a laborious and time-consuming process. Another method of identifying methanogen is to use rRNA targeted archeabacteria specific PCR primers or methanogen specific group 16s rDNA probes. These methods suffer from a limitation wherein the probes cross-react with organisms of other physiological or even phylogenetic groups when applied to environmental samples containing unknown sequences.

Methanogens is among the fastidious microorganisms which attracts attention of researchers due to its challenging laboratory analysis and its impact on global warming due to generation of methane gas. Over 75% of CH_4 released from natural sources such as wetlands (including bogs, peat lands, swamps, and marshes) (Summanen *et al*, 1993) can be attributed to the activities of the methanogenic communities in them (Shanmon, 2003 and Utsumi *et al*., 2003;). As global conditions continue to change, wetlands may play an increasingly important role in CH_4 release.

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CHAPTER THREE

METHODOLOGY

3.1 Study design

The study was experimental and laboratory based involving pre-treatment, anaerobic digestion, biochemical tests and microbiological examination. Different types of organic wastes such as pig dung, water hyacinth and maize cob were utilized in the experiment. The experiment was divided into six (6) treatment groups:

Treatment A = Pig dung (PD)
Treatment B = Water Hyacinth (WH)
Treatment C = Maize cob (MC)
Treatment D = 1:1 of Pig dung + Maize cob (PM)
Treatment E = 1:1 of Pig dung + Water hyacinth (PW)
Treatment F = 1:1:1 of Pig dung + Maize cob + Water hyacinth (PMW)

The experiment employed a complete randomized design with three replicate of each of the sample biomass. An evaluation of the biogas yielding capacity and microbial load of the different biomass was carried out.

3.2 Description of study area

The study area of this research was in Ibadan. Ibadan is the capital city of Oyo State of Nigeria. It is the third largest city in Nigeria by population and geographical area. It is located in south-west Nigeria and according to 2006 census results; Ibadan has a total population of 2,258,625 inhabitants, made up of 1,125,843 urban and 1,132,728 rural populations (Omonijo *et al*, 2007). It is located along the rainforest belt in the humid tropical region with an annual rainfall of about 2,500mm and temperature below 53^0F. The major occupation of the inhabitants are mostly farming and trading.

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The choice of Ibadan as a study area is because of large scale Agricultural activities, which is evident by the presence of research institute viz: International Institute of Tropical Agriculture (IITA), National Institute for Horticultural Research and Training (NIHORT), Agricultural Plantation (Government and private owned), Bodija Abattoir Centre, University of Ibadan Teaching and Research Farm, Institute of Agricultural Research and Training (IART) and it also serves as a market nerve centre for Agricultural produce such as maize, millet, yam and other tuber crops brought from the Northern part of Nigeria. This has led to the generation of enormous animal and agro base waste which pose a great concern to the government because of poor waste management practice in the state.

3.3 Sample Source:

The different organic wastes utilized in this study were obtained from the following locations in Ibadan:

1. **University of Ibadan Teaching and Research Farm (UITRF)** where PD and MC were obtained is located in the northern end of the University Campus and was established in the year 1950. It covers approximately a land area of hundred and sixty hectares (160 ha) [400acres] which is used for both livestock (cattle, pig, poultry and sheep) and crop (maize, cassava etc.) production. Piggery unit occupies an area of acre while the maize plantation unit covers an area 0.65 hectare. UITRF was established primarily; (a) to provide teaching and research facilities for the staff and students of the Faculties of Agriculture, Forestry and Veterinary Medicine, (b) to demonstrate to the general public, where feasible, the commercial potential of advanced farming techniques and in the nearest future, to operate on a commercial scale the findings of research, and (c) to serve as source of knowledge and provide a body of technical and economic data for the extension services and the farming population. Large quantities of animal and crop wastes are generated from these production units which are left to litter the environment.

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Plate 3.1: Piggery unit of UITRF where samples of PD was collected.

Plate 3.2: Maize unit of UITRF where MC was collected.

Oba-Dam: Water Hyacinth was obtained from Oba-Dam which is located in the out outskirt of the University of Ibadan very close to Ibadan Polytechnic. The Dam was established in 1964 to supply water to the entire university (water for domestic use, laboratory use, fish culture etc.) It is about 130 metres in length, 12.2 metres wide at the top, about 27.4 metres wide at the deepest portion and has a maximum depth of about 5.5 metres. It has a capacity to hold about 227 million litres of water.

Plate 3.3: Oba-Dam, where samples of the Water Hyacinth was collected

3.4 Morphological description of material sample

The morphological description of the different biomass feedstock materials utilized in this study is presented in the following sections

3.4.1 Pig dung

Pig dung is dark grey waste product from pig. It is solid in nature though of a high moisture content. It produces very offensive odour if left in the environment. It is always associated with the proliferation of flies and very pathogenic in nature.

Plate 3.4: Pig dung from piggery unit of UITRF

3.4.2 Maize cob:

Maize cob is a major by product of maize processing centres where maize is grown and the grain is processed into different foodstuff. For example, it can be cooked, roasted, used for making pap, corn flakes while the cobs from these processes are left to litter the environment.

Plate 3.5: Maize cob from maize unit of UITRF

3.4.3 Water hyacinth:

Water hyacinth (Eichhornia crassipes) is one of the world's worst aquatic weeds. It infests rivers, dams, lakes and irrigation channels on every continent except Antarctica. It devastates aquatic environments and costs billions of dollars every year in control costs and economic losses (Queensland, 2001). It is a floating waterweed up to 65 cm tall, with an extensive (up to 1m) feathery, black to purple coloured, root system. Its leaves are round, bright to dark green and up to 5-10cm in diameter. The leaf stalks of young plants are swollen into spongy, bulbous structures; mature plants have elongated leave stalks (Queensland, 2001).

Environmental Health Impacts:

- Destroys native wetlands and waterways, killing native fish and other wildlife.
- Depletes water bodies of oxygen.
- Increases water loss.
- Provides breeding ground for mosquitoes

(Water hyacinth fact sheet, 2004)

Plate 3.6: Water hyacinth from Oba-Dam

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3.5 Feasibility Study on Sample Collection Areas

A feasibility test was carried out on the sample collection areas to estimate the sample population and to determine the amount of wastes being generated from the parent materials as shown below:

PHASE ONE: Estimation of Sample Population

The various sample populations were determined as follows using the following methods below: PD was determined by counting the population of pigs; MC by using the formula below

$$\textbf{Plant population} = \frac{\textbf{Area of land used}}{\textbf{Feeding area (spacing)}}$$

While WH was estimated by throwing a quadrat of area 2.5m^2 in five (5) different places and the mean weight value was recorded.

PHASE TWO: Quantification of Biomass Feedstock from the Parent Sources

Thus for any known amounts of animal and plant production, it is possible to estimate the amounts of by-product generated using the method of Vimal and Tyagi (1984) which utilizes the residue to biomass feedstock ratio approach.

(i) The weight of the waste of the sample population was determined using the Top-Load and Silvano Weighing Balances respectively.

(ii) The volume of the waste of the sample population was determined using a 500 ml calibrated beaker.

(iii) The density of the waste of the sample population was calculated using the formula:

$$\text{Density} = \frac{\text{Mass (in Kg)}}{\text{Volume (in } m^3\text{)}}$$

3.5.1 Sample Collection and Transport

Considerable quantities of the samples utilized were packed in sack bags and then transported directly to the laboratory for processing. Grab samples were taken and their wet and dry weights

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were determined. This was used in estimating the quantity of dry matter in the raw wet samples that was utilized in the experiment.

3.6 Materials and methods

3.6.1 Materials

The following materials were utilized in this study: Six 10 litres black plastic kegs, twelve 5litres transparent plastic kegs, delivery tubes (pipes), weighing balance, one 10 litres calibrated bucket, measuring cylinder, bowl, tongit gum, iron rods, fire, conical flasks, beakers, test tubes, measuring cylinders, bunsen burner, lighter, cotton wool, incubator, oven, foil-paper, analytical balance (± 0.001g), pH meter, glass funnel, burette, pipette, glass bottles, reagent bottles, tripod stand.

3.6.2 Consumables

The following consumables were utilized in the course of the experiment: Nutrient Agar, Potato Dextrose Agar (PDA), Distilled water, Methylated spirit, and Detergent.

3.6.3 Collection of Materials

The materials used in this study were obtained from the Department of Environmental Health Science laboratory, Faculty of Public health and IMRAT both in the College of Medicine, UCH, Ibadan. The weighing balance, pipes, Bunsen burner, kegs, tongit gum, trading tape, were purchased.

3.6.4 Quality Control and Quality Assurance

All the glass-wares used for this study were thoroughly washed with detergent, rinsed with distilled water and then allowed to dry in a hot- air oven. The process of sterilization of the equipment was to safeguard against possible contamination of the sample under study.

Disinfection was carried out by cleaning the whole surface of the working bench with cotton wool soaked in methylated spirit before and after each process.

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3.7 EXPERIMENTAL PROCEDURE

1. **Construction of anaerobic digesters.**
2. **Pre-treatment of the sample.**
3. **Charging of anaerobic digester/anaerobic digestion**.
4. **Physical, chemical and microbiological characterization of the various treatment groups** for pH, temperature, total solids, Total Organic Carbon (TOC), Total Nitrogen (TN), Total Phosphorus (TP), Total Potassium (TK), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total aerobic organisms, total fungi organisms, total anaerobic organisms and total coliform organisms.
5. **Quantification of biogas yield and microbial species across the digesters.**

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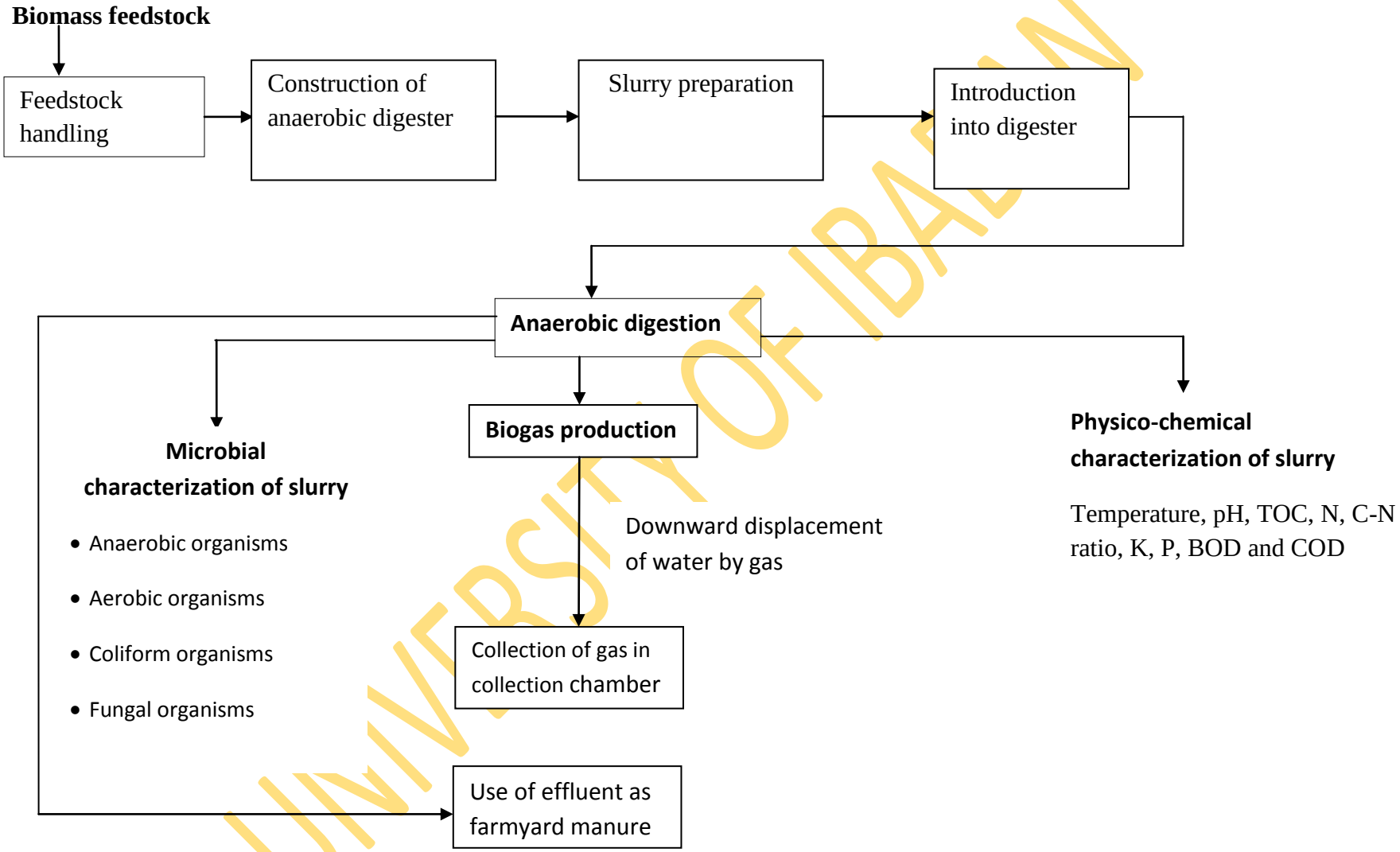

Figure 3.1: Experimental flow chart

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3.7.1 Construction of the anaerobic digesters

Each digesting vessel that was used in the various experiments consists of a black 10-litre water dispensing plastic keg (Keg 1). A plastic tap with open and lock system was connected to the base of the digester (Keg 1) which served as the outlet pipe. A short tube was connected to the top of the keg which served for testing when gas generation started. This tube was properly stoppered with nut, pipe clip and treading tape to avoid gas leakage. Another delivery tube was used to connect keg1 to a 5-litre transparent plastic keg (keg 2) which served as the gas collection chamber. This keg was filled with water. A third delivery tube was used to connect keg 2 to another 5-litre transparent plastic keg (keg 3) which served for the collection of water displaced from keg 2 by the gas generated (Archimedes' Principle). The whole system was made airtight using tongit gum. The above process was repeated for the remaining five different treatments.

Testing for leakages:

Water was poured into all the kegs and left overnight. After this period, there were no signs of leakages found. For proper confirmation, an omo-suspension (detergent) was made and applied to all the jointed areas and left for 10minutes. Absence of bubbles in the jointed areas after 10 minutes was a clear confirmation of the absence of leakages.

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Plate 3.7: Picture of pig dung digester, 1; gas displacement chamber, 2; and water collection chamber, 3.

3.7.2 Pre-treatment of Sample

- **Pig dung:** This was collected from the piggery unit of the University of Ibadan Teaching and Research Farm.
- **Water Hyacinth:** This was collected from Oba Dam in the University of Ibadan. It was grounded using a local mortar to increase the surface area for microbial activity.
- **Maize Cob:** This was collected from piggery unit in UITRF. It was grounded to increase the surface area.

Wet and dry weight determination

Grab samples of each substrate was wrapped in a known weight of aluminum foil,

weighed using a digital sensitive balance, and dried in a hot air oven for 8hrs at 85^0C. They were immediately reweighed to know their dry weight. This process was found very important before commencement of the experiment so as to be able to ascertain the amount of dry matter present in a kg of the wet sample. This was very helpful during the measurement of the quantity of each substrate used for biogas production and in making inferences on the amount of gas yield by single substrates and their mixtures.

Mathematically, the weights were determined as follows:

Drying

Wt of Aluminium foil = W_1

Wt of Aluminium foil + pig dung (wet) = W_2

Wt of pig dung (wet) = $W_3 = W_2 – W_1$

Wt of Aluminium foil + pig dung (dry) = W_4

Wt of pig dung (dry) = $W_5 = W_4 - W_1$

Pig Dung:

Wt of Aluminium foil = 0.0016kg

Wt of Aluminium foil + PD (wet) = 0.1342kg

Wt of PD (wet) = 0.1342 – 0.0016 = 0.1326kg

Wt of Aluminium foil + PD (dry) = 0.05464kg

Wt of pig dung (dry) = 0.05464 – 0.0016 = 0.05304kg

Moisture content = 0.1326 – 0.05304 = 0.07956kg

Therefore, percentage moisture content = $\frac{0.07956}{0.1326} \times 100\% = 60\%$

Therefore, percentage dry weight = 100 – 60 = 40%

Since 0.1326kg of wet Pig Dung contains 0.05304kg of dry matter of Pig Dung,

Therefore, 1kg wet pig dung contains (0.05304/0.1326)kg dry matter = 0.4kg dry matter.

1kg of dry Pig dung were contained in 2.5kg of wet Pig Dung

0.75kg of dry Pig dung was contained in 1.875kg of wet Pig dung.

0.5kg of dry Pig dung was contained in 1.25kg of wet Pig dung.

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0.25kg of dry Pig dung was contained in 0.625kg of wet Pig dung.

Maize cob

Wt. of Aluminium foil = 0.0014kg

Wt. of Aluminium foil + Maize Cob (wet) = 0.0983kg

Wt. of Maize Cob only (wet) = 0.0983 – 0.0014 = 0.0969kg

Wt. of Aluminium foil + Maize Cob (dry) = 0.02355kg

Wt. of Maize Cob (dry) = 0.02355 – 0.0014 = 0.02215kg

Moisture content = 0.0969 – 0.02215 = 0.07475kg

Therefore, percentage moisture content = $\frac{0.07475}{0.0969} \times 100\%$

= 77.14%

Therefore, percentage dry weight = 100 – 77.14 = 22.86%

Since 0.0969 kg of wet Maize Cob contained 0.0222kg of dry matter of Maize Cob,

Therefore, 1kg wet Maize Cob contained (0.02215/0.0969)kg dry matter = 0.2286kg dry matter.

1kg of dry matter of Maize Cob was therefore contained in 4.4kg of wet Maize Cob

0.75kg of dry Maize Cob was contained in 3.3kg of wet Maize Cob.

0.5kg of dry Maize Cob was contained in 2.2kg of wet Maize Cob.

0.25kg of dry Maize Cob was contained in 1.1kg of wet Maize Cob.

Water hyacinth

Wt. of Aluminium foil = 0.0014kg

Wt. of Aluminium foil + Water Hyacinth (wet) = 0.0882kg

Wt. of Water Hyacinth (wet) = 0.0882 – 0.0014 = 0.0868kg

Wt. of Aluminium foil + Water Hyacinth (dry) = 0.0193kg

Wt. of Water Hyacinth (dry) = 0.0193 - 0.0014 = 0.0179kg

Moisture content = 0.0868 – 0.0179 = 0.0689kg

Therefore, percentage moisture content = $\frac{0.0689}{0.0868} \times 100\% = 79.4\%$

Therefore, percentage dry weight = 100 – 79.4 = 20.6%

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Since 0.0868kg of wet Water Hyacinth contains 0.0179kg of dry matter of Water Hyacinth,

Therefore, 1kg wet Water Hyacinth contained (0.0179/0.0868)kg dry matter = 0.206kg dry matter.

1kg of dry Water Hyacinth was contained in 4.85kg of wet Water Hyacinth.

0.75kg of dry Water Hyacinth was contained in 3.638kg of wet Water Hyacinth.

0.5kg of dry Water Hyacinth was contained in 2.425kg of wet Water Hyacinth.

0.25kg of dry Water Hyacinth was contained in 1.213kg of wet Water Hyacinth.

3.7.3 Charging of anaerobic digester/anaerobic digestion

All slurry mixtures were made in ratio 1:11 (0.75kg: 8.25kg) of substrate and water.

Single Substrates:

- **Treatment 1-Pig dung:** 1.88 kg of wet pig dung was weighed and 7.12 litres of water was added to form slurry (9 litres).
- **Treatment 2-Water Hyacinth:** 3.64 kg of wet water hyacinth was weighed out and 5.36 litres of water was added to form slurry (9 litres).

  Treatment 3-Maize Cob: 3.30kg of wet water hyacinth was weighed out and 5.7 litres of water was added to form slurry (9 litres).

Mixtures of substrates:

- **Treatment 4-Pig Dung/Maize Cob:** 0.94 kg of wet pig dung and 1.65 kg of wet maize cob were weighed out and 6.41litres of water was added to form slurry (9 litres).
- **Treatment 5-Pig Dung/Water Hyacinth:** 0.94kg of wet pig dung and1.82kg of wet water hyacinth was weighed out and 6.24litres of water was added to form slurry (9litres).

Treatment 6-Pig Dung/Maize Cob/Water Hyacinth: 0.63kg of wet pig dung, 1.10kg of wet maize cob and 1.22kg of wet water hyacinth were weighed and 6.05 litres of water

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was added to form slurry (9 litres). All mixtures of slurry were poured into their respective digesters and were properly sealed for anaerobic digestion process to begin.

3.7.4 Physical and chemical characterization of the various treatment groups:

The pH and temperature of all slurry mixtures were determined using pH meter and thermometer while 200 ml of each slurry mixture was collected into clean bottle water container and were immediately taken to the laboratory for analysis of the following parameters; Physical characteristics (total solids), chemical characteristics (TOC, TN, TP, TK, BOD, COD and microbial (aerobic, anaerobic, coliform and fungal) characteristics. Samples were also taken to the laboratory for analysis on days 7, 14, 21, 28 and 35.

3.7.4.1 Physical Characterization:

1. Determination of temperature:

Aim

To determine the temperature of given samples using thermometer.

Procedure:

1. The electrode of the thermometer was submerged into the slurry inside the digester through the tap opening. The solution was stirred for a few seconds.
2. The readings were allowed to stabilize (a minute or so) and results, recorded.

Probe was always rinsed off with deionized water after using.

2. **Determination of pH**

Aim

pH determination to check the level of acidity and alkalinity of the medium.

Principle

pH value of water indicates the hydrogen ion concentration in water and concept of pH was put forward by Sorenson (1909). pH is expressed as the logarithm of the reciprocal of the hydrogen ion concentration in moles/litre at a given temperature. The pH scale extends from 0 (very

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acidic) to 14 (very alkaline) with 7 corresponding to exact neutrality at 25°C. While the alkalinity or acidity measures the total resistance to the pH change or buffering capacity, the pH gives the hydrogen ion activity. pH can be measured colorimetrically or electrometrically. Colorimetric method is used only for rough estimation. It can be done either by using universal indicator or by using pH paper. The hydrogen electrode is the absolute standard for the measurement of pH. They range from portable battery operated units to highly precise instruments.

Apparatus

1. pH meter with electrode 2. Beaker

Reagents

Buffer solution 7.01 and 4.01

Procedure

Using Hanna pH Meter

1. Before use, the pH metre was calibrated, using buffers 7.01 and 4.01acording to the manufacturer's instructions.
2. The metre was switched on which automatically entered into pH mode.
3. The electrode of the pH meter was submerged into the sample to be tested.
4. Reading on the metre was allowed to stabilize (a minute or so) and results were recorded.

3.7.4.2 Chemical characterization

Samples were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C. 1990). All analysis were carried out in triplicate.

1. Determination of Total Organic Carbon

The Total Organic Carbon was determined by using the Walkey Black Method**.**

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Reagents and Methods of Preparation

1. Standard Normal Potassium Dichromate

K_2CrO_7 was oven dried at 130-150^{O}C for 2 to 3 hours. It was cooled in a desiccator, weighed at exactly 49.035g of the dried salt, dissolved in about 950ml of the distilled water, and placed in a cool place or room overnight. When cool, it was made up to 1000ml with distilled water (cold).

2. Standard Normal Ferrous Ammonium Sulphate.

156.86g of Fe (NH_4) $(SO_4)_2$ was weighed out and dissolved in about 900ml of distilled water. 25ml Con. H_2SO_4 was added and allowed to cool. It was made up to the mark with distilled water and standardized using the Normal Potassium dichromate

3. Diphenylamine Indicator

1g of diphenylamine was dissolved in 200ml of 1 to 1 solution of H_20 to H_2SO_4.

Procedure

1. Between 0.1- 3.0gm of the sample was weighed; depending on how dark the colour of the analyte is.

2. 10ml of the 1N $K_2Cr_2O_7$ was added from an automatic burette, then added to this very carefully was appropriately 20M Conc. H_2SO_4 from an acid dispensing burette. Shake gently and left to cool.

3. Distilled water was added to make up to approximately 150ml mark on the conical flask

4. Added to it was 8-10 drops of diphenylamine- indicators; the colour was now dark violet.

5. It was titrated with 0.4N Ferrous Ammonium Sulphate until the voilet colour changed to green.

6. A duplicate blank determination was carried out on 10ml of the Normal $K_2Cr_2O_7$using all the reagents each time a set of determination was done.

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Calculation

Let y be the vol. in millimeters of 0.4N Ferrous ammonium sulphate used to react with the remaining in $K_2Cr_2O_7$ is O.4y e.g since 10ml of $K_2Cr_2O_7$ were used in the first place, then the amount used to oxidize any carbon in the sample will be (10.0 – 0.4y). 1ml of $K_2Cr_2O_7$ = 0.003g carbon. However, the reaction is only approximately 75% complete.

Therefore, 1ml of $K_2Cr_2O_7$= 0.003×100/75= 0.004gC.

That is % Total organic carbon in the sample (hydrolysate)

$$= \frac{(10.0-0.4y) \times 0.004 \times 100}{\text{Wt of sample taken}}$$

Since y is Titre value (T.V) used for the titration

Hence

$$\% \text{ Total Organic carbon} = \frac{(10.0-0.4\times T.V) \times 0.004 \times 100}{\text{Wt of sample taken}}$$

Where T.V= Titre Value.

2. Determination of Total Nitrogen (%)

The total nitrogen (%) in the samples was determined by the routine semi- micro Kjeldahl procedure/ technique. This consists of three techniques namely Digestion, Distillation and Titration.

Apparatus: Analytical balance, Digestion tubes, Digestion Block Heater, 50ml Burette, 5ml Pipette, 10ml pipette, 10ml Measuring Cyclinder, 100ml Beakers, Fume cupboard

Reagents: Conc H_2SO_4, 0.01N HCl, 40% (w/v) NaOH, 2% Boric Acid Solution, Methyl Red-Bromocresol green mixed indicator, Kjeldahl catalyst tablet.

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Digestion

0.5g of each sample was weighed carefully into the kjeldahl digestion tubes to ensure that all sample materials got to the bottom of the tubes. To this were added 1 Kjeldahl catalyst tablet and 10ml of Conc H_2SO_4. These were set in the appropriate hole of the Digestion Block Heaters in a fume cupboard. The digestion was left on for 4 hours, after which a clear colourless solution was left in the tube. The digest was cooled and transferred into 100ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

Distillation

The distillation was done with Markham Distillation Apparatus which allows volatile substances such as ammonia to be steam distilled with complete collection of the distillate. The apparatus was steamed out for about ten minutes. The steam generator was then removed from the heat source to the developing vacuum to removed condensed water. The steam generator was then placed on the heat source (i.e heating mantle) and each component of the apparatus was fixed up appropriately.

Determination: 5ml portion of the digest above was pipetted into the body of the apparatus via the small funnel aperture. To this was added 5ml of 40% (w/v) NaOH through the same opening with the 5ml pipette.

The mixture was steam- distilled for 2 minutes into a 50ml conical flask containing 10ml of 2% Boric Acid plus mixed indicator solution placed at the receiving tip of the condenser. The Boric Acid plus indicator solution changed colour from red to green showing that all the ammonia liberated had been trapped.

Titration

The green colour solution obtained was then titrated against 0.01N HCl contained in a 50ml Burette. At the end point or equivalent point, the green colour turned to wine colour which indicated that all the Nitrogen trapped as Ammonium Borate [$(NH_4)_2$ BO_3] was removed as Ammonium chloride (NH_4Cl).

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Calculation

The percentage nitrogen in this analysis was calculated using the formula:

$$\% N = \frac{\text{Titre value} \times \text{Normality of HCl used} \times \text{Atomic mass of N} \times \text{Volume of Flask containing the digest} \times 100}{2000}$$

3. Determination of Total Phosphorus (%)

Phosphorus was determined routinely by the Vanado-Molybdate colourimeter or spectrophotometric method.

Apparatus: Spectrophotometer or colourimeter, 50ml volumetric flask, 10ml Pipette, Whatman filter paper, Funnel, Wash bottle, Glass rod, Heating mantle, Crucibles, Flame photometer, Analytical balance.

Reagents: Vanadate- molybdate yellow solution, 2M HCl.

Preparation of Standard Phosphate Solution: 219.5mg anhydrous $KH_2PO_4$was dissolved in distilled water and diluted to 1000ml; 1.00ml = 50.0ug PO_4^{3-} P.

Preparation of Calibration Curve: 10ml of the standard phosphate solution was placed in a 50ml volumetric flask. 10ml vanadate- molybdate yellow solution was added and diluted to the mark with distilled water, stoppered and left for 10mins for full yellow development. After 10mins or more, the absorbance was measured versus a blank solution (using 15ml, 20ml, 25ml 30ml). A graph of Absorbance against Concentration was drawn and the slope was calculated.

Procedure: 20mg (0.02g) of each sample was digested by adding 5ml of 2 M HCl solution to the hydrolysate in the crucible and heated to dryness on a heating mantle. 5ml of 2M HCl was added again, heated to boil, and filtered through a whatman No 1 filter paper. 10ml of the filterate solution was pipetted into 50ml standard flask and 10ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density

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(OD) or absorbance of the solution on a Spectronic 20 spectrophotometer or colourimeter at wavelenght of 470nm.

NOTE: A wavelenght of 470nm is usually used because ferric ion causes interference at low wavelenghts, particularly at 400nm.

Calculation

The percentage phosphorus was calculated from using the formula:

$$\% P = \frac{\text{Absorbance reading} \times \text{Slope} \times \text{Dilution factor}}{1000}$$

Where

Absorbance× Slope× Dilution faction=PPM/10,000

Hence

% P = PPM/10,000

Where

Absorbance = Reading obtained from the spectrophotometer.

Slope = Result of the Standard curve
Dilution factor = Volume of the extract/ weight of the sample.

4. Determination of Potassium

Principle:

Trace amounts of potassium can be determined by flame photometry at a wavelength of 766.5 nm. The sample was sprayed into a gas flame and excitation was carried out under carefully controlled and reproducible conditions. The desired spectral line was isolated by the use of interference filters or by a suitable slit arrangement in light dispersion devices such as prisms or gratings. The intensity of light was measured by a phototube potentiometer or other appropriate

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circuit. The intensity of light at 766.5nm was approximately proportional to the concentration of the element.

Procedure:

A blank and Sodium calibration standards in stepped amounts in any of the following applicable ranges: 0-1.0, 0-100mg/L was prepared. Starting with the highest calibration standard and working towards the most dilute, measure emission at 766.5nm. The operation was repeated with both calibration standard and samples enough times to secure a reliable average reading for each solution. A calibration curve was plotted from the potassium standards. The concentration of potassium in the sample was determined from the calibration curve.

Calculation:

$$\text{mg Na/l} = (\text{mg Na/l in portion}) \times D$$

$$D = \text{dilution ratio} = \frac{\text{ml sample} + \text{ml distilled water}}{\text{ml sample}}$$

5. Determination of BOD

Aim

To determine the amount of BOD exerted by the given samples

Principle

The Biochemical Oxygen Demand (B.O.D.) of sewage or of polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under aerobic condition and at the standardized time and temperature. Usually, the time is taken as 5 days and the temperature 20°C as per the global standard.

Apparatus

B.O.D. bottles 300mL capacity, B.O.D. incubator, burette, Pipette, air compressor, measuring cylinder etc.

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Reagents

Distilled water, phosphate buffer solution, Magnesium sulphate solution, Calcium chloride solution, Ferric chloride solution, acid and alkali solution, Seeding, Sodium sulphite solution and reagents required for the determination of D.O.

Procedure

1. The desired volume of distilled water was placed in a 5 litre flask (usually about 3 litres of distilled water was needed for each sample).
2. 1mL each of phosphate buffer, magnesium sulphate solution, calcium chloride solution and ferric chloride solution were added for every litre of distilled water.
3. The sample was seeded with 1–2 mL of settled domestic sewage.
4. The dilution water in the flask was saturated by aerating with a supply of clean compressed air for at least 30 minutes.
5. Highly alkaline or acidic samples were neutralised to pH 7.
6. The chlorine residual in the sample was destroyed by keeping the sample exposed to air for 1 to 2 hours or by adding a few mL of sodium sulphite solution.
7. Sample was taken in the required concentrations as follows:

   Strong industrial waste: 0.1, 0.5 and 1 per cent

   Raw and settled sewage: 1.0, 2.5 and 5 per cent

   Oxidised effluents: 5, 12.5 and 25 per cent

   Polluted river water: 25, 50 and 100 per cent
8. The required quantity of sample (calculate for 650 mL dilution water the required quantity of sample for a particular concentration) was placed in 1000 mL measuring cylinder. The dilution water was added to the 650mL mark.
9. The contents were mixed in the measuring cylinder.
10. The solution was added into two B.O.D. bottles, one for incubation and the other for determination of initial dissolved oxygen in the mixture.
11. The other concentrations and for all the other samples were prepared in the same manner.
12. Lastly the dilution water alone was filled into two B.O.D. bottles. One was kept for incubation and the other for determination of initial dissolved oxygen.

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13. The set of bottles to be incubated were placed in a B.O.D. incubator for 5 days at 20°C. Care was taken to maintain the water seal over the bottles throughout the period of incubation.
14. The initial dissolved oxygen contents in the other set of bottles were determine and the results were noted.
15. The dissolved oxygen content in the incubated bottles at the end of 5 days were determined and results noted down.
16. The B.O.D. of the given sample was calculated.

Sample calculation

To determine the value of the BOD in mg/L the following formula was used:

BOD, mg/L = [(Initial DO - Final DO) x 300]/V (mL) sample

D1 = Initial Dissolved Oxygen = ... mg/L

D2 = Dissolved Oxygen at the end of 5 days = ... mg/L

V= volume of sample used

6. Determination of COD

Aim

To determine the Chemical Oxygen Demand (C.O.D.) for given sample.

Principle

The organic matter present in sample gets oxidized completely by potassium dichromate ($K_2Cr_2O_7$) in the presence of sulphuric acid (H_2SO_4), silver sulphate ($AgSO_4$) and mercury sulphate ($HgSO_4$) to produce CO_2 and H_2O. The sample is refluxed with a known amount ofpotassium dichromate ($K_2Cr_2O_7$) in the sulphuric acid medium and the excess potassium dichromate ($K_2Cr_2O_7$) is determined by titration against ferrous ammonium sulphate, using ferroin as an indicator. The dichromate consumed by the sample is equivalent to the amount of O_2 required to oxidize the organic matter.

Apparatus

Reflux apparatus, burettes, pipettes

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Reagents

Standard potassium dichromate solution 0.25N., Sulphuric acid reagent, standard ferrous ammonium sulphate (approximately 0.1N), Ferroin indicator solution, Mercuric sulphate, Sulphuric acid crystals.

Procedure

1. 50.0 mL of sample was placed in a 500 mL refluxing flask.
2. 1g mercuric sulphate and a few glass beads were added.
3. Sulphuric acid was added to dissolve the mercuric sulphate and cooled.
4. 25.0 ml 0.25 N potassium dichromate solution was added and mixed.
5. The flask was attached to the condenser and the cooling water was started.
6. The remaining acid reagent (70 mL) was added through the open end of condenser and mixed properly.
7. Heat was applied and the system was refluxed for 5 hours.
8. The condenser was then cooled and washed down with distilled water.
9. The mixture was diluted to about twice its volume and cooled to room temperature.
10. The excess dichromate was titrated with standard ferrous ammonium sulphate using ferroin indicator (2 to 3 drops).
11. The colour changed from blue green to reddish indicating the end point.
12. A blank consisting of distilled water of equal volume as that of the sample was refluxed in the same manner.

Calculation

$$\text{mg/L C.O.D.} = \frac{(V1 - V2)\ N \times 80000}{V}$$

where,

V1 = mL ferrous ammonium sulphate used for blank

V2 = mL ferrous ammonium sulphate used for sample

N = normality of ferrous ammonium sulphate

V = volume of sample used.

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3.7.5 Microbiological examination

Isolation of organisms from the slurry samples:

The 6 samples listed above were diluted using the tenfold serial dilution technique to reduce the microbial concentration in the samples step wisely. 1ml of the appropriate dilutions was pipetted (aseptically) into various Petri-dishes and already sterilized molten agar at 45^{o}C were poured on them, swirled gently and allowed to solidify using the method of Harrigan and Mc Cance (1966). The culture media used include: Potato Dextrose agar (PDA) for fungal isolation, MacConkey agar for coliforms, Nutrient Agar for Total Heterotrophic count, and DE Man Sharpe Rogosa (MRS) Agar for anaerobic organisms. Fungal plates were incubated at 30^{o}C for 3 to 5 days, coliforms and aerobic plates were incubated at 30^{o}C for 24-48 hours while anaerobic plates were incubated in an anaerobic jar containing a moistened pack of gas generating kit (Oxoid BR, Basrugstoke, England) at 30^{0}C for 72 hours. After the incubation period, colonies of organisms were counted using a colony counter and the total count for each target organism was enumerated by multiplying with the corresponding dilution factor.

Characterization of isolates

The obtained microorganisms were characterized using macroscopic, microscopic and biochemical methods. The results were compared with the scheme of Bergey's Manual of Determinative Bacteriology and Cowan and Steel.

Methods

Gram staining

This was done to classify the isolated organisms into Gram positive or gram negative based on their reaction to the Gram staining technique of Christian Gram.

Procedure: A heat fixed smear from a 18-24 hr old culture was prepared for each organism. They were stained with crystal violet solution for between 1-2 minutes and poured off. The slides were rinsed with Gram's Iodine solution and the iodine was allowed to react for 1 minute. The iodine solution was subsequently rinsed off and the slides washed off with 95% alcohol until no more violet colour runs from the slide. They were then rinsed with water and counterstained with

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Safranin for 1-2 minutes. Finally, the slides were washed with water, dried and examined under the oil immersion power of the light microscope.

Catalase test

This test was carried out to detect the production of the enzyme, catalase by an organism. The enzyme converts Hydrogen peroxide to Water and Oxygen as shown in the equation:

$2H_2O_2$ …… $2H_2O + O2$

Procedure: 3% Hydrogen peroxide was prepared by adding 97ml of distilled water to 3ml of concentrated H_2O_2. A drop of the 3% H_2O_2 was placed on a clean glass slide and an 18-24 hour old culture of each organism was smeared on the slide and observed. Effervescence, caused by the liberation of Oxygen gas indicated Catalase production, a positive result while the absence indicated a negative reaction (Seeley and Van Denmark, 1972).

Oxidase test

The Oxidase test was carried out to detect the presence of Cytochrome C in the organisms under study. The test is very sensitive and of importance in taxonomic and identification studies.

Procedure: A few drops of tetramethyl-p-phenylenediamine hydrogen chloride was added to a piece of Whatman filter paper in a petri dish. 18-24 hr old cultures of the organisms were smeared onto the impregnated filter paper. A purple colouration within 10-15 seconds indicated an Oxidase positive culture while a negative result was indicated by no colour change.

Citrate utilization test

This was carried out to differentiate the isolated organisms by their ability to utilize citrate as a sole carbon source.

Procedure: Citrate agar slants were prepared according to the Manufacturer's instruction, and inoculated with a peptone water culture of the organisms using an inoculating loop. They were then incubated for 2-5 days at 37^{o}C. A change in the indicator from green to blue indicated utilization of the citrate (Olutiola, 1991).

Indole production

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This test was carried out to detect the production of indole from tryptophan by the organisms.

Procedure: Tubes of tryptone water were inoculated with a loopful broth culture of the organisms under study. The set up was incubated for 5-7 days at 37^{o}C in the incubator. 0.5ml Kovac's reagent was then added to each tube, shaken gently and allowed to stand. A deep red colour/ring which separates out in the alcohol layer indicated the production of Indole.

Sugar fermentation tests

The ability of an organism to ferment several sugars is demonstrated by this test. The sugars utilized may be characteristic of a particular microorganism and hence such organisms can be identified on the basis of the type of sugar they ferment.

Procedure: To the sugar fermentation medium, 0.10% phenol red (indicator) was added, 10ml of the prepared medium was dispensed into the test tubes. A Durham tube was inverted into each tube to trap any evolved. They were then sterilized at 121^{o}C for 15 minutes. The tube for each sugar was then inoculated with the isolates and incubated for 5-7 days. A change of in the colour of the indicator from red to yellow indicated acid production while accumulation of bubble in the Durham tube was an indicator of gas production.

Data Management & Statistical Analysis

Data was recorded at every given step in the process. This was achieved by measurement of weight, volumes, density, pH, temperature, TOC, TN, TP, TK, BOD, COD, microbial (aerobic, anaerobic, coliform and fungal) characteristics and biogas yield.

All data was summarized using descriptive statistics such as proportions, means and standard deviation.

The results of the physico-chemical analysis, microbial analysis and biogas yields from the various slurries were subjected to One-Way Analysis of Variance (ANOVA) at 5% level of Precision (α=5%) to compare their various means.

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CHAPTER FOUR

RESULTS

This chapter presents the results of the pilot/feasibility study which includes the estimation of quantity of samples from source, quantification of the biomass feedstocks (weight, volume, density) as well as results of the physico-chemical properties (Temperature, pH,% Total Organic Carbon (T.O.C), Total Nitrogen (%), Carbon to Nitrogen Ratio (C/N), Total Phosphorus (%),Total Potassium), Microbial characteristics and biogas yield of the slurries.

4.1 Source availability of feedstock biomass

A feasibility study was carried out on the sample collection areas to determine the sample populations and the quantity of agro by-product generated from the parent food materials. **Table 4.1** shows the estimation of the quantity of sample generated from the various sources. The 1acre of pig farm at University of Ibadan (UI) rear 215 pigs. The 0.65 hectares maize plantation at Abadina Quarters in UI produces 24074 to 40,000 maize cobs per harvest. The Oba-dam at UI occupy a land area of about 1586m^2 which is covered with 126880 to 190320 water hyacinth (WH) at the river bank.

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Table 4.1: Source availability of feedstock biomass

SAMPLE	LOCATION	PARENT SOURCE	Estimated quantity
Pig dung	University Of Ibadan Pig Farm	1 acre	215 pigs
Maize cob	Abadina Quarters	0.65 hectare maize plantation	24074 to 40,000 maize cobs per harvest
Water hyacinth	Oba-Dam	1586m^2 area of land covered	126880 to 190320 Water hyacinth

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4.2 Quantification of biomass feedstock materials.

Tables 4.2a-4.2b shows the quantity of biomass wastes generated from a unit parent source. One pig generates pig dung of mean weight 0.495±.048 kg and a mean volume of 0.0006±.0002 m^3 per defecation. The mean density was estimated as 717.8000±28.8156 kg/m^3. The mean weight, volume and density of a single strand of water hyacinth were estimated as 0.0307±0.0054 kg, 0.0003±0.00005 m^3 and 100.6133±1.4161 kg/m^3 respectively while the mean weight, volume and density of a single maize cob were estimated as 0.127±0.023 kg, 0.00042±0.000073 m^3 and 303.120±15.655 kg/m^3 respectively.

Tables 4.2c-4.2d shows the quantity of biomass wastes generated from the parent source. The 1 acre of pig farm at UI generates pig dung of mean weight 128.0±16.0kg and a mean volume of 0.179±0.023m^3 per day. The mean density was estimated as 717.083±15.181kg/m^3. The 0.65 hectare of maize plantation at Abadina Quarters in UI generates MC with a mean weight ranging from 1163.6±36.8 to 1933.3±61.1kg and a mean volume ranging from 0.71±0.02 to 1.02±0.3 m^3 per harvest, while the mean density was estimated as 1641.443±36.240181kg/m^3. The Oba-dam at UI occupy a land area of about 1586m^2 which are covered with WH of mean weight ranging from 3895.2±681.4 to 5842.82±1022.1 kg and mean volume which ranges from 38.7±6.7 to 58.05±10.0 m^3. Its mean density was estimated as 100.6±1.4kg/m^3.

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Table4.2a: The weight, volume and density of the various biomass wastes from a unit parent source.

	Experiment	Pig dung	Maize cob	Water hyacinth
Weight of waste	1	0.550	0.050	0.0365
(Kg)	2	0.475	0.047	0.0297
	3	0.460	0.048	0.0259
Volume of	1	0.0008	0.00003	0.000363
Waste	2	0.00067	0.00002835	0.000291
(m^3)	3	0.0004	0.00003	0.000261
Density of	1	694.44	1657.85	100.55
Waste	2	708.96	1666.67	102.06
(Kg/m^3)	3	750.00	1600.00	99.23

Table 4.2b: Mean and SD of weight, volume and density of the various biomass wastes from a unit parent source.

	PD	WH	MC
Weight (g)	495.00 ± 48.00	30.70 ± 5.40	127.00 ± 23.00
Volume (l)	0.6 ± 0.2	0.3 ± 0.05	0.42 ± 0.07
Density (g/l)	717.8 ± 28.82	100.61 ± 1.42	1641.50 ± 36.20

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Table 4.2c: Estimation of weight, volume and density of the various biomass wastes from the parent source.

	Experiment	Pig dung	Water hyacinth		Maize cob	
			Min	Max	Min	Max
Weight of waste (Kg)	1	144	4631.12	6946.68	1203.7	2000
	2	112	3768.34	5652.50	1131.5	1880
	3	128	3286.19	4929.29	1155.6	1920
Volume of Waste (m^3)	1	0.199	46.06	69.09	0.72	1.20
	2	0.154	36.92	55.38	0.68	1.13
	3	0.183	33.12	49.67	0.72	0.72
Density of Waste (Kg/m^3)	1	722.22	100.55		1657.85	
	2	729.03	102.06		1666.67	
	3	700.00	99.23		1600.00	

Table 4.2d: Mean and SD of weight, volume and density of the various biomass wastes from parent source

Sample	Mean Weight (Kg) Mean ± S.D		Mean Volume (m^3) Mean ± S.D		Density (Kg/m^3) Mean ± S.D
	Min. Limit	Max. Limit	Min. Limit	Max. Limit	
Pig dung	128.0±16.0		0.179±0.023		717.1±15.2
Water hyacinth	3895.2±681.4	5842.82±1022.1	38.7±6.7	58.05±10.0	100.6±1.4
Maize cob	1163.6±36.8	1933.3±61.1	0.71±0.02	1.02±0.3	1641.5±36.2

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4.3 The Physico-Chemical Characterization of the Slurry Mixtures:

Tables 4.3-4.8 show the mean values obtained from the physico-chemical characterization of the different slurry mixtures at weekly interval. The mean ambient and slurry temperatures of all the slurry mixtures were within the mesophilic range of 25.25 ± 0.4^{o}C to 26.25 ± 0.4^{o}C and 25.75 ± 0.4^{o}C to 28.75 ± 0.4^{o}C respectively throughout the duration of study. pH of all the slurries from day 0 to day 35 were within the range of 5.80 ± 0.0 to 7.85 ± 0.1. The Total Solids (TS) obtained from all the slurries ranged from 8.25 ± 0.1 to 20.57 ± 0.15.

The mean T.O.C (%) decreased as the anaerobic digestion progressed and vice versa. Among the biomass, MC recorded the highest T.O.C (%) at different days of anaerobic digestion while the least mean T.O.C (%) was found in PD. The T.O.C of each of the biomass were significantly different from each other ($p<0.05$). For the Total Nitrogen, TN (%); it was evident that the mean TN (%) increased as the days of anaerobic digestion increased and vice versa. The mean TN (%) was found to be greatest in PD for the whole duration of the study and least for MC. Thus, the mean TN (%) of the various substrates were significantly different from each other ($p<0.05$). The mean Total Phosphorus, TP (%) increased as the biodegradation process progressed in the anaerobic digester. It was obvious from the table below that PD had the highest TP (%) throughout the course of the experiment while MC recorded the least TP (%). The mean TP of the various substrates were significantly different from each other ($p<0.05$). Lastly, it was observed that the mean Total Potassium, TK (%) increased as the time of anaerobic digestion increased. Among the biomass, PW recorded the highest TK (%) at different days of anaerobic digestion while the least mean TK (%) was found in MC. The TK (%) of each of the biomass were significantly different from each other at $p<0.05$.

The mean Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) decreased sharply in the first three (3) weeks [days 0, 7 and 14] of the experiment and slowly for the remaining weeks of the experiment [days 21, 28 and 35]. PD had the highest BOD and COD value compared to the other biomasses (WH, MC, PM, PW, PMW) [$p<0.05$].

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Table 4.3: Pattern of Physico-Chemical Properties of the slurry mixtures at weekly interval (day 0)

DAY 0	PD	WH	MC	PD/MC	PD/WH	PMW
Parameters						
Temperature of slurry	25.8±.39	25.9±.32	26.2±.29	25.9±.40	26.1±.17	26.1±.23
Ambient Temperature	26.2±.29	26.2±.29	26.2±.29	26.2±.29	26.2±.29	26.2±.29
pH	6.6±.00	7.9±.06	5.8±.00	6.0±.00	7.3±.06	6.2±.00
Total Solids %	13.6±.21	11.3±.15	16.4±.15	20.6±.15	15.6±.27	14.1±.15
TOC (%)	41.7±4.86	45.9±1.27	63.4±1.09	58.0±.58	56.5±8.10	56.9±3.75
Total Nitrogen %	2.1±.09	2.0±.05	0.7±.02	1.0±.01	2.0±.01	1.1±.02
C/ N ratio	20.1±2.10	23.3±.09	97.5±3.29	57.3±.23	28.5±4.22	49.9±2.85
TP as phosphate (mg/l)	208.3±2.89	191.7±2.89	153.3±2.89	198.3±5.77	198.3±2.89	203.3±2.89
Potassium (mg/l)	29.7±.58	28.7±.58	23.0±1.00	29.7±.58	30.7±1.16	30.3±.58
BOD5 (mg/l)	2533.3±15.28	2223.3±12.58	1456.7±16.07	2336.7±15.28	2250.0±8.66	2448.3±25.66
COD (mg/l)	4675.0±15.00	3925.0±21.80	2630.0±10.00	4248.3±20.82	4130.0±13.23	4653.3±22.55

Note: PD = Pig Dung
WH = Water Hyacinth
MC = Maize Cob
PD/MC = Pig Dung + Maize Cob
PD/WH = Pig Dung + Water Hyacinth
PMW = Pig Dung + Maize Cob + Water Hyacinth

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Table 4.4: Pattern of Physico-Chemical Properties of the slurry mixtures at weekly interval (day 7)

DAY 7	PD	WH	MC	PD/MC	PD/WH	PMW
Temperature of slurry	28.0±.50	28.2±.38	28.3±.17	28.4±.10	27.9±.10	28.2±.29
Ambient Temperature	25.7±.29	25.7±.29	25.7±.29	25.7±.29	25.7±.29	25.7±.29
pH	6.4±.06	7.3±.10	4.0±3.46	6.1±.00	7.2±.00	6.2±.00
Total Solids %	11.6±.12	9.8±.15	14.7±.27	18.7±.15	13.5±.15	12.6±.15
TOC (%)	41.7±3.97	44.1±.11	60.4±1.35	55.1±1.53	56.1±2.28	55.9±1.04
Total Nitrogen %	2.1±.03	2.0±.01	0.7±.01	1.1±.02	2.0±.01	1.2±.02
C/ N ratio	20.0±1.81	21.7±.15	90.2±1.39	52.5±2.35	27.9±1.13	48.2±1.02
P as phosphate (mg/l)	225.0±5.00	205.0±.00	166.7±2.89	211.7±2.89	213.3±2.89	218.3±2.89
Potassium (mg/l)	31.0±1.00	29.0±1.00	26.0±1.00	31.3±.58	32.3±.58	32.0±.00
BOD5 (mg/l)	2263.3±15.28	2040.0±20.00	1226.7±15.28	2166.7±41.63	2098.3±47.52	2168.3±38.19
COD (mg/l)	4323.33±12.58	3783.3±07.64	2185.0±13.23	4051.7±2.89	4005.0±31.23	4228.3±17.56

Note: PD = Pig Dung
WH = Water Hyacinth
MC = Maize Cob
PD/MC = Pig Dung + Maize Cob
PD/WH = Pig Dung + Water Hyacinth
PMW = Pig Dung + Maize Cob + Water Hyacinth

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Table 4.5: Pattern of Physico-Chemical Properties of the slurry mixtures at weekly interval (day 14)

DAY 14	PD	WH	MC	PD/MC	PD/WH	PMW
Temperature of slurry	28.5±.00	28.3±.58	28.0±.00	28.7±.29	28.6±.14	28.0±.50
Ambient Temperature	26.0±.50	26.0±.50	26.0±.50	26.0±.50	26.0±.50	26.0±.50
pH	6.5±.06	7.3±.06	6.2±.00	6.2±.00	7.3±.00	6.4±.00
Total Solids %	10.3±.17	8.7±.40	13.6±.15	17.6±.15	12.5±.10	11.6±.25
TOC (%)	41.3±3.28	39.0±2.83	55.6±.75	50.9±.00	55.4±3.24	54.8±1.34
Total Nitrogen %	2.1±.03	2.1±.01	0.7±.01	1.1±.00	2.1±.03	1.2±.03
C/ N ratio	19.6±1.79134	18.8±1.34	78.3±.06	47.6±.00	26.8±1.92	46.4±.10
P as phosphate (mg/l)	235.0±.00	210.0±5.00	176.7±5.77	218.3±2.89	225.0±.00	230.0±.00
Potassium (mg/l)	32.0±.00	29.3±.58	27.7±.58	32.3±1.16	33.7±.58	33.3±.58
BOD5 (mg/l)	2140.0±10.00	1988.3±18.93	1041.7±17.56	2073.3±20.82	1980.0±5.00	2088.3±33.29
COD (mg/l)	4013.3±22.55	3570.0±00.00	1813.3±2.89	3981.7±10.41	3506.7±25.17	3766.7±15.28

Note: PD = Pig Dung
WH = Water Hyacinth
MC = Maize Cob
PD/MC = Pig Dung + Maize Cob
PD/WH = Pig Dung + Water Hyacinth
PMW = Pig Dung + Maize Cob + Water Hyacinth

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Table 4.6: Pattern of Physico-Chemical Properties of the slurry mixtures at weekly interval (day 21)

DAY 21	PD	WH	MC	PD/MC	PD/WH	PMW
Temperature of slurry	28.2±.32	28.5±.29	28.5±.09	28.4±.17	28.3±.29	28.4±.20
Ambient Temperature	26.0±.00	26.0±.00	26.0±.00	26.0±.00	26.0±.00	26.0±.00
pH	6.4±.06	7.3±.00	6.1±.06	6.1±.06	7.2±.00	6.3±.00
Total Solids %	10.0±.15	8.6±.15	13.2±.15	17.3±.12	12.2±.06	11.4±.21
Total Organic Carbon %	43.8±3.61	33.9±.27	47.0±1.45	44.2±1.17	54.5±.00	52.3±2.25
Total Nitrogen %	2.2±.01	2.1±.03	0.8±.00	1.2±.03	2.1±.00	1.2±.01
C/ N ratio	19.8±1.73	15.9±.13	62.7±1.93	38.5±1.43	26.0±.00	42.2±1.51
P as phosphate (mg/l)	273.3±2.89	231.7±2.89	196.7±5.77	250.0±5.00	260.0±5.00	270.0±.00
Potassium (mg/l)	33.7±.58	29.7±.58	28.0±1.00	35.0±.00	36.0±1.00	34.7±1.53
BOD5 (mg/l)	2051.7±12.58	1893.3±15.28	996.7±12.58	1996.7±20.82	1913.3±7.64	1995.0±25.00
COD (mg/l)	3888.3±67.89	3456.7±16.07	1733.3±36.86	3875.0±96.57	3485.0±21.80	3723.3±12.58

Note: PD = Pig Dung
WH = Water Hyacinth
MC = Maize Cob
PD/MC = Pig Dung + Maize Cob
PD/WH = Pig Dung + Water Hyacinth
PMW = Pig Dung + Maize Cob + Water Hyacinth

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Table 4.7: Pattern of Physico-Chemical Properties of the slurry mixtures at weekly interval (day 28)

DAY 28	PD	WH	MC	PD/MC	PD/WH	PMW
Temperature of slurry	28.1±.48	28.6±.23	28.8±.38	27.6±1.84	28.3±.29	28.6±.24
Ambient Temperature	25.9±.32	25.9±.32	25.9±.32	25.9±.32	25.9±.32	25.9±.32
pH	6.6±.06	7.2±.06	6.2±.00	6.3±.06	7.0±.00	6.2±.00
Total Solids %	9.7±.06	8.5±.06	12.8±.17	16.9±.10	11.9±.06	10.9±.10
TOC %	38.4±2.64	31.5±.08	39.2±.28	35.7±.77	52.5±4.79	50.9±3.63
Total Nitrogen %	2.3±.02	2.2±.01	.8±.02	1.2±.04	2.1±.01	1.3±.04
C/ N ratio	16.9±1.28	14.1±.07	51.0±.80	29.7±.25	24.6±2.25	38.8±1.91
TP as phosphate (mg/l)	285.0±5.00	238.3±2.89	208.3±2.89	258.3±7.64	268.3±2.89	276.7±2.89
Potassium (mg/l)	34.3±.58	30.7±.58	29.3±.58	35.7±.58	36.7±.58	35.0±1.00
BOD5 (mg/l)	2010.0±10.00	1853.3±11.55	976.7±7.64	1963.3±05.77	1888.3±2.89	1976.7±10.41
COD (mg/l)	3790.0±10.00	3371.7±7.64	1631.7±16.07	3681.7±30.14	3393.3±30.55	3668.3±16.07

Note: PD = Pig Dung
WH = Water Hyacinth
MC = Maize Cob
PD/MC = Pig Dung + Maize Cob
PD/WH = Pig Dung + Water Hyacinth
PMW = Pig Dung + Maize Cob + Water Hyacinth

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Table 4.8: Pattern of Physico-Chemical Properties of the slurry mixtures at weekly interval (day 35)

DAY 35	PD	WH	MC	PD/MC	PD/WH	PMW
Temperature of slurry	28.0±.46	28.0±.00	28.2±.25	28.3±.12	27.9±.17	28.3±.25
Ambient Temperature	25.8±.29	25.8±.29	25.8±.29	25.8±.29	25.8±.29	25.8±.29
pH	6.5±.00	7.2±.00	6.1±.00	6.1±.06	7.0±.06	6.2±.06
Total Solids %	9.5±.06	8.3±.06	12.3±.12	16.5±.06	11.5±.06	10.3±.06
TOC %	37.8±2.71	29.1±2.38	37.2±.67	34.9±2.92	52.0±4.23	49.5±2.99
Total Nitrogen %	2.3±.01	2.3±.03	0.8±.01	1.2±.03	2.2±.00	1.3±.02
C/ N ratio	16.3±1.13	13.0±1.19	47.7±1.29	28.3±2.12	24.2±1.97	37.2±2.35
TP as phosphate (mg/l)	301.7±2.89	251.7±2.89	221.7±2.89	273.3±2.89	281.7±2.89	290.0±5.00
Potassium (mg/l)	35.0±1.00	31.0±1.00	29.5±.58	36.0±.00	36.7±.58	35.7±1.00
BOD5 (mg/l)	1995.0±10.00	1846.7±2.89	968.3±10.41	1956.7±7.64	1870.0±5.00	1961.7±7.64
COD (mg/l)	3698.3±25.66	3360.0±13.23	1576.7±25.17	3588.3±28.43	3355.0±5.00	3526.7±12.58

Note: PD = Pig Dung
WH = Water Hyacinth
MC = Maize Cob
PD/MC = Pig Dung + Maize Cob
PD/WH = Pig Dung + Water Hyacinth
PMW = Pig Dung + Maize Cob + Water Hyacinth

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4.4 Biogas yield

Figure 4.1 a & b shows the daily biogas production obtained from the different slurries of the various biomasses from day 20 to day 33. The initiation time for biogas production was observed on day 20 (PD, PD/MC and PD/WH) and day 22 (WH, MC and PMW). Peak biogas production was observed on day 23 for PD (987.50±3.5ml); day 24 for PW (1095.00 ± 7.1ml), and PM (732.50 ± 17.7ml); day 25 for MC (560.00 ± 7.1ml), day 26 for WH (635.00 ± 7.1ml) and PMW (662.50 ± 10.6ml). Group PW had the highest biogas yield of 6067.00 ± 38.2ml for the entire duration of the study. There was a significant difference between the mean biogas yields of the various feedstock groups ($p<0.05$)

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Plate 4.1: Showing biogas flame from bursen burner

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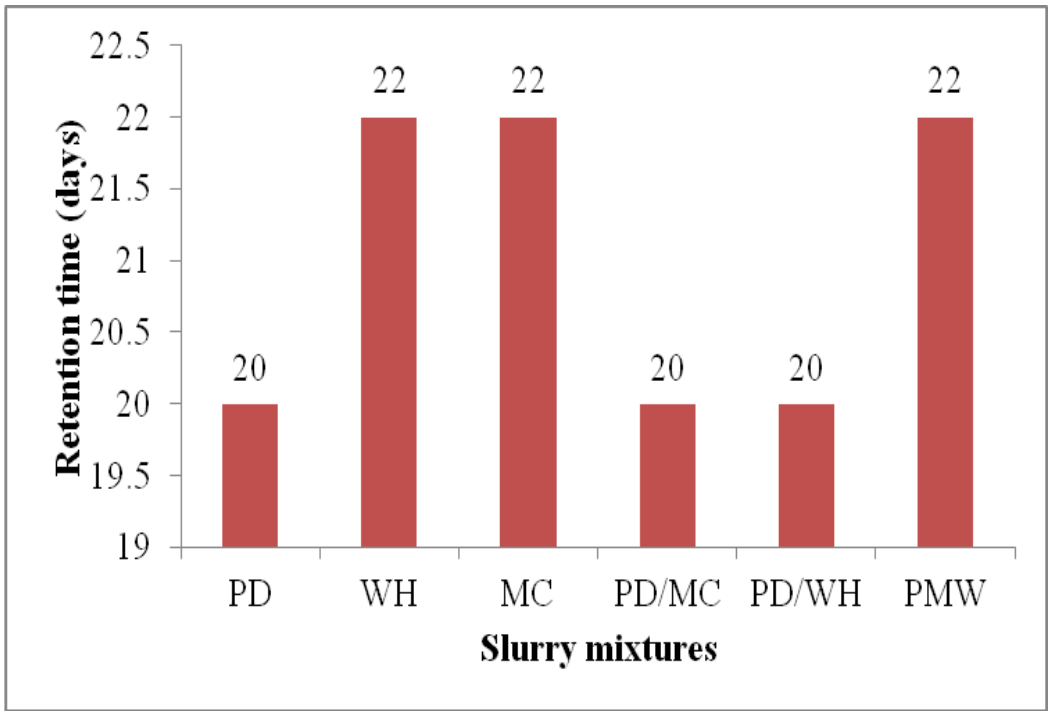

Figure 4.1: Showing day of commencement of biogas production in each slurry.

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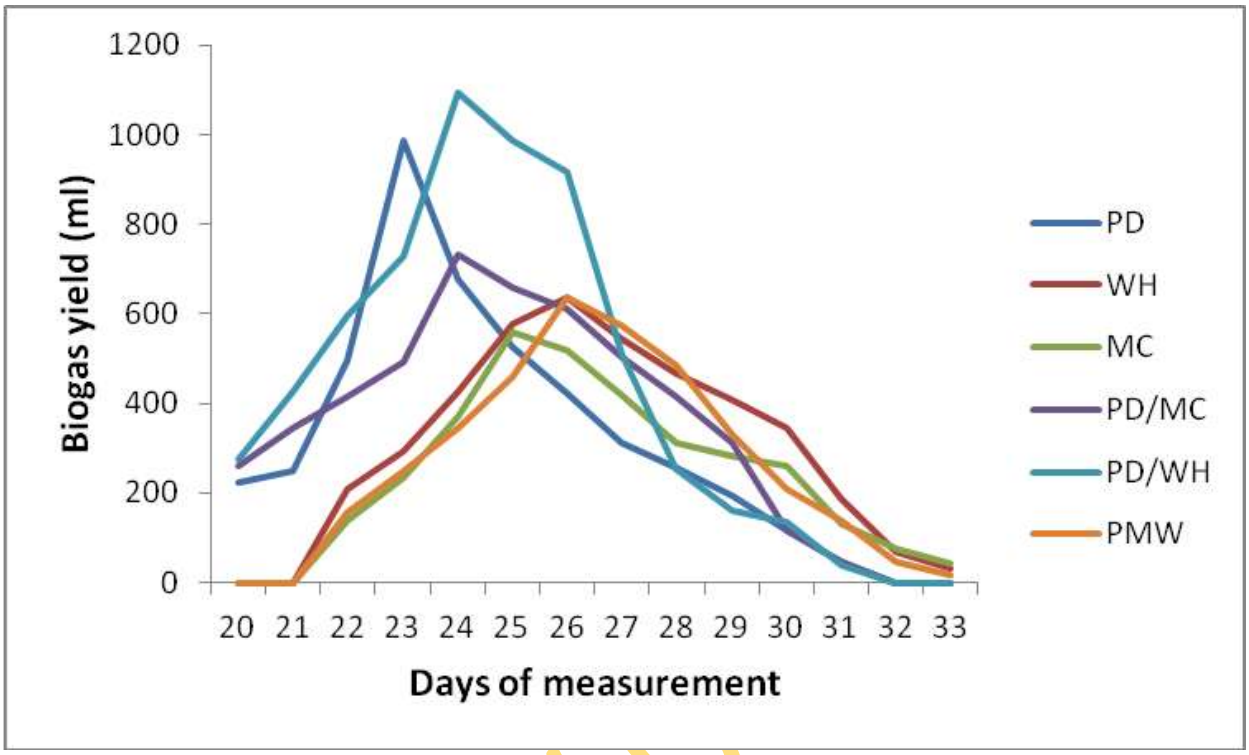

Figure 4.2: Biogas yield over the entire sampling period.

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4.5 Microbial load in slurries

Figures 4.3-4.6 show results of the microbiological examination of slurries obtained from the anaerobic digestion of the various biomass feedstocks. The organisms identified were **aerobes (***Bacillus spp; Flavobacterium sp; Micrococcus sp; Pseudomonas sp; Staphylococcus sp),* **coliform groups (***E.coli; Enterobacter sp; Aeromonas sp; Proteus sp),* **anaerobes (***Lactobacillus spp; Methanobacterium spp.),* and **Fungi (***Aspergillus sp; Candida spp***).**

The anaerobic, coliform and fungal counts ranged from 6.80×10^{2} to 1.0×10^{5}cfu/g, 4.3×10^{4} to 6.2×10^{6}cfu/g, and 9.1×10^{3} to 6.3×10^{6}cfu/g respectively throughout the duration of the study. The highest anaerobic count ($1.0\times10^{5} \pm 0.03\times10^{5}$cfu/g) was recorded in PW on day 28. **Figure 4.3** shows that the mean Total Anaerobic count (TANC) increased steadily from day 0-14 and sharply from day 14-28 before declining from day 28-35. In **Figures 4.4-4.6,** the mean Total Aerobic count (TAC), Total coliform count (TCC) and Total fungal count (TFC) [cfus/g] decreased significantly throughout the duration of the study ($p<0.05$).

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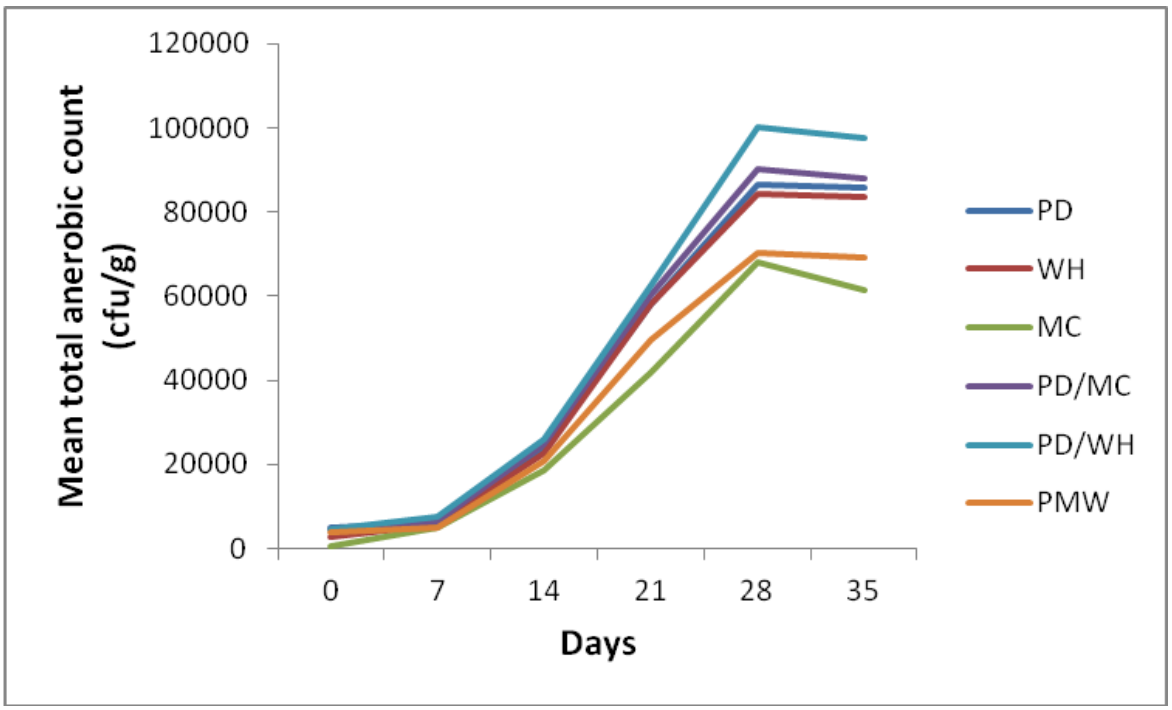

Figure 4.3: Mean total anaerobes from slurries over the entire sampling period.

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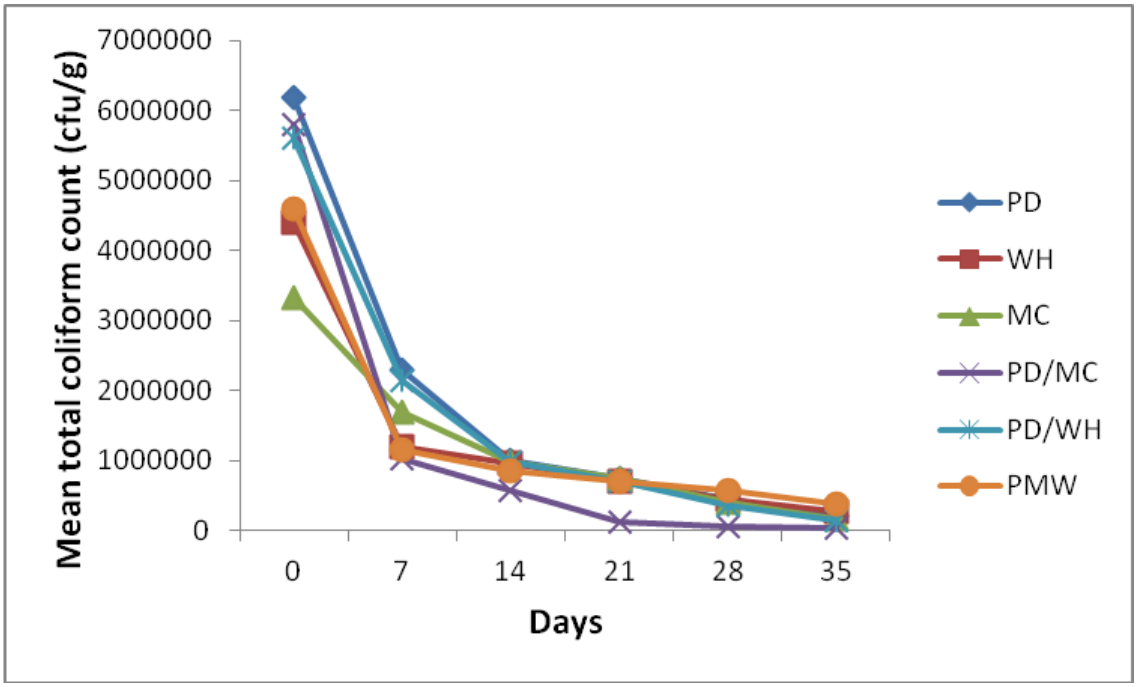

Figure 4.4: Mean total coliform count from slurries over the entire sampling period.

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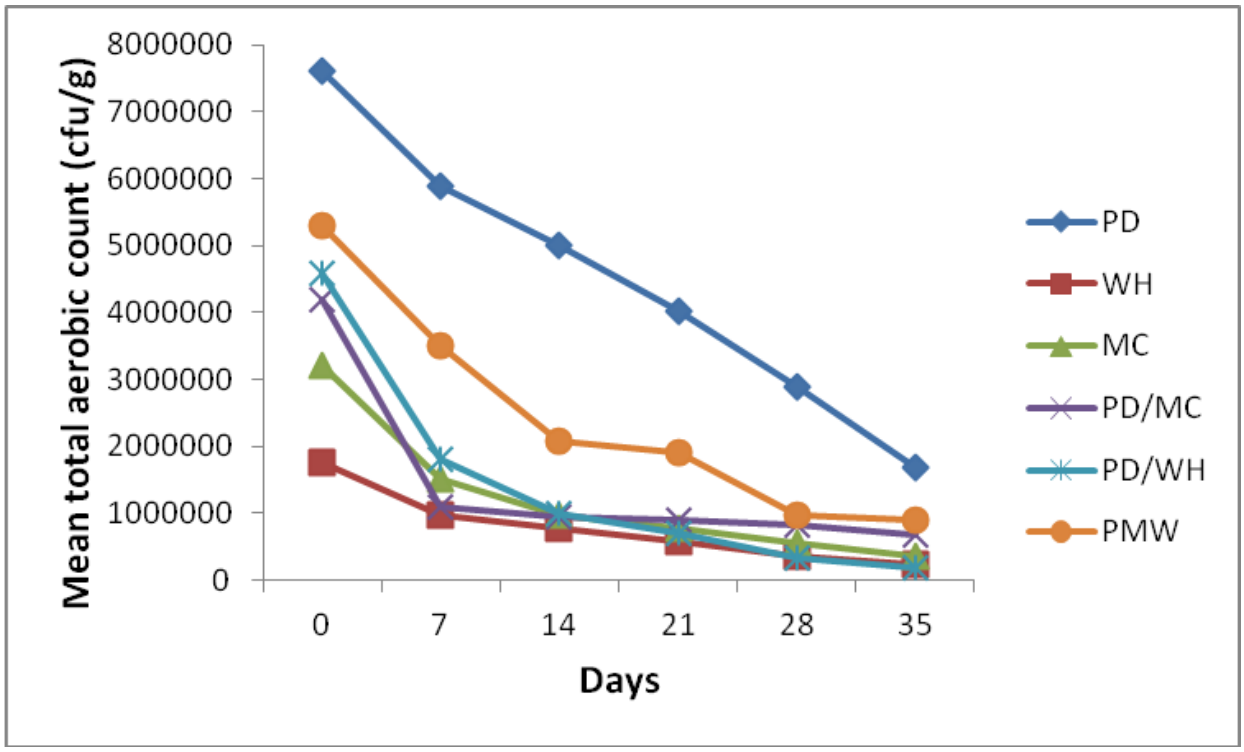

Figure 4.5: Mean total aerobic count from slurries over the entire sampling period.

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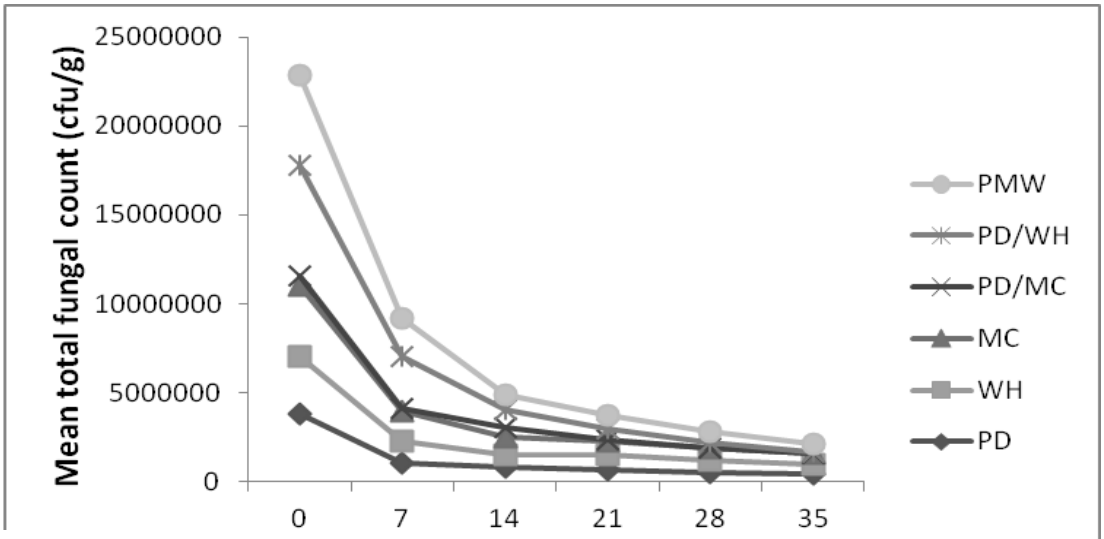

Figure 4.6: Mean total fungal count from slurries over the entire sampling period.

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4.6 Anaerobic count and biogas yield

Figure 7 shows the relationship between the mean anaerobic count and the sum of biogas yield for the entire duration of the study. The anaerobic count is in direct proportion to the biogas yield, that is as the anaerobic count increases, the biogas production also increases for the whole substrates.

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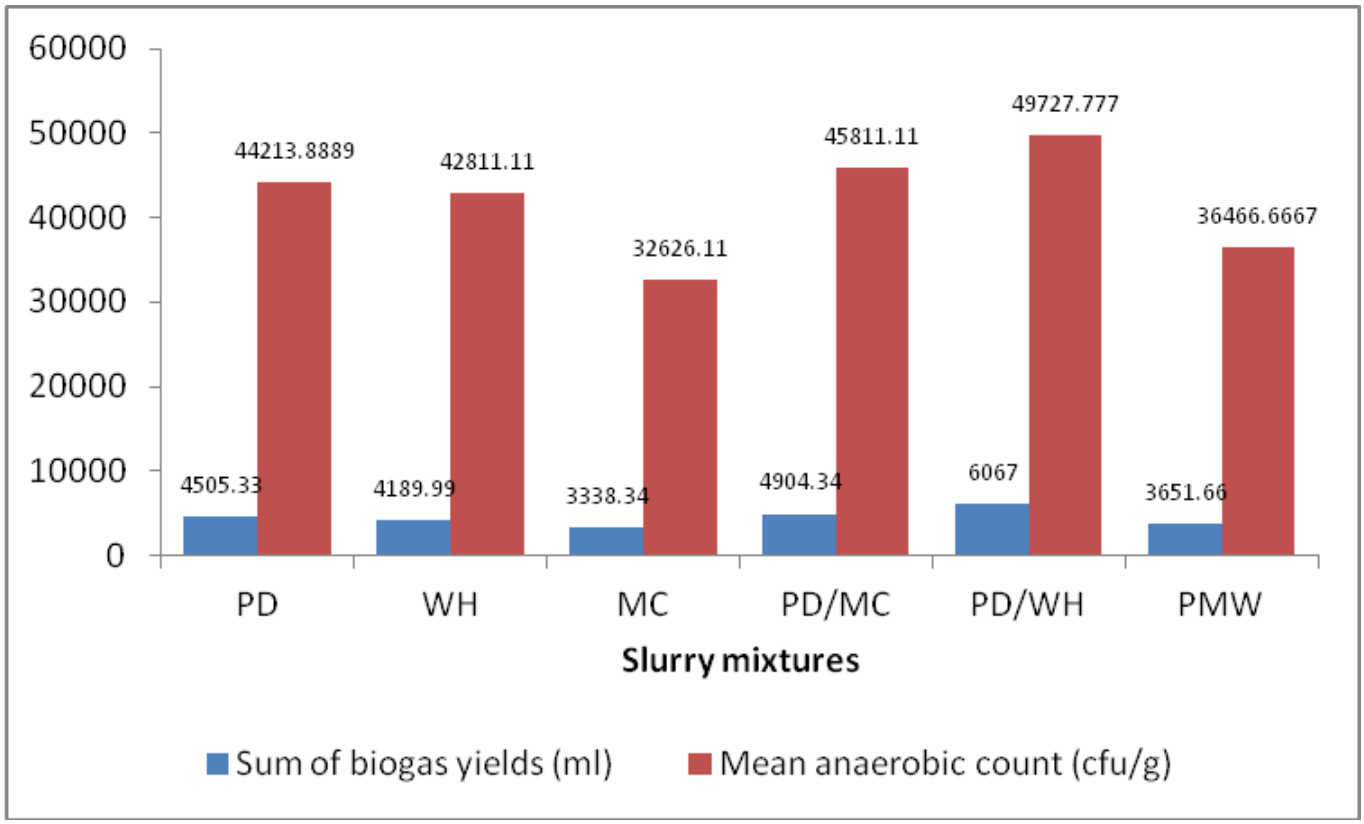

Figure 4.7: Relationship between anaerobic count and biogas yield

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4.7 Projected yields of biogas from parent source

Developing countries, such as Nigeria are rich in biomass and wastes materials that are suitable precursors for biofuel, yet this has not been fully explored. From this study, it is possible to estimate the biogas yield that will be produce from the parent source. **Table 4.9** shows an estimate of the projected quantity of biogas which will be yielded if all wastes generated is digested anaerobically. The pig farm generates 128.0±16.0kg of pig dung per day which if digested anaerobically will yield an estimated biogas of 306.69±37.90L. The 1586m^2 area of Oba-Dam if completely covered with water hyacinth will produce water hyacinth of mean weight ranging from 3.90±0.68 to 5.84±1.02tons which when put into biogas production will generate mean biogas of 4482.29±769.52 to 6723.43±1154.27L. While the 0.65 hectare maize plantation at Abadina Quarters generates maize cob of mean weight 1.16±0.04 to 1.93±0.06tons per harvest which if totally utilized for biogas production will yield 1177.04±33.42 to 1955.66±55.56L of biogas.

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Table 4.9: Projected mean value of biogas yield of pig dung, water hyacinth and maize cob that will be generated from the parent sources.

Sample	Experimental quantity of waste used and its corresponding total biogas yield			Total wastes generated from parent source and its projected total biogas yield					
	Waste (kg)		Biogas yield (ml)	Mean weight of waste (tons) Mean ± SD				Mean Biogas yield (L) Mean ± SD	
				Wet Weight (WW)		Dry weight (DW)			
	WW	DW		Min. limit	Max. limit	Min. limit	Max. limit	Min. limit	Max. limit
PD	1.88	0.75	4505.3±35.50	0.13±0.02	0.13±0.02	0.05±0.006	0.05±0.006	306.69±37.90	306.69±37.90
WH	3.64	0.75	4190.0±21.10	3.90±0.68	5.84±1.02	0.80±0.14	1.20±0.21	4482.29±769.52	6723.43±1154.27
MC	3.30	0.75	3338.3±10.60	1.16±0.04	1.93±0.06	0.27±0.008	0.44±0.01	1177.04±33.42	1955.66±55.56

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CHAPTER FIVE

DISCUSSION

5.1 Source of Substrates

From the feasibility study carried out on some selected locations in Ibadan viz, pigery unit at UI farms, Oba-dam at UI and maize plantation at UI farms, it is evident that huge amount of wastes are generated from these production centres. A similar study conducted by Omonijo *et al*., (2007) reported that huge amount of lignocellulosic wastes from agricultural activities are in Ibadan; and this posses a great burden on the environment, thus needs urgent attention.

Many sources of biomass, such as agricultural and forestry residues, pulp and paper streams, and municipal solid waste are abundant and underutilized resources, which can be converted to biogas. Woody and herbaceous energy crops, such as hybrid poplar and switchgrass can also be used as renewable resources for biogas production. In developing countries, biomass accounts for approximately 35% of the affordable source of energy (Mc Gowan 1991; Hall, Rosillo – calle, and de Groot 1992). Dedicated energy crops, grown on our nation's wastelands, could possibly represent one of the largest biomass sources. Advances in plant sciences and process technologies promise to revolutionize production of energy and other products from biomass. However it appears that the first materials used for biogas production will most probably be waste materials and residues (Kumar and Deobagkar, 1996).

5.2 Physico-Chemical Characteristics of Substrates

5.2.1 pH

Anaerobic bacteria, especially the methanogens, are sensitive to the acid concentration within the digester and their growth can be inhibited by acidic conditions. It has been reported (RISE-AT, 1998) that an optimum pH value for anaerobic digestion (AD) lies between 5.5 and 8.5. In this study, a pH range of about 5.80 ± 0.0 to 7.85 ± 0.1 was observed which conforms with the reported range. Several authors (Farrel *et al*, 2006; Gungor and Karthikeyan, 2005) have also reported that highest biogas yields were observed at digester pH 8. Acetogenesis can lead to accumulation of large amounts of organic acids resulting in pH below 5. Excessive generation of

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acid can inhibit methanogens, due to their sensitivity to acid conditions. Reduction in pH can be controlled by the addition of lime or recycled filtrate obtained during residue treatment. In fact, the use of recycled filtrate can even eliminate the lime requirement.

5.2.2 Temperature

The temperature of the digester in this study remained constant at mesophilic range (25.75 ± 0.4^{o}C to 28.75 ± 0.4^{o}C) throughout the digestion period. Temperature has been observed by most researchers to be quite critical for anaerobic digestion, since methane – producing bacteria operate most efficiently at temperatures 30.0 – 40.0^{o}C or 50.0 – 60.0oC (Ilori *et al*, 2007). The low methane production observed during the experimental period (March to July) could be attributed to the low temperature (below 30^{o}C) associated with the rainy season which consequently led to low methanogen growth. This is similar to the report of (Ilori *et al*, 2007) that the recovery time for biogas production as well as the quality and quantity of biogas produced from agricultural materials are a function of the nature, and composition of the digester feedstock.

5.2.3 Total Solids

Total solids are dissolved solids plus suspended and settleable solids in water. There are three different ranges of solid content viz: low solid (LS) systems which contain less than 10% Total Solid (TS), medium solid (MS) which lies within 15-20% and high solid systems (HS) which ranges from 22-40%. In this study, it was observed that the total solid content of the various slurries were 8.25 ± 0.1 to 20.55 ± 0.2% which is within the low and medium solid range (ISWM, 2012).

5.2.4 Total Organic Carbon

Organic matter content is typically measured as total organic carbon and dissolved organic carbon, which are essential components of the carbon cycle. The Total Organic Carbon test measures all organic carbon as CO_2. From **Tables 4.3 to 4.8**, the mean T.O.C decreased as the duration of anaerobic digestion increased, meaning that the organic bonded carbon in the slurries

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were oxidized to carbon dioxide (CO_2) and other inorganic Carbon (IC) such as carbonate, bicarbonate etc (Lal and Reddy, 2005).

Among the various substrates, the mean T.O.C was greatest for the **MC** throughout the duration of the anaerobic digestion while the least mean T.O.C was found in **PD**; with the implication that **MC** had a high quantity of organic bonded carbon in its composition than other wastes. Environmentally, this implies that the natural degradation of these wastes contributes a substantial amount of greenhouse gases such as CO_2, CH_4 etc to the environment. This was in agreement with Lal and Reddy (2005) who also reported that natural degradation of lignocellulosic wastes by anaerobic digestion of methanogenic bacteria, generate about 25 million tons of methane gas annually worldwide.

5.2.5a Total Nitrogen, Phosphorus and Potassium (NPK) content of the various slurries

Nitrogen that enters a digester from dairy manure is either in the inorganic ammonium or organic form. Much of the organic nitrogen is converted via nitrogen mineralization during the digestion process to ammonium, raising the overall level of ammonium in the effluent (Field *et al.,* 1984). Although a small amount of ammonia gas will be lost to biogas, the total nitrogen leaving the digester is generally considered equal to that added to the digester. (Topper *et al,* 2006) In this study, it was observed that the total nitrogen content of the slurries increased steadily as the day of anaerobic digestion progressed and this was in agreement with other studies. Some studies, (Rubaek *et al*, 1996; Chantigny et al., 2007; de Boer, 2008) have found increased yield and nitrogen availability with application of anaerobically digested material as compared to non-digested material, possibly due to increased nitrogen content and reduced carbon content, which can result in nitrogen mineralization by microbes. In addition, manure applications to soils have enhanced soil quality and fertility compared to soils receiving synthetic fertilizers (de Boer, 2008; Arthurson, 2009).

Acid hydrolysis at boiling-water temperature converts dissolved and particulate condensed phosphates to dissolved orthosphosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a minimum by judicious selection of acid

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strength and hydrolysis time and temperature. In this study, it was found that the Total Phosphate of all the slurries increased throughout the experiment. Nutrient speciation data collected from previous AD studies suggest that a high percentage of the P can be found in the inorganic form in the AD effluent (Wrigley *et al*., 1992; Bowers et al., 2007; Marti et al., 2008; Moody *et al*, 2009). Moody *et al* (2009) and colleagues demonstrated a 26% increase of inorganic P ($PO4^3$) in digested swine slurry compared to the raw swine slurry (1591 mg/L and 1256.2 of $PO4^3$- respectively). Inorganic P is comprised of soluble and insoluble orthophosphates and polyphosphates.

Potassium is an important element which plays a vital role in intracellular cell division of microbes in an anaerobic digestion process (Barth and Kroes, 1985). In this study, it was observed that potassium increased steadily in all slurries throughout the duration of the anaerobic digestion. PD had the highest potassium while MC had the least. Tchobanoglous *et al.,* (2003) reported that for the proper functioning and continuous reproduction of methanogens in the anaerobic digestion process, there is a need for synthesis of new cellular materials, of which inorganic elements such as potassium play a key role.

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5.2.5b Carbon-Nitrogen Ratio (C:N)

The relationship between the amount of carbon and nitrogen present in organic materials is expressed in terms of the Carbon/Nitrogen, C-N ratio. A C-N ratio ranging from 20 to 30 is considered optimum for anaerobic digestion. Mean C-N ratio of the various slurries decreased from day 0 to day 35 as follows: 20.05 ± 2.1 to 16.27 ± 1.1, 23.28 ± 0.1 to 12.95 ± 1.2, 97.54 ± 3.3 to 47.70 ± 1.3, 57.27 ± 0.2 to 28.34 ± 2.1, 28.52 ± 4.2 to 24.19 ± 2.0 and 49.86 ± 2.9 to 37.24 ± 2.4 for PD, WH, MC, PM, PW, and PWM respectively. In this study, it was observed that the values obtained for the C:N of PM and PW lies within the optimum range while those of the other substrates (PD, WH,MC, and PWM) lies outside the optimum value of C:N for biogas generation from biomass. This high biogas production observed C:N in PM and PW may be attributed to their C:N which lie within the optimum range (20:1-30:1). Several authors have also reported that the substances with carbon-to-nitrogen (C/N) ratio of 20-30:1 were found to be ideal for gas producton (Fernando and Dangogga, 1986; Lapp *et al;*1975 and Goldstein, 2000). Too much of carbon may have retarded effective gas generation at some point during the digestion. Anaerobic digestion facilitates nitrogen mineralization, while carbon is converted to biogas. Additionally, carbon is partially removed from the digested material, reducing the C:N ratio (Kirchmann and Witter, 1992; Moller *et al*., 2008).

5.2.5c Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

The biochemical oxygen demand (BOD) test tries to closely model an aerobic wastewater treatment system and the natural aquatic ecosystem. It measures oxygen taken up by the bacteria during the oxidation of organic matter. While COD test measures all organic carbon with the exception of certain aromatics (benzene, toluene, phenol, etc.) which are not completely oxidized in the reaction.

The result of the analysis of the feedstocks during the anaerobic digestion revealed that there is reduction in BOD and COD indicating that anaerobic digestion is a potent way of reducing these parameters from sludge or wastewater. The reduction in BOD observed in this study agrees with (House, 2007) that treating human waste through anaerobic digestion is a credibly ethical

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sanitation technology and removes Biochemical Oxygen Demand (BOD) from sewage, conserves nutrients (especially nitrogen compounds) and most importantly reduces pathogens. From the environmental point of view, anaerobic digestion treatment help to avert the serious public health risk posed by these wastes, which if discharged directly into water bodies can contribute to algal blooms and cyanobacterial growth thus destroying the aquatic ecosystem. Also, the reduction in BOD and COD is in agreement with Wei et al, 2011 who reported a high BOD and COD removal from supernatant of hydrothermally treated municipal sludge by up-flow anaerobic sludge blanket reactor (UASD). In a similar study, Yoneyama *et al., (*2006) reported the recovery of bioenergy from hydrothermally heated cow manure with COD removal rate reaching up to 75.9%.

5.2.6 Microbial Load

The groups of bacteria isolated from the digester feedstock include *Bacillus, Escherichia, Clostridium, Klebsiella, Proteus and Bacteroides* some of which are acid-formers and a methane former *Methanococcus* species, the correct balance between these two groups of microorganisms determines the successful operation of anaerobic digesters for biogas production. The methane formers however multiply at a slower rate than acid formers and are very sensitive to environmental changes as seen in this research. Fungal isolates includes *Aspergillius, Rhizopus, Penicillium and Mucor* whose source could be the feedstock. Pritchard *et al., 2009* reported a similar result when he isolated *E. coli, Aspergillius, Clostridium botulinum, C. chavoie* and others from water contaminated by human excreta in Malawi. The decreasing trend seen in the aerobic count could be attributed to the increasing anaerobiosis. The acidic nature of the feedstock over the first four weeks of digestion could have supported the growth of acid producing organisms despite the anaerobic condition. Decrease in fungal isolates over the first three weeks even as the digestion becomes more anaerobic is in support with fungal general physiology and metabolism which is known to be purely aerobic. In support of this, Triolo, 2011 in his research, reported that the acidic condition of his digester could be a support for fungi which are known to be acid loving.

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5.2.7 Biogas yield

The identification of feedstock substrate for an economically feasible biogas production in Nigeria, to include water lettuce, water hyacinth, dung, cassava leaves and processing waste, urban refuse, solid (including industrial) waste, agricultural residues and sewage have been made (Akinbami *et al.*, 1996; Akinbami *et al.*, 2001; Okagbue, 1988; Ubalua, 2008). Many other raw materials available in Nigeria have been critically assessed for their possible use in biogas production by Odeyemi (1983). They include refuse and sewage generated in urban areas, agricultural residues and manure. It was concluded that poultry manure generated in Nigerian homes and in commercial poultry farms could be economically feasible substrates for biogas production. The potential to utilize poultry, cow and kitchen wastes for biogas production was demonstrated by other investigators including (Akinluyi and Odeyemi, 1986; Abubakar, 1990; Lawal *et al.*, 1995; Mathew, 1982; Ojolo *et al.*, 2007; and Zuru *et al.*, 2002).

Gas generation commenced on the twentieth (20th) [PD, PD/MC and PD/WH] and twenty second (22nd) [WH, MC and PMW] days, it kept a steady increased steadily and reached the peak on the 23rd (PD); 24th (PW and PM); 25th (MC), and 26th (WH and PMW) days before dropping. This result agrees with the findings of Wei (2011) who reported an increasing trend of biogas production from commencement and a drop after 30 days from supernatants of hydrothermally treated municipal sludge by up-flow anaerobic sludge blanket reactor. This is also similar to the report of Ilori (2007) that the recovery time for biogas production as well as the quality and quantity of biogas produced from agricultural materials are a function of the nature, and composition of the digester feedstock. Alkan-Ozkaynak and Karthikayan (2011) also reported a high rate of biogas production from treated thin sillage with a drop towards the end of the experiment.

Atuanya and Aigbirior (2002) reported the feasibility of biogas production using an upflow anaerobic sludge blanket reactor (UASB) of 3.50 L capacity. Seeding of co-digested pig waste and cassava with wood ash was reported to result in significant increase in biogas production compared with unseeded mixture of pig waste and cassava peels (Adeyanju, 2008). Fariku and Kidah (2008) reported good biogas production from anaerobic digestion of waste shells of *Lophira lanceolata* fruit. The potential use of local algal biomass for biogas production in

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Nigeria was recognised by Weerasinghe and Naqvi (1983). Odeyemi, (1981) identified four other substrates, namely *Eupatorium odoratum*, water lettuce, water hyacinth and cow dung as potential substrates for biogas production. *Eupatorium odoratum* gave the highest yield of biogas and cow dung was the poorest substrate. He concluded that *E. odoratum* was a cheap source of biogas in Nigeria because of its luxuriant and ubiquitous growth. These laboratory studies demonstrated the potential of biogas production from agricultural, industrial, urban and animal wastes in Nigeria.

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CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The purpose of this study was to evaluate the biogas yielding potential of mixtures of some selected biomass feedstocks and their corresponding microbial load in the respective biodigesters. The findings from this research show that biomass wastes which are the substrates for anaerobic digestion are abundant and readily available in the country. The study also indicates that methanogens which are the culprits in biogas production are affected by pH and temperature of the biodigester.

There was reduction in the BOD, COD and microbial load (most human pathogens) of the slurries after undergoing anaerobic digestion. Conclusively, among the substrates utilized in the study, co-digestion of pig dung with water hyacinth had the highest number of anaerobes and biogas yield as compared to single feedstocks. Therefore, the use of multi-biomass feedstocks for biogas production as a source of alternative energy production should be fully optimized.
Bioconversion offers a cheap and safe method of not only disposing the agricultural residues, but also it has the potential to convert agro-based waste into usable forms such as bioenergy that could be used for domestic and industrial activities. Hence the conversion of agro-based "wastes" into bioenergy such as biogas will help reduce environmental pollution, contribute toward the mitigation of greenhouse gases emissions and serve as a sustainable solid waste management strategy.

Conclusively, the utilization of lignocellulosic wastes in biotechnological process has cut across wide range of product which has potential for industrial application and commercialization. However, the economic viability depends solely on efficient use of the agro-waste. The seasonal harvesting of the parent source of these wastes remained an impediment to constant availability of the feedstock for biogas production thereby damping its viability for industrial production of the renewable energy. In addition, the present environmental legislation in most developing countries is not efficient for effective collection of these agro-based residues from major

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producers. If such situation prevails for long it may remain a lifetime challenge for commercialization of biogas from the waste.

5.2 Recommendations

The following recommendations are hereby suggested as strategy of minimizing waste generation and mitigate greenhouse gases emissions:

- Public enlightenment and environmental education should be undertaken to create awareness in individuals/community on environmental and health hazards associated with indiscriminate disposal of wastes.
- Dissuade people from the open burning of biomasses.
- Waste minimization and segregation at point source should be encouraged for all farmers who engage in subsistence farming and animal husbandry for easy collection for biogas production.
- Research should be focused in the direction of evolving microbes that can convert the complex biomass materials to simpler metabolizable sugars and then to biogas.
- Develop engineering systems with improved activities suitable for industrial-scale application through specially designed equipment and control systems.
- Technological advancements in genomics and proteomics areas that will be able to overcome the feedstock inhibition of the hydrolyzed products, non-specific reactions and harmful by-products.

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APPENDICES

Appendix 1:

Table 4.2c: Estimation of the quntity of pig dung generated per day

Experiment	Weight (Kg)	Volume (m^3)	Density (Kg/m^3)
1	144	0.199	722.22
2	112	0.154	729.03
3	128	0.183	700.00
Mean ± SD	128.0±16.0	0.179±0.023	717.083±15.181

Table 4.2d: Estimation of the quantity of water hyacinth in 1586 m^2 area cover of Oba-Dam

Experiment	Weight (Kg) per 2.5 m^2 area	Weight (Kg) per 1586 m^2 area	Volume (m^3)	Density (Kg/m^3)
1	7.78	4935.63	0.722	1667.18
2	7.29	4624.78	0.936	1657.15
3	7.43	4713.59	1.200	1600.00
Mean ± SD	7.500±0.252	4758.000±160.113	0.953±0.239	1641.443±36.240

Table 4.2e: Estimation of the quantity of maize cob generated per harvest

Experiment	Number of cobs per harvest	Weight (Kg)	Volume (m^3)	Density (Kg/m^3)
1	24074	1203.70	0.722	1667.18
2	33002	1551.09	0.936	1657.15
3	40000	1920.00	1.200	1600.00
Mean ± SD	32358.667±7982.467	1558.263±358.204	0.953±0.239	1641.443±36.240

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Table 1.1: SUPPLIMENTARY RESULT OF LABORATORY ANALYSIS OF SAMPLES

PARAMETER	PD	WH	MC	PD/MC	PD/WH	PMW
Day 0						
Total Aerobic Count (cfus/g)	7620000.0	1080000.0	3210000.0	4220000.0	4580000.0	5330000.0
	7570000.0	2120000.0	3190000.0	4190000.0	4620000.0	5230000.0
	7620000.0	2090000.0	3210000.0	4190000.0	4580000.0	5340000.0
Organisms Isolated	*Bacillus spp; Flavobacterium sp; Micrococcus sp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp; Micrococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp*	*Bacillus spp; Pseudomonas sp; Micrococcus sp*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp; Micrococcus sp;*	*Bacillus spp; ; Pseudomonas sp; Staphylococcus sp*
Total Coliform Count (cfus/g)	6200000.0	4410000.0	3330000.0	5830000.0	5580000.0	4580000.0
	6200000.0	4390000.0	3320000.0	5770000.0	5600000.0	4600000.0
	6180000.0	4420000.0	3300000.0	5810000.0	5620000.0	4620000.0
Organisms Isolated	*E. coli; Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; ; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp Proteus sp*
Total Anaerobic Count (cfus/g)	5300.0	2500.0	720.0	4800.0	4600.0	4000.0
	5200.0	2900.0	600.0	5000.0	4700.0	3700.0
	5000.0	2600.0	850.0	4600.0	4900.0	3700.0
Organisms Isolated	*Lactobacillus spp;*	*Lactobacillus spp;*	*Lactobacillus spp;*	*Lactobacillus sp;*	*Lactobacillus spp;*	*Lactobacillus spp;*

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	Methanobacterium spp	*Methanobacterium spp*	*Methanobacterium spp*	*Methanobacterium spp*	*Methanobacterium spp*	*Methanobacterium spp*
Total Fungal Count (cfus/g)	3760000.0	3180000.0	4020000.0	520000.0	6290000.0	4950000.0
	3810000.0	3230000.0	4000000.0	519000.0	6300000.0	5030000.0
	3830000.0	3220000.0	4010000.0	522000.0	6260000.0	5020000.0
Organisms Isolated	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida sp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*
pH	6.6	7.8	5.8	6.0	7.3	6.2
	6.6	7.9	5.8	6.0	7.4	6.2
	6.6	7.9	5.8	6.0	7.3	6.2
Temperature	26.0	26.1	26.0	26.3	26.0	26.0
	26.2	26.0	26.5	26.0	26.0	26.0
	26.0	26.1	26.0	26.0	26.3	26.4
Ambient Temperature	26.0	26.0	26.0	26.0	26.0	26.0
	26.5	26.5	26.5	26.5	26.5	26.5
	26.0	26.0	26.0	26.0	26.0	26.0
Total Solids %	13.8	11.3	16.4	20.7	15.5	14.0
	13.4	11.1	16.2	20.4	15.9	14.1
	13.5	11.4	16.5	20.6	15.4	14.3
Total Organic Carbon %	40.82	45.49	63.84	57.4	52.08	58.2
	46.96	47.36	64.13	58.54	51.48	59.74
	37.37	44.95	62.12	58.14	65.8	52.62
Total Nitrogen %	2.16	1.95	0.63	1.0	1.99	1.13
	2.09	2.03	0.67	1.02	1.98	1.16

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	1.99	1.94	0.65	1.02	1.97	1.13
C/N Ratio	18.9	23.33	101.33	57.4	26.17	51.5
	22.47	23.33	95.71	57.4	26.0	51.5
	18.78	23.17	95.57	57.0	33.4	46.57
*P as PO_4^{---}(mg/L)	205.0	195.0	150.0	195.0	200.0	205.0
	210.0	190.0	155.0	195.0	200.0	205.0
	210.0	190.0	155.0	205.0	195.0	200.0
*K+ (mg/L)	30.0	28.0	23.0	29.0	30.0	30.0
	29.0	29.0	24.0	30.0	30.0	31.0
	30.0	29.0	22.0	30.0	32.0	30.0
BOD_5 (mg/L)	1450.0	2210.0	2550.0	2320.0	2260.0	2420.0
	1445.0	2235.0	2530.0	2340.0	2245.0	2470.0
	1475.0	2225.0	2520.0	2350.0	2245.0	2455.0
COD (mg/L)	2620.0	3900.0	4660.0	4225.0	4145.0	4655.0
	2640.0	3935.0	4690.0	4265.0	4120.0	4630.0
	2630.0	3940.0	4675.0	4255.0	4125.0	4675.0
Day 7						
Total Aerobic Count (cfus/g)	5930000.0	971000.0	1480000.0	1090000.0	1780000.0	3510000.0
	5900000.0	971000.0	1510000.0	1080000.0	1830000.0	3500000.0
	5870000.0	968000.0	1520000.0	1130000.0	1800000.0	3500000.0
Organisms Isolated	*Bacillus spp; Flavobacterium sp; Pseudomonas sp.; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp; Micrococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp*	*Bacillus spp; Pseudomonas sp; Micrococcus sp*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp; Micrococcus sp;*	*Bacillus spp; ; Pseudomonas sp; Staphylococcus sp*

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Total Coliform Count (cfus/g)	2310000.0	1170000.0	1660000.0	1040000.0	2160000.0	1130000.0
	2300000.0	1200000.0	1700000.0	1020000.0	2140000.0	1170000.0
	2290000.0	1240000.0	1740000.0	1010000.0	2120000.0	1150000.0
Organisms Isolated	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; ; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp Proteus sp*
Total Anaerobic Count (cfus/g)	5900.0	5700.0	5000.0	6900.0	7400.0	5100.0
	6350.0	6000.0	4700.0	6700.0	7900.0	4700.0
	6100.0	5500.0	4900.0	6300.0	7600.0	5200.0
Organisms Isolated	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus sp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*
Total Fungal Count (cfus/g)	1030000.0	1240000.0	1700000.0	202000.0	2880000.0	2210000.0
	1010000.0	1220000.0	1700000.0	200000.0	2910000.0	2200000.0
	1020000.0	1180000.0	1670000.0	203000.0	2890000.0	2180000.0
Organisms Isolated	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida sp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*
pH	6.4	7.2	6.0	6.1	7.2	6.2
	6.4	7.4	6.0	6.1	7.2	6.2
	6.5	7.3	6.0	6.1	7.2	6.2
Temperature	28.0	28.2	28.2	28.5	27.8	28.0
	28.5	28.5	28.5	28.3	28.0	28.5
	28.0	28.2	28.2	28.4	27.9	28.0

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Ambient Temperature	25.4	25.5	25.5	25.5	26.0	25.5
	25.5	26.0	26.0	26.0	26.0	26.0
	26.0	25.5	25.5	25.5	25.5	25.5
Total Solids %	11.7	9.6	14.5	18.6	13.4	12.8
	11.5	9.8	15.0	18.9	13.7	12.6
	11.7	9.9	14.6	18.7	13.5	12.5
TOC %	39.09	44.17	59.99	54.28	54.5	56.75
	39.71	44.16	59.36	54.16	55.05	56.16
	46.26	43.98	61.95	56.87	58.69	54.73
Total Nitrogen %	2.06	2.03	0.66	1.06	2.0	1.15
	2.11	2.02	0.67	1.06	2.02	1.18
	2.1	2.04	0.68	1.03	2.01	1.15
C/N Ratio	19.04	21.76	90.89	51.21	27.25	49.35
	18.88	21.86	88.6	51.09	27.25	47.59
	22.09	21.56	91.1	55.21	29.2	47.59
*P as PO_4^{--}(mg/L)	220.0	205.0	165.0	210.0	215.0	220.0
	230.0	205.0	165.0	215.0	215.0	220.0
	225.0	205.0	170.0	210.0	210.0	215.0
*K+ (mg/L)	32.0	30.0	26.0	31.0	32.0	32.0
	31.0	28.0	27.0	31.0	32.0	32.0
	30.0	29.0	25.0	32.0	33.0	32.0
BOD_5 (mg/L)	1210.0	2020.0	2260.0	2180.0	2145.0	2210.0
	1230.0	2040.0	2280.0	2120.0	2100.0	2160.0

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	1240.0	2060.0	2250.0	2200.0	2050.0	2135.0
COD (mg/L)	2175.0	3775.0	4110.0	4050.0	4040.0	4230.0
	2180.0	3785.0	4135.0	4055.0	3995.0	4245.0
	2200.0	3790.0	4125.0	4050.0	3980.0	4210.0
Day 14						
Total Aerobic Count (cfus/g)	5010000.0	778000.0	977000.0	951000.0	989000.0	2080000.0
	5000000.0	781000.0	980000.0	951000.0	990000.0	2100000.0
	5020000.0	781000.0	978000.0	952000.0	992000.0	2090000.0
Organisms Isolated	*Bacillus spp;; Pseudomonas sp.; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp*	*Bacillus spp; Pseudomonas sp; Micrococcus sp*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; ; Pseudomonas sp; Staphylococcus sp*
Total Coliform Count (cfus/g)	1010000.0	958000.0	976000.0	570000.0	985000.0	842000.0
	1000000.0	960000.0	980000.0	570000.0	991000.0	842000.0
	1010000.0	961000.0	981000.0	563000.0	991000.0	841000.0
Organisms Isolated	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; ; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp Proteus sp*
Total Anaerobic Count (cfus/g)	23800.0	22300.0	18400.0	24900.0	25500.0	20700.0
	23600.0	22900.0	18700.0	24900.0	25900.0	20700.0
	23500.0	22600.0	19100.0	24800.0	26100.0	20800.0
Organisms Isolated	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus sp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*

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Total Fungal Count (cfus/g)	813000.0	978000.0	975000.0	570000.0	987000.0	840000.0
	811000.0	98000.0	967000.0	570000.0	990000.0	840000.0
	813000.0	983000.0	967000.0	569000.0	989000.0	837000.0
Organisms Isolated	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida sp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*
pH	6.6	7.3	6.2	6.2	7.3	6.4
	6.5	7.4	6.2	6.2	7.3	6.4
	6.5	7.3	6.2	6.2	7.3	6.4
Temperature	28.5	28.0	28.0	28.5	28.5	28.0
	28.5	28.0	28.0	28.5	28.5	27.5
	28.5	28.0	28.0	29.0	28.5	28.5
Ambient Temperature	25.5	25.5	25.5	25.5	25.5	25.5
	26.5	26.5	26.5	26.5	26.5	26.5
	26.0	26.0	26.0	26.0	26.0	26.0
Total Solids %	10.2	8.3	13.5	17.5	12.5	11.9
	10.5	8.7	13.6	17.8	12.6	11.6
	10.2	9.1	13.8	17.6	12.4	11.4
Total Organic Carbon %	41.38	39.07	56.35	50.89	53.69	54.34
	38.02	41.85	54.86	50.89	59.18	53.75
	44.57	36.2	55.57	50.89	53.44	56.31
Total Nitrogen %	2.12	2.07	0.72	1.07	2.09	1.17
	2.13	2.08	0.7	1.07	2.04	1.16
	2.08	2.09	0.71	1.07	2.08	1.21

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C/N Ratio	19.52	18.87	78.26	47.56	25.69	46.44
	17.85	20.06	78.37	47.56	29.01	46.34
	21.43	17.39	78.27	47.56	25.69	46.54
*P as PO_4^{---}(mg/L)	235.0	205.0	170.0	215.0	225.0	230.0
	235.0	215.0	180.0	220.0	225.0	230.0
	235.0	210.0	180.0	220.0	225.0	230.0
*K+ (mg/L)	32.0	30.0	28.0	31.0	33.0	33.0
	32.0	29.0	27.0	33.0	34.0	34.0
	32.0	29.0	28.0	33.0	34.0	33.0
BOD_5 (mg/L)	1040.0	1975.0	2130.0	2090.0	1975.0	2125.0
	1060.0	1980.0	2150.0	2050.0	1980.0	2060.0
	1025.0	2010.0	2140.0	2080.0	1985.0	2080.0
COD (mg/L)	1810.0	3750.0	4015.0	3985.0	3480.0	3750.0
	1815.0	3750.0	3990.0	3970.0	3510.0	3780.0
	1815.0	3750.0	4035.0	3990.0	3530.0	3770.0
Week Day 21						
Total Aerobic Count (cfus/g)	4030000.0	565000.0	767000.0	888000.0	691000.0	1910000.0
	4010000.0	570000.0	770000.0	893000.0	690000.0	1900000.0
	4010000.0	567000.0	772000.0	891000.0	689000.0	1910000.0
Organisms Isolated	*Bacillus spp;; Pseudomonas sp.; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp*	*Bacillus spp; Pseudomonas sp; Micrococcus sp*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; ; Pseudomonas sp; Staphylococcus sp*
Total Coliform Count (cfus/g)	739000.0	701000.0	746000.0	123000.0	688000.0	710000.0

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	740000.0	700000.0	750000.0	120000.0	690000.0	710000.0
	739000.0	699000.0	747000.0	117000.0	693000.0	711000.0
Organisms Isolated	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; ; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp Proteus sp*
Total Anaerobic Count (cfus/g)	58200.0	58000.0	42300.0	59900.0	63000.0	49800.0
	58000.0	57800.0	42500.0	60000.0	62300.0	49000.0
	58500.0	58400.0	41100.0	61000.0	62000.0	50000.0
Organisms Isolated	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus sp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*
Total Fungal Count (cfus/g)	670000.0	800000.0	820000.0	64000.0	580000.0	820000.0
	670000.0	802000.0	824000.0	64000.0	581000.0	818000.0
	672000.0	798000.0	816000.0	63800.0	580000.0	818000.0
Organisms Isolated	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida sp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*
pH	6.4	7.3	6.2	6.1	7.2	6.3
	6.5	7.3	6.1	6.2	7.2	6.3
	6.4	7.3	6.1	6.1	7.2	6.3
Temperature	28.1	28.3	28.6	28.5	27.9	28.5
	28.1	28.3	28.6	28.5	27.9	28.5
	28.1	28.3	28.6	28.5	27.9	28.5
Ambient Temperature	25.9	25.9	25.9	25.9	25.9	25.9

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	25.9	25.9	25.9	25.9	25.9	25.9
	25.9	25.9	25.9	25.9	25.9	25.9
Total Solids %	9.9	8.5	13.0	17.2	12.2	11.6
	10.2	8.6	13.2	17.4	12.3	11.3
	10.0	8.8	13.3	17.2	12.2	11.2
Total Organic Carbon %	40.23	33.73	48.66	45.34	54.52	49.73
	47.45	33.78	46.23	44.34	54.52	53.23
	43.71	34.22	46.07	43.0	54.52	53.94
Total Nitrogen %	2.22	2.1	0.75	1.13	2.1	1.23
	2.2	2.14	0.75	1.18	2.1	1.24
	2.21	2.15	0.75	1.14	2.1	1.25
C/N Ratio	18.12	16.05	64.88	40.12	25.96	40.43
	21.57	15.8	61.64	37.58	25.96	42.93
	19.78	15.92	61.43	37.72	25.96	43.15
*P as PO_4^{---}(mg/L)	270.0	230.0	200.0	250.0	260.0	270.0
	275.0	235.0	190.0	255.0	265.0	270.0
	275.0	230.0	200.0	245.0	255.0	270.0
*K+ (mg/L)	34.0	29.0	29.0	35.0	36.0	33.0
	33.0	30.0	28.0	35.0	36.0	35.0
	34.0	30.0	27.0	35.0	36.0	36.0
BOD_5 (mg/L)	985.0	1880.0	2050.0	1990.0	1905.0	2020.0
	1010.0	1910.0	2065.0	1980.0	1920.0	1970.0
	995.0	1890.0	2040.0	2020.0	1915.0	1995.0

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COD (mg/L)	1720.0	3475.0	3875.0	3855.0	3500.0	3725.0
	1775.0	3445.0	3760.0	3790.0	3495.0	3735.0
	1705.0	3450.0	3880.0	3980.0	3460.0	3710.0
Day 28						
Total Aerobic Count (cfus/g)	2900000.0	351000.0	547000.0	879000.0	328000.0	973000.0
	2900000.0	350000.0	550000.0	800000.0	330000.0	972000.0
	2880000.0	352000.0	550000.0	800000.0	331000.0	972000.0
Organisms Isolated	*Bacillus spp;; Pseudomonas sp.; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp*	*Bacillus spp; Pseudomonas sp; Micrococcus sp*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; ; Pseudomonas sp; Staphylococcus sp*
Total Coliform Count (cfus/g)	441000.0	452000.0	394000.0	557000.0	346000.0	572000.0
	441000.0	450000.0	392000.0	560000.0	351000.0	572000.0
	442000.0	448000.0	393000.0	556000.0	348000.0	569000.0
Organisms Isolated	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; ; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp Proteus sp*
Total Anaerobic Count (cfus/g)	87000.0	84100.0	68700.0	89000.0	102000.0	70800.0
	86000.0	84900.0	68000.0	90500.0	101500.0	70000.0
	86400.0	84100.0	68000.0	91000.0	96500.0	70200.0
Organisms Isolated	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus sp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*
Total Fungal Count (cfus/g)	483000.0	676000.0	7160000.0	29900.0	250000.0	632000.0

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	480000.0	680000.0	7140000.0	29800.0	253000.0	633000.0
	477000.0	678000.0	7150000.0	29700.0	251000.0	634000.0
Organisms Isolated	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida sp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*
pH	6.6	7.1	6.2	6.3	7.0	6.2
	6.5	7.2	6.2	6.3	7.0	6.2
	6.6	7.2	6.2	6.2	7.0	6.2
Temperature	28.4	28.5	28.7	28.3	28.1	28.4
	28.4	28.5	28.7	28.3	28.1	28.4
	28.4	28.5	28.7	28.3	28.1	28.4
Ambient Temperature	26.1	26.1	26.1	26.1	26.1	26.1
	26.1	26.1	26.1	26.1	26.1	26.1
	26.1	26.1	26.1	26.1	26.1	26.1
Total Solids %	9.7	8.4	12.6	17.0	11.9	11.0
	9.7	8.5	12.9	16.8	11.9	10.9
	9.8	8.5	12.9	16.9	12.0	10.8
Total Organic Carbon %	40.82	31.51	38.9	35.05	46.92	51.91
	38.78	31.38	39.38	35.45	54.95	46.87
	35.59	31.52	39.38	36.53	55.47	53.91
Total Nitrogen %	2.26	2.23	0.75	1.17	2.13	1.3
	2.26	2.24	0.78	1.19	2.12	1.28
	2.29	2.25	0.78	1.24	2.14	1.35
C/N Ratio	18.06	14.13	51.87	29.96	22.03	39.93

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	17.16	14.01	50.49	29.79	25.92	36.62
	15.54	14.01	50.49	29.46	25.92	39.93
*P as PO_4^{---}(mg/L)	290.0	240.0	210.0	265.0	265.0	275.0
	280.0	240.0	205.0	260.0	270.0	280.0
	285.0	235.0	210.0	250.0	270.0	275.0
*K+ (mg/L)	34.0	31.0	29.0	36.0	37.0	34.0
	34.0	31.0	29.0	36.0	36.0	36.0
	35.0	30.0	30.0	35.0	37.0	35.0
BOD_5 (mg/L)	975.0	1860.0	2010.0	1960.0	1885.0	1985.0
	970.0	1860.0	2020.0	1960.0	1890.0	1965.0
	985.0	1840.0	2000.0	1970.0	1890.0	1980.0
COD (mg/L)	1650.0	3380.0	3780.0	3710.0	3400.0	3675.0
	1620.0	3365.0	3800.0	3685.0	3360.0	3680.0
	1625.0	3370.0	3790.0	3650.0	3420.0	3650.0
Day 35						
Total Aerobic Count (cfus/g)	1680000.0	231000.0	361000.0	669000.0	187000.0	896000.0
	1700000.0	230000.0	360000.0	670000.0	186000.0	903000.0
	1680000.0	230000.0	362000.0	671000.0	188000.0	901000.0
Organisms Isolated	*Bacillus spp;; Pseudomonas sp.; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp*	*Bacillus spp; Pseudomonas sp; Micrococcus sp*	*Bacillus spp; Pseudomonas sp; Staphylococcus sp;*	*Bacillus spp; ; Pseudomonas sp; Staphylococcus sp*
Total Coliform Count (cfus/g)	203000.0	273000.0	169000.0	43000.0	146000.0	378000.0
	202000.0	271000.0	170000.0	43100.0	147000.0	380000.0

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	201000.0	272000.0	169000.0	43100.0	148000.0	379000.0
Organisms Isolated	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; ; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp; Proteus sp*	*Enterobacter sp; Aeromonas sp Proteus sp*
Total Anaerobic Count (cfus/g)	86000.0	83300.0	60700.0	88000.0	98200.0	70000.0
	85000.0	84000.0	62000.0	87500.0	97000.0	69000.0
	86000.0	83000.0	61000.0	88800.0	98000.0	69000.0
Organisms Isolated	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus sp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*	*Lactobacillus spp; Methanobacterium spp*
Total Fungal Count (cfus/g)	392000.0	567000.0	613000.0	9100.0	82200.0	470000.0
	390000.0	570000.0	612000.0	9110.0	82000.0	470000.0
	388000.0	568000.0	610000.0	9100.0	82200.0	468000.0
Organisms Isolated	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida sp*	*Aspergillus sp; Candida spp*	*Aspergillus sp; Candida spp*
Ph	6.5	7.2	6.1	6.2	7.1	6.2
	6.5	7.2	6.1	6.1	7.0	6.2
	6.5	7.2	6.1	6.1	7.0	6.3
Temperature	28.0	28.0	28.2	28.4	27.8	28.5
	28.1	28.0	28.2	28.4	27.8	28.3
	28.4	28.0	28.2	28.4	27.8	28.0
Ambient Temperature	26.0	26.0	26.0	26.0	26.0	26.0
	25.5	25.5	25.5	25.5	25.5	25.5

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	26.0	26.0	26.0	26.0	26.0	26.0
Total Solids %	9.4	8.3	12.2	16.6	11.6	10.3
	9.5	8.2	12.4	16.5	11.5	10.4
	9.5	8.3	12.4	16.5	11.5	10.3
Total Organic Carbon %	35.1	30.22	37.87	37.17	54.14	52.96
	37.63	30.76	36.53	31.59	47.13	48.06
	40.52	26.4	37.19	35.85	54.74	47.53
Total Nitrogen %	2.32	2.23	0.77	1.26	2.15	1.33
	2.31	2.24	0.78	1.22	2.15	1.31
	2.33	2.28	0.79	1.21	2.15	1.35
C/N Ratio	15.13	13.55	49.18	29.5	25.18	39.82
	16.29	13.73	46.83	25.89	21.92	36.69
	17.39	11.58	47.08	29.63	25.46	35.21
*P as PO_4^{---}(mg/L)	300.0	250.0	225.0	275.0	280.0	285.0
	305.0	255.0	220.0	270.0	285.0	295.0
	300.0	250.0	220.0	275.0	280.0	290.0
*K+ (mg/L)	35.0	31.0	29.6	36.0	37.0	36.0
	36.0	30.0	29.0	36.0	37.0	36.0
	34.0	32.0	30.0	36.0	36.0	35.0
BOD_5 (mg/L)	960.0	1845.0	2005.0	1965.0	1870.0	1970.0
	965.0	1845.0	1985.0	1950.0	1875.0	1955.0
	980.0	1850.0	1995.0	1955.0	1865.0	1960.0
COD (mg/L)	1550.0	3365.0	3720.0	3620.0	3360.0	3540.0

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	1600.0	3370.0	3670.0	3580.0	3355.0	3525.0
	1580.0	3345.0	3705.0	3565.0	3350.0	3515.0

***To convert to % divide by10, 000**

Table 1.2: DAILY BIOGAS YIELD

Pig Dung		**Water Hyacinth**		**Maize Cob**		**Pig Dung/Maize cob**		**Pig dung/Water Hyacinth**		**Pig dung/Maize Cob/Water Hyacinth**	
Day	**Biogas Yield**	**Day**	**Biogas Yield**	**Day**	**Biogas Yield**	**Day**	**Biogas Yield**	**Day**	**Biogas Yield**	**Day**	**Biogas Yield**
20	280	20	220	22	150	20	260	22	200	22	130
21	430	21	245	23	255	21	350	23	305	23	235
22	600	22	500	24	350	22	400	24	420	24	380
23	744	23	990	25	460	23	500	25	580	25	555
24	1100	24	695	26	655	24	725	26	630	26	530
25	1000	25	510	27	570	25	665	27	550	27	415
26	910	26	430	28	480	26	600	28	475	28	305
27	500	27	320	29	330	27	505	29	405	29	280
28	250	28	250	30	210	28	415	30	335	30	255
29	155	29	190	31	140	29	310	31	180	31	130
30	125	30	120	32	40	30	110	32	65	32	75
31	40	31	50	33	20	31	50	33	25	33	40

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Apendix 2

Table 2.1: Determination of Total Organic Carbon (T.O.C)

PARAMETER	Pig Dung	Water Hyacinth	Maize Cob	Pig Dung/ Maize Cob	Pig Dung/ Water Hyacinth	Pig Dung/ Maize Cob/ Water Hyacinth
Weight of sample taken (g)	0.05	0.05	0.05	0.05	0.05	0.05
Day 0						
Rep 1: TV (cm^3)	12.24	10.78	5.05	7.06	8.73	6.81
Rep 2: TV (cm^3)	10.33	10.20	4.96	6.71	8.91	6.33
Rep 3: TV (cm^3)	10.32	10.95	5.59	6.83	4.44	8.56
Day 7						
Rep 1: TV (cm^3)	12.78	11.20	6.25	8.04	7.97	7.27
Rep 2: TV (cm^3)	12.59	11.2	6.45	8.08	7.80	7.45
Rep 3: TV (cm^3)	10.54	11.26	5.64	7.23	6.66	7.90
Day 14						
Rep 1: TV (cm^3)	12.07	12.79	7.39	9.10	8.22	8.02
Rep 2: TV (cm^3)	13.12	11.92	7.86	9.10	6.51	8.20
Rep 3: TV (cm^3)	11.07	13.69	7.63	9.10	8.3	7.40
Day 21						
Rep 1: TV (cm^3)	12.43	14.46	9.79	10.83	7.96	9.46
Rep 2: TV (cm^3)	10.33	14.44	10.55	11.14	7.96	8.37
Rep 3: TV (cm^3)	11.34	14.31	10.60	11.56	7.96	8.14
Day 28						
Rep 1: TV (cm^3)	12.24	15.15	12.84	14.05	10.34	8.78
Rep 2: TV (cm^3)	12.88	15.19	12.69	13.92	7.83	10.35
Rep 3: TV (cm^3)	13.88	15.15	12.69	13.58	7.67	8.15
Day 35						
Rep 1: TV (cm^3)	14.03	15.56	13.17	13.38	8.08	8.45
Rep 2: TV (cm^3)	13.24	15.39	13.58	15.13	10.27	9.98
Rep 3: TV (cm^3)	12.34	16.75	13.38	13.80	7.89	10.15

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Table 2.2: Determination of Total Nitrogen (%)

PARAMETER	Pig Dung	Water Hyacinth	Maize Cob	Pig Dung/ Maize Cob	Pig Dung/ Water Hyacinth	Pig Dung/ Maize Cob/ Water Hyacinth
WT of sample taken	0.05	0.05	0.05	0.05	0.05	0.05
Day 0						
Rep 1: TV (cm^3)	3.09	2.79	0.90	1.43	2.85	1.62
Rep 2: TV (cm^3)	2.99	2.90	0.96	1.46	2.83	1.66
Rep 3: TV (cm^3)	2.85	2.77	0.93	1.46	2.82	1.62
Day 7						
Rep 1: TV (cm^3)	2.95	2.90	0.94	1.52	2.86	1.65
Rep 2: TV (cm^3)	3.02	2.89	0.96	1.52	2.89	1.69
Rep 3: TV (cm^3)	3.00	2.92	0.97	1.47	2.87	1.65
Day 14						
Rep 1: TV (cm^3)	3.03	2.96	1.03	1.53	2.99	1.67
Rep 2: TV (cm^3)	3.05	2.97	1.00	1.53	2.92	1.66
Rep 3: TV (cm^3)	2.97	2.99	1.02	1.53	2.97	1.73
Day 21						
Rep 1: TV (cm^3)	3.18	3.00	1.07	1.62	3.00	1.76
Rep 2: TV (cm^3)	3.15	3.06	1.07	1.69	3.00	1.77

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Rep 3: TV (cm^3)	3.16	3.08	1.07	1.63	3.00	1.79
Day 28						
Rep 1: TV (cm^3)	3.23	3.19	1.07	1.67	3.05	1.86
Rep 2: TV (cm^3)	3.23	3.20	1.12	1.70	3.03	1.83
Rep 3: TV (cm^3)	3.28	3.22	1.12	1.77	3.06	1.93
Day 35						
Rep 1: TV (cm^3)	3.32	3.19	1.10	1.80	3.08	1.90
Rep 2: TV (cm^3)	3.30	3.20	1.12	1.75	3.08	1.87
Rep 3: TV (cm^3)	3.33	3.26	1.13	1.73	3.08	1.93

Where Rep = Replicate and TV = Titre value

Table 2.3: Determination of Total Phosphorus (%)

PARAMETER	Pig Dung	Water Hyacinth	Maize Cob	Pig Dung/ Maize Cob	Pig Dung/ Water Hyacinth	Pig Dung/ Maize Cob/ Water Hyacinth
Absorbance reading (AB) (470nm)						
Day 0						
Rep 1: AB	0.33	0.31	0.24	0.31	0.32	0.33
Rep 2: AB	0.34	0.30	0.25	0.31	0.32	0.33
Rep 3: AB	0.34	0.30	0.25	0.33	0.31	0.32

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Day 7						
Rep 1: AB	0.35	0.33	0.26	0.34	0.34	0.35
Rep 2: AB	0.37	0.33	0.26	0.34	0.34	0.35
Rep 3: AB	0.36	0.33	0.27	0.34	0.34	0.34
Day 14						
Rep 1: AB	0.38	0.33	0.27	0.34	0.36	0.37
Rep 2: AB	0.38	0.34	0.29	0.35	0.36	0.37
Rep 3: AB	0.38	0.34	0.29	0.35	0.36	0.37
Day 21						
Rep 1: AB	0.43	0.37	0.32	0.40	0.42	0.27
Rep 2: AB	0.44	0.38	0.30	0.41	0.42	0.43
Rep 3: AB	0.44	0.37	0.32	0.39	0.41	0.43
Day 28						
Rep 1: AB	0.46	0.38	0.34	0.42	0.42	0.44
Rep 2: AB	0.45	0.38	0.33	0.42	0.43	0.45
Rep 3: AB	0.46	0.38	0.34	0.40	0.43	0.44
Day 35						
Rep 1: AB	0.48	0.40	0.36	0.44	0.45	0.46
Rep 2: AB	0.49	041	0.35	0.43	0.46	0.47
Rep 3: AB	0.48	0.4	0.35	0.44	045	046

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Table 2.4: Determination of Total Potassium (%)

PARAMETER	Pig Dung	Water Hyacinth	Maize Cob	Pig Dung/ Maize Cob	Pig Dung/ Water Hyacinth	Pig Dung/ Maize Cob/ Water Hyacinth
Absorbance reading (AB) (470nm)						
Day 0						
Rep 1: AB	0.05	0.05	0.04	0.05	0.05	0.05
Rep 2: AB	0.05	0.05	0.04	0.05	0.05	0.05
Rep 3: AB	0.05	0.05	0.04	0.05	0.05	0.05
Day 7						
Rep 1: AB	0.05	0.05	0.04	0.05	0.05	0.05
Rep 2: AB	0.05	0.05	0.04	0.05	0.05	0.05
Rep 3: AB	0.05	0.05	0.04	0.05	0.05	0.05
Day 14						
Rep 1: AB	0.05	0.05	0.05	0.05	0.05	0.05
Rep 2: AB	0.05	0.05	0.04	0.05	0.05	0.05
Rep 3: AB	0.05	0.05	0.05	0.05	0.05	0.05
Day 21						
Rep 1: AB	0.05	0.05	0.05	0.06	0.06	0.05
Rep 2: AB	0.05	0.05	0.05	0.06	0.06	0.06
Rep 3: AB	0.05	0.05	0.04	0.06	0.06	0.06
Day 28						
Rep 1: AB	0.05	0.05	0.05	0.06	0.06	0.05
Rep 2: AB	0.05	0.05	0.05	0.06	0.06	0.06
Rep 3: AB	0.06	0.05	0.05	0.06	0.06	0.06
Day 35						
Rep 1: AB	0.06	0.05	0.05	0.06	0.06	0.06
Rep 2: AB	0.06	0.05	0.05	0.06	0.06	0.06

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Rep 3: AB	0.05	0.05	0.05	0.06	0.06	0.06

Table 2.5: Determination of COD

PARAMETER	Pig Dung	Water Hyacinth	Maize Cob	Pig Dung/ Maize Cob	Pig Dung/ Water Hyacinth	Pig Dung/ Maize Cob/ Water Hyacinth
Day 0						
Rep 1: V_2	28.49	20.43	15.64	18.38	18.89	15.67
Rep 2: V_2	28.37	20.21	15.45	18.13	19.04	15.67
Rep 3: V_2	28.43	20.18	15.55	18.19	19.01	15.55
Day 7						
Rep 1: V_2	31.30	21.22	19.11	19.49	19.55	18.35
Rep 2: V_2	31.27	21.16	18.95	19.45	19.83	18.26
Rep 3: V_2	31.14	21.12	19.01	19.49	19.93	18.48
Day 14						
Rep 1: V_2	33.60	21.38	19.71	19.90	23.08	21.38
Rep 2: V_2	33.57	21.38	19.86	19.99	22.89	21.19
Rep 3: V_2	33.57	21.38	19.58	19.86	22.76	21.25
Day 21						
Rep 1: V_2	34.16	23.11	20.59	20.71	22.95	21.53
Rep 2: V_2	33.82	23.30	21.31	21.12	22.98	21.47
Rep 3: V_2	34.26	23.27	20.56	19.93	23.20	21.63
Day 28						
Rep 1: V_2	34.61	23.71	21.19	21.63	23.58	21.85
Rep 2: V_2	34.79	23.80	21.06	21.79	23.83	21.82
Rep 3: V_2	34.76	23.77	21.12	22.01	23.45	22.01
Day 35						
Rep 1: V_2	35.24	23.80	21.56	22.19	23.83	22.70
Rep 2: V_2	34.92	23.77	21.88	22.45	23.86	22.79
Rep 3: V_2	35.05	23.93	21.66	22.54	23.90	22.86

Where V_2 = Volume of Ferrous Ammonium Sulphate used for sample

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Volume of sample used (V_1) = 50ml

Normality of Ferrous Ammonium Sulphate (N) = 0.1

Table 2.6: Determination of BOD

PARAMETER	Pig Dung	Water Hyacinth	Maize Cob	Pig Dung/ Maize Cob	Pig Dung/ Water Hyacinth	Pig Dung/ Maize Cob/ Water Hyacinth
Day 0						
Rep 1: D1	272	244	181	223	227	211
D2	248	207	139	184	189	171
Rep 2: D1	273	239	185	220	229	203
D2	249	202	143	181	192	162
Rep 3: D1	267	241	188	218	229	205
D2	242	204	146	178	192	164
Day 7						
Rep 1: D1	289	273	227	249	255	244
D2	269	239	189	213	219	207
Rep 2: D1	284	270	223	260	262	252
D2	264	236	185	225	227	216
Rep 3: D1	286	266	229	246	268	257
D2	265	232	192	209	234	221
Day 14						
Rep 1: D1	296	278	258	264	278	259
D2	279	245	223	229	245	224
Rep 2: D1	292	277	254	268	277	265
D2	274	244	218	234	244	231
Rep 3: D1	299	273	256	266	276	266
D2	282	240	220	231	243	231
Day 21						
Rep 1: D1	316	301	268	279	296	273
D2	299	270	234	246	264	239
Rep 2: D1	311	295	265	281	293	283
D2	294	263	231	248	261	250

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Rep 3: D1	314	299	270	273	294	278
D2	297	268	236	239	262	245
Day 28						
Rep 1: D1	318	305	273	285	300	280
D2	302	274	240	252	269	247
Rep 2: D1	319	305	273	285	299	284
D2	303	274	239	252	268	251
Rep 3: D1	316	309	275	283	299	281
D2	309	278	242	250	268	248
Day 35						
Rep 1: D1	321	308	276	284	304	283
D2	305	277	243	257	272	257
Rep 2: D1	320	308	280	287	303	286
D2	304	277	247	254	271	253
Rep 3: D1	317	307	278	286	305	285
D2	301	276	245	253	273	252

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