COMPARATIVE STUDY OF EFFLUENT FROM ANAEROBIC DIGESTERS FOR HUMAN AND FRUIT WASTE

by

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DELCLARATION

I, Abdul Aziz Issah hereby declare that this submission is my own work for the award of M.Sc. Agro-Environmental Engineering and that to the best of my knowledge, it contains no material previously published by another person or group of people or material that has been accepted for the award of any other degree by the university, except where due acknowledgement has been made in the text.

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ABSTRACT

The study examined comparatively the levels of macronutrients, pollution monitoring parameters, metals and pathogenic indicator organisms from effluent of two anaerobic digesters for human waste and fruit waste with respect to the influent. Both digesters were operated within mesophilic conditions at very short hydraulic retention time. Laboratory analysis of these parameters (macronutrients, pollution monitoring indicators and pathogenic indicator organisms) was therefore important to ensure that utilisation of the effluent was environmentally friendly and acceptable to farmers, the food industry, consumers, the environmental protection agency and the World Health Organisation (WHO). Results of this study found that, pH and macronutrients (NH_4-N, P_2O_5 and K_2O) were higher in the effluent than in the influent for both human and fruit waste. For instance, ammonium-N was about 25.1% higher in the effluent with respect to the influent for human waste and 19.1% higher in the effluent of fruit waste. Total Solids (TS), Volatile Solids (VS), BOD and COD were lower in the effluent than in the influent for both wastes (human and fruit wastes). Reduction of 51.6% Total Coliforms (TC), 53.4% Faecal Coliform (FC) and 58.9% *E. coli* were found in the digested effluent of fruit waste. 62.8% TC, 64.4% FC and 60.6% *E.coli* were found in the digested effluent of the human excreta. Heavy metals in the respective effluents were within tolerable limits that are acceptable to the Ghana Environmental Protection Agency (GEPA). The results of this study therefore join other studies to show that the process of anaerobic digestion causes reduction in pollution indicators, destruction of pathogenic organisms and increases the availability of macronutrients.

Keywords: Anaerobic digestion, Influent, Effluent, Macronutrients, Metals, Pathogens, Pollution indicators.

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AD	Anaerobic digestion
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DM	Dry Matter
EC	Electrical Conductivity
FC	Faecal Coliform
FRI	Food Research Institute
FSM	Faecal Sludge Management
GEPA	Ghana Environmental Protection Agency
HRT	Hydraulic Retention Time
ODM	Organic Dry Matter
SE	Standard Error
TKN:	Total Kjeldahl Nitrogen
TS	Total Solids
TC	Total Coliform
VS	Volatile Solids
WHO	World Health Organisation

CHAPTER ONE

1. INTRODUCTION

1.1 Background

The worldwide interest in Anaerobic Digestion (AD) in biogas plants in recent time is due to current issues such as global warming, demand for renewable energy, landfill tax on wastes, high fossil fuel and inorganic fertiliser prices, legislation relating to the treatment and disposal of organic wastes (Lukehurst *et al.*, 2010), on-site sanitation systems associated with faecal sludge management (FSM) especially in relation to difficulties with emptying, transportation and disposal (Boot and Scott, 2008). Anaerobic digestion in biogas plants consists of several interdependent, complex sequential and parallel biological reactions in the absence of oxygen in which the products from one group of microorganisms serve as substrates for the next resulting in transformation of organic matter into biogas and nutrient rich effluent called digestate (Parawira, 2004).

Anaerobic digestion is one of the oldest technologies used for waste treatment. The utilisation of anaerobic digestion started hundreds of years ago from sewer systems to provide lighting by the ancient Egyptians. In the 1930s, small groups in India and elsewhere began to develop early versions of the biodigester systems that are in use around the world today. After World War II there was renewed interest in the technology for redevelopment efforts in Europe, as well as some small projects in the United States (Mueller,2007) and other countries including, China, Nepal, Germany, Sweden among others (Heegde and Sonder, 2007).

Today anaerobic digestion is being used to improve sanitation and provide organic fertiliser. For instance, in Sweden, an estimated amount of 200,000–220,000 tonnes per annum digestate is produced in large-scale biogas plants and more than 90% is currently disposed on arable land as biofertiliser (Berglund, 2006). Also, in Germany, annual production of biogas effluent has been estimated at 8.7 million tonnes (Al Seadi *et al.*, 2006).

In Africa, the interest in biogas technology is in the infant stage. Nonetheless, the technology has been promoted and stimulated by the efforts of various international organisations and foreign aid agencies through publications, meetings and visits. Currently, some digesters have been installed in several Sub-saharan African countries, utilising a variety of wastes such as slaughter house waste, municipal waste, industrial waste, animal dung and human excreta with the ultimate aim of improving sanitation, generating energy and providing biofertiliser (Mshandete and Parawira, 2009).

In the Ghanaian perspective, the use of biogas to generate energy for street and household lighting, heating, sanitation and agriculture started in 1987 at Appolonia in the Greater Accra Region (Bensah and Brew-Hammond, 2008). Today, the focus of biogas plants in Ghana has shifted from energy to sanitation. Notwithstanding this, Ghana has the potential to generate energy and biofertiliser from organic waste. For example, Anaman (2011) estimated Ghana's energy potential from biogas at about 88,144m^3 per day, capable of generating about 107.7 MWh of electricity daily to support the energy needs of 300 households annually and a grand total of 121,700 m^3 to replace 138.8 tonnes of firewood daily. In addition, the country has the potential of

realizing about 280,000 biogas plants capable of producing about 6000m^3 of liquid biofertiliser daily by 2020 and this is expected to increase agricultural production by 25%, if 80% of the bio-slurry is use as organic fertiliser (The Biogas for Better Life Initiative Action Plan Document 2006-2020). Also, Bensah and Brew-Hammond (2008) estimated biogas effluent potential of Ghana at about 360,000 tonnes per year, capable of fertilising about 70,000 hectares of irrigated farmland or 140,000 hectares of dry farmland.

There are several advantages of using biogas effluent as fertiliser in agriculture. All the plant nutrients in the raw materials digested are preserved in the effluent. Characteristically, it has no smell, does not attract flies (less odour), low pathogens, does not pollute the atmosphere(less green house gases emission) during its application and does not pose health hazards to the user and animals (Sasse, 1988 and Smith *et al* 2007). Anaerobic digestion can therefore allow for recirculation of plant nutrients in urban waste products, and potentially reduce the demand for chemical fertilisers.

However, depending on the source of organic waste used and the prevailing digestion conditions, biogas effluent after anaerobic digestion may contain chemical pollutants (nutrients, metals, persistent organic compounds etc.) and pathogens that may be above the Ghana Environmental Protection Agency (GEPA) or the World Health Organisation (WHO) maximum permissible limits for disposal or agricultural utilisation. It is against this backdrop that this research sought to analyse the extent of changes in physicochemical and sanitary parameters of undigested and anaerobic digested slurries of two biogas plants treating human excreta and fruit wastes(

pineapple and mango peels) and to ensure that the changes in the effluent is environmentally acceptable for land disposal or agricultural utilisation.

1.2 Problem Statement

Sanitation is inextricably linked with not only good drinking water but the source and kind of food eaten. For example, Seidu *et al.* (2008) reported that people in urban Ghana commonly consume 10–12 g of lettuce in 'fast food' on each of four days per week. The cholera outbreak in the Greater Accra region in recent times has been linked to poor sanitary habits and contaminated foods. In Ashaiman and the Food Research Institute (FRI), the biogas plants installed are designed mainly to convert human faecal matter and fruit waste (mostly pineapple and mango peels) into energy within short Hydraulic Retention Time (HRT). But the resulting effluent is expected to be used by nearby farmers as liquid bio-fertiliser for irrigation. However, onion and vegetable farmers in Ashaiman using an irrigation canal in Figure1.1 are hesitant in using the effluent because of risk of pollutants, pathogens and cultural reasons regarding human excreta. Also the effluent from the fruit waste is expected to be used by the Food Research Institute (FRI) to fertilise a pineapple demonstration farm closer to the digester. Nonetheless, this has not been scientifically tested to ascertain the level of nutrients, pollution indicators, heavy metals and pathogens.

However, the source of the input wastes (anthropogenic and industrial) and the adopted short retention time at both projects sites presupposes that the raw waste may contain macronutrients (N, P, K), heavy metals (Cd, Cu, Pb, and Zn) and pathogenic indicator bacteria such as Total coliform (TC), *Fecal Coliform (FC)* and *Escherichia coli* that may pass into the effluent. This is because the process of anaerobic digestion

has transformation effect, not purifying effect (In source energy- ISE, 2010). It is therefore necessary to perform laboratory analysis of the effluent with respect to the influent to ascertain the level of nutrients, pollution indicators and pathogens acceptable to farmers, consumers, the food industry and the environmental protection agency.

Figure 1.1: An irrigation canal used for onion and vegetable cultivation at Ashaiman

1.3 Objective(s)

1.3.1 General Objective(s)

The general objective of the research was to determine the level of macronutrients, pollution monitoring indicators and pathogenic organisms in the effluent with respect to the influent of two biogas plants treating human waste and fruit waste for energy.

1.3.2 Specific objectives

The specific objectives of the research were to determine the level of:

1. Nutrient values in terms of Total Nitrogen, NH_4-N, P_2O_5 and K_2O.
2. Reduction of pollution indicators such as Total Solids (TS), VS, BOD, COD, pH, Turbidity and Conductivity.
3. Metals such as Cd, Pb and Zn in the effluent with respect to the influent.
4. Reduction in pathogenic indicator organisms such as TC, FC, *E.coli*, and *Salmonella* in the effluent with respect to the influent.

1.4 Scope and Delimitations

1. The research was conducted within the Ghanaian context. Nevertheless, the results and discussions may be applicable to other countries or regions with similar conditions.

2. The study was conducted under short Hydraulic Retention Time (HRT).

3. All the biogas digesters were studied under mesophilic temperature.

4. The research focused mainly on chemical pollutants (nutrients and heavy metals), pollution monitoring indicators and pathogenic bacteria in the influent and how they are transformed in the effluent after anaerobic digestion process.

5. Testing for all possible pathogens was complex, laborious, time-consuming, and expensive.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction

This section reviews previous works done by researchers on anaerobic digestion. It includes basic definitions of key concepts, general description of anaerobic digestion process, process control parameters that influence anaerobic digestion, products resulting from the digestion process, advantages and disadvantages of anaerobic digestion, transformation of physicochemical (nutrients, metals) and guidelines for indicator organisms.

2.2 Basic definitions

Acetogens: These are microorganisms that use hydrogen and carbon for building organic molecules to generate acetate as a product of anaerobic respiration (Drake *et al.*, 2008).

Ammonium (NH_4^+): This is the ionic form of ammonia following dissolution of ammonia gas in aqueous solution (Smith *et al.*, 2007).

Batch-load digester: This type of digester is filled with organic substrates, sealed and emptied when the substrate become completely digested and stop producing gas.

Carbon Nitrogen ratio (C/N ratio): C/N is defined as the amount of total carbon divided by the amount of total nitrogen contained in organic waste (Smith *et al.*, 2007).

Continuous load digester: This type of digester is filled and emptied regularly, normally daily. Gas production is constant and slightly higher than in batch digesters (Sasse, 1988).

Effluent: Liquid (e.g. treated or untreated wastewater) that flows out of a process or confined space (WHO, 2006).

Indicator organisms: Microorganisms whose presence is indicative of faecal contamination and possibly of the presence of more harmful microorganisms (WHO, 2006).

Methanogens: These are archaeans and obligate anaerobes that produce methane as metabolic by-product in anoxic condition (Joseph, 1999).

Non-Point Source Pollution: Pollution from sources that cannot be precisely identified such as runoff from agricultural or mining operations.

Nutrient: Any substance such as N, P, K and essential trace elements in soil and water used by living things to promote growth (Smith *et al.*, 2007).

Pathogen: A disease-causing organism (e.g. bacteria, helminths, protozoa and viruses (WHO, 2006).

Point Source Pollution: Include pollution sources that can be specifically identified such as factories, refineries or outfall pipes.

pH: An expression of the intensity of the basic or acid condition of a liquid (WHO, 2006).

Sanitation: Sanitation literally means measures necessary for improving and protecting health or any system that promotes proper disposal of human and animal wastes, proper use of toilet and avoiding open space defecation (WHO, 2006).

Total Kjeldahl Nitrogen (TKN): The sum of N and $NH4^{+}$, but does not include the oxidized forms of NO_2^- and NO_3^- (Holstege *et al.*, 2010).

Sewage – Mixture of human excreta and water used to flush the excreta from the toilet and through the pipes; may also contain water used for domestic purposes (WHO, 2006).

2.3 General Description of AD Process

Anaerobic digestion(AD) in biogas plants is a biochemical process that consists of the degradation of organic materials like sewage sludge, organic fraction of municipal solid wastes (OFMSW), cattle manure, pig slurries, slaughter waste (rumen content and blood), fruit waste, human excreta etc. into biogas and nutrient rich effluent called digestate (Lukehurst, 2010). The feedstock can be a single input or a mixture of two or more feedstock types called co-digestion as shown in figure 2.1.

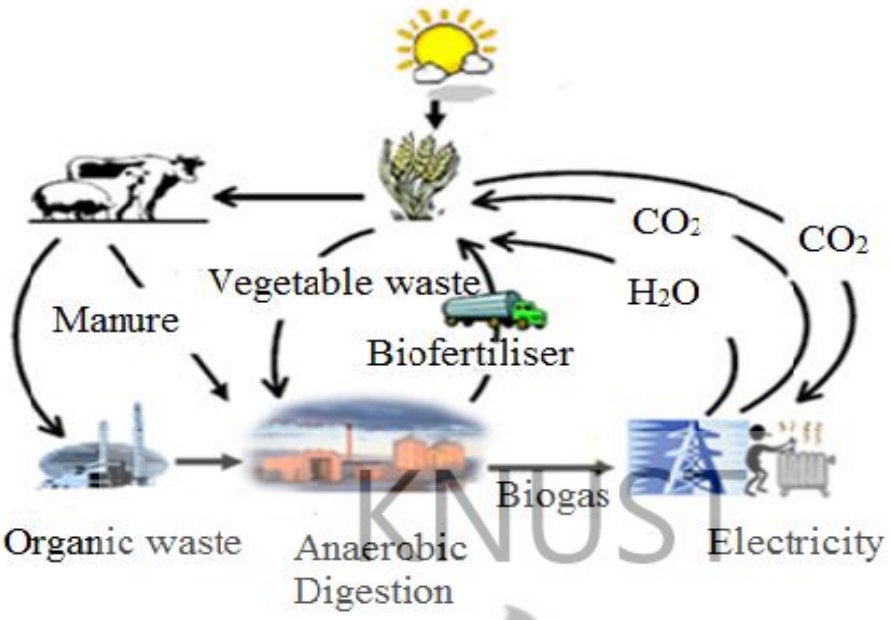

Figure 2.1: Co-digestion of organic wastes (modified from Al Seadi *et al.*, 2006)

The process of AD takes place in four consecutive stages (Serna, 2009) namely;

- Hydrolysis,
- Acidogenesis,
- Acetogenesis,
- Methanogenesis.

2.3.1 Hydrolysis

The first step in the anaerobic digestion process is hydrolysis. Hydrolysis is often the slowest rate limiting step in fermentation, particularly if the influent contains particulate or large complex molecules in large quantities (Schieder *et al.*, 2000 and Torondel, 2010). It occurs when complex polymeric substrates are hydrolysed into their respective monomers by hydrolytic enzymes. More precisely, xylanase and amylase degrade polysaccharides into sugars, protease degrades protein into amino

acid and lipase degrades lipids into glycerol and long chain fatty acid-LCFA (Boe, 2006).

2.3.2 Acidogenesis

This is the second step in AD process. It involves the conversion of the products of hydrolysis into hydrogen, acetate, carbon dioxide and volatile fatty acids by acidogenic bacteria. Acidogenic products in a well-operating anaerobic digester are approximately 51% acetate, 19% hydrogen and carbon dioxide and 30% intermediate products mostly propionate and butyrate (Ahring, 2003). Among these products, hydrogen, carbon dioxide and acetate are used directly during methanogenesis. The intermediate products play a minor role and need to be degraded during acetogenesis (Schink, 1997).

2.3.3 Acetogenesis

Acetogenesis is the conversion of certain acidogenic products such as butyrate, propionate, alcohols and aromatic fatty acids into acetate and hydrogen by obligate hydrogen producing bacteria (Boe, 2006). Nevertheless, for the conversion to take place, a low hydrogen pressure is necessary in order for the acetogenic reactions to be thermodynamically possible. Such lowering of the hydrogen partial pressure is carried out by hydrogen scavenging bacteria, thus the hydrogen concentration of a digester is an indicator of its health (Serna, 2009).

2.3.4 Methanogenesis

The terminal stage of anaerobic digestion is methanogenesis**.** Here archaeal methanogens utilise the intermediate products of the preceding stages and convert them into methane, carbon dioxide and water. It is these components that make up the

majority of the biogas emitted from the system. Methanogenesis is sensitive to both high and low pH and occurs between pH 6.5 and pH 8. The remaining non digestible material which the microbes cannot utilise along with any dead bacterial forms the digestate (effluent).

Methanogenic archaea are diverse and are classified into five well established orders. Representatives of the orders *Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinale*s are common in anaerobic environments where they serve as partners for anaerobic bacteria (Gill *et al.*, 2006). The microorganisms that form methane are physiologically specialized. They can grow only with a few simple substrates, such as H_2 and CO_2, formate, methanol and acetate, and they therefore depend on the other microorganisms that degrade more complex organic compounds for their substrate supply.

It is generally accepted that two thirds or more of the methane produced in an anaerobic bioreactor is derived from acetate. Of the many methanogenic genera, only two, *Methanosaeta* and *Methanosarcina* are known to grow by an acetoclastic reaction, producing methane from acetate. *Methanosarcina* can also form methane from hydrogen and carbon dioxide (hydrogenothrophs), from methanol and methylamines (methylotrophs), and from acetate (acetoclasts) (Rocheleau *et al.*, 1999).

2.4 Process Control Parameters of AD

Process control parameters that favour biogas digestate production are categorized into the so-called operational and environmental factors. They are required at optimum conditions to promote and maintain steady digester performance.

Concentrations above certain limits will result in digester failure (FAO/CM, 1996). Table 2.1 is a summary of some recommended process control parameters of AD in a digester.

Table 2.1: Recommended limits of process control parameters of AD

Parameter	Unit	Limit
pH		7.0 -7.5
TS	%	8.0 -15.0
NH_4-N	mg/l	50 -2000
Alkalinity	mg/l	2000-3500
VFA's:		
Acetic acid	mg/l	< 3,000
propanoic acid	mg/l	< 1000
*TF Acids	mg/l	4,000

Source: Renewable Energy Projects, EnD-I AG (2011)

***TF Acids: Total Fatty Acids

2.4.1 Operational parameters/sludge characteristics

Operational parameters/sludge characteristics of AD are Total Solids (TS), Volatile Solids (VS), Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT).

2.4.1.1 Total solids (TS)/dry matter

This is the residue left in a vessel after evaporation of a liquid from a sample and subsequent drying in an oven at 105^0C (US-EPA, 2001). According to Erickson *et al.* (2004), solids can be classified into low-solid anaerobic digestion with dilute

feedstock less than 8%, semi-liquid AD with solid content of 10-15% (wet fermentation) and high-solid AD with solid content of 20-40% (dry fermentation).

2.4.1.2 Organic Dry Matter (ODM)

Organic Dry Matter is the loss in weight by ignition of a sample previously dried at 105^0C at 550^0C for 2 hours in a muffle furnace (APHA, 1999). It describes the TS fraction of organic feedstock used to approximate the biodegradability of the substrate. For example, VS/TS reflects the rate of substrate biodegradability (Biogas Conversion Primer, 2005).

2.4.1.3 Organic Loading Rate (OLR)

OLR is the amount of raw materials added to the digester per day per unit volume of digester without the digester being overfed or underfed. An overfed reactor decreases pH below 6.0 due to accumulation of VFA's and underfed reactor decreases bacteria food requirements. Therefore, normal organic loading rate at 35^0C is estimated at 2 and 3kg ODM/m^3/day or 3.3kgVS/m^3/day (Biogas conversion primer, 2005). Organic loading rate can be calculated from equation 2.0.

OLR = Volatile solids/day/Volume of digester ……Equation 2.0

2.4.1.4 Hydraulic Retention Time (HRT)

Hydraulic retention time is the average period that a given quantity of input material remains in the digester to be acted upon by bacteria. HRT depends on temperature and substrates flow rate. For example, HRT at psychrophilic, mesophilic and thermophilic temperatures lie between 30-40days or longer, 10-20 days and 5-10 days respectively. Nevertheless, long HRT decreases NH_4-N and DM content in the effluent (PF 'FLUID', 2010; Smith *et al.*, 2007). HRT can be calculated from equation 2.1.

HRT = digester volume/Daily feed rate…….. ……Equation 2.1

2.4.1.5 Agitation/mixing

In practice, digester content is stirred between 4-6 hours/day (PF "FLUID" 2010).This is done to remove metabolites produced by the methanogens, mixing of fresh substrate and bacterial population (inoculation), preclusion of scum formation and sedimentation, avoidance of pronounced temperature gradients within the digester and provision of uniform bacteria population density.

2.4.2 Environmental parameters

Environmental factors that influence digester performance include nutrients or C: N ratio, pH, temperature, alkalinity, NH_3, VFA and lack of toxicity (Bouallagui *et al.*, 2004a). These are important in maintaining process stability and bacteria performance.

2.4.2.1 C: N ratio/Nutrients

AD proceeds best when the input material contains carbon (carbohydrates) for energy and nitrogen (as protein, nitrates, ammonia, etc) for building cell structures (Fry, 1973). For high carbon substrates (high C/N ratio), nitrogen is rapidly used up by bacteria leaving much of the carbon. In excess nitrogen (low C/N ratio), the carbon soon becomes exhausted and the remaining nitrogen lost as ammonia gas (NH_3). These conditions slow down the digestion process and consequently decrease the fertility of the effluent sludge (Fry, 1973). Therefore, a C/N ratio of 20:1 to 30:1 is considered optimum for AD (FAO/CMS, 1996). Vitamins such as Fe, Ni, Mg, Ca, Na, Mo, Se, P, S, K and Co also stimulate bacteria growth (Kossmann *et al.*, 1999).

2.4.2.2 Temperature range

Temperature required for the process of AD in a biogas plant can be classified into psychrophilic (< 25 ºC), mesophilic (30-40 ºC) and thermophilic (50-60 ºC) However, bacteria that produce methane in the range of 32-35°C are more stable to environmental changes and produce quality biogas and effluent (digestate) (Fry, 1973). Thermophilic temperature on the other hand increases substrates degradation, destruction of pathogens and decreases retention but produces poor quality effluent compared to mesophilic anaerobic digestion (Chen *et al.,* 2008; Song *et al.,* 2004).

2.4.2.3 pH

pH stability is of great importance to the anaerobic digestion process. It is reported that for appropriate microbiology activity, pH should be within a range of 6.5 - 8.0 (Bhagwan *et al*, 2008). If the pH value drops below 6.2, the medium will have a toxic effect on the methanogenic bacteria (Kossmann, 1999). In anaerobic digesters a process imbalance may lead to a decrease in methane gas production and accumulation of organic acids (produced during acidogenesis). This provokes a decrease in the pH of the system, causing inhibition of methanogenesis and cessation of the degradation process (Anderson *et al.,* 2003). Thus in many systems, adjustment of the pH buffering capacity through addition of an external source of alkalinity such as lime dosing may result in improved process stability and increase rate of anaerobic stabilisation. However, the combined effect of CO_2, NH_3 and H_2O to form NH_4HCO_2, may also provide the main buffering capacity to maintain neutral pH in the medium even when large amounts of acid or alkali are added (Kossmann *et al.,* 1999). pH also plays an important role in determining the concentration of inhibitory substances which can cause imbalance in the anaerobic digestion process (Chen *et al.,* 2008).

2.4.2.4 Ammonia

This is produced by the biological degradation of nitrogenous matter, mostly in the form of proteins and urea (Kayhanian, 1999). The two principal forms of inorganic ammonia nitrogen in aqueous solution are ammonium ion (NH_4^+) and free ammonia (NH_3). Free ammonia has been suggested to be the main cause of inhibition since it is freely membrane-permeable (de Baere, 1984) and may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency. Among the four types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition (Kayhanian, 1994). It is generally believed that ammonia concentrations below 200 mg/L are beneficial to the anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms (Liu and Sung, 2002). However, there is a large variation in the inhibitory total ammonia nitrogen concentrations reported in the literature ranging from 1.7 to 14g/L (Zeeman *et al.* 1985).

2.5 Products of Anaerobic Digestion

Anaerobic digestion is the breakdown of organic matter by putrefactive bacteria under airless condition. The main products of anaerobic digestion are

- Biogas as renewable energy
- Biogas effluent known as digestate

According to Al Seadi *et al.* (2006), 96-98% of raw substrate fed into a biogas plant is recovered as digestate. Figure 2.2 is a simplified mass balance of the products of AD.

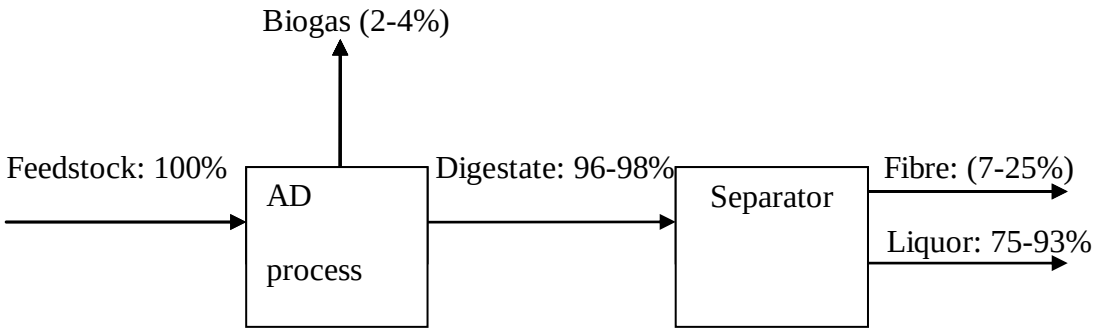

Figure 2.2: Simplified mass balance of AD (Adopted and modified from Al Seadi *et al.*, 2006).

2.5.1 Biogas

Biogas is an extremely useful source of renewable energy. It consists principally of methane: 50–75%, carbon dioxide: 25–50%, nitrogen: 0–10%, hydrogen: 0–1%, hydrogen sulphide: 0–3% and oxygen: 0–2%. The calorific value of biogas is about 20-26 MJ/m^3 (5.6 -7.2KWh/m^3; heating oil equivalent is approximately 0.5 – 0.7 liters/m^3 (Frost and Gilkinson, 2010).

2.5.2 Biogas Effluent (Digestate): Macronutrients and pollution indicators

Anaerobic digestion draws carbon, hydrogen and oxygen from feedstock to generate biogas, leaving the essential plant nutrients N, P, K almost the same but bio-available in the effluent (Berglund, 2006; Frost and Gilkinson, 2010; NNFCC, 2010; Monnet, 2003 and Smith *et al.*, 2007). The composition of the fertilizing constituents (N, P, K) however, depend on the type of feedstock used and digestion technology applied and can therefore vary (Berglund, 2006 and Monnet, 2003).

Virtually all digestates from large-scale biogas plants used in agriculture are liquid and contain approximately 2–7% dry matter and high proportions of nitrogen (typically >100 kg N per dry tonne, of which about 75 kg is in the form of

ammonium, phosphorus (about 15 kg per dry tonne) and potassium (about 50 kg per dry tonne) (Berglund, 2006). Table 2.2 is a nutrient range for all digestate proposed by Drouillon *et al.* (1997).

Table 2.2: Nutrients standards for all biogas effluent (digestates)

Parameter	Unit	Minimum	Maximum
TN	g/kg	2.8	10.7
NH_4-N	g/kg	1.8	2.4
NH_4-N(% of TN)		46	82
P_2O_5	g/kg	0.6	4.3

Source: Drouillon *et al.* (1997).

Furthermore, the anaerobic digestion process causes substantial changes in slurry composition. These include significant reduction in BOD and solid content and consequential increase in ash content due to the conservation of minerals against a background of reducing slurry carbon and organic matter (Smith *et al.*, 2007). Increases in slurry pH (up to 0.5 pH units) and NH_4-N content (up to 20 - 25%) may also occur (Monnet, 2003; Smith *et al.* 2007 and Frost and Gilkinson, 2010). Table 2.3 presents an example of changes between influent and effluent of some nutrients and pollution indicators found in cow slurry (Martin, 2004).

Table 2.3 Comparison of undigested and digested cow slurry

Parameter	Unit	Influent	Effluent
TN	kg/m^3	4.63	5.11
NH_4-N	kg/m^3	2.16	1.88
%NH_4-N(%TN)		46.7	56.4
Total P_2O_5	kg/m^3	1.86	1.92
Ortho P_2O_5	kg/m^3	1.05	1.29
%Ortho (total P_2O_5)		56.5	67.2
pH		7.4	7.9
DM	%	11.32	8.47
COD	mg/l	69,923	43,000

Source: Martin (2004)

2.5.2.1 TN and NH_4-N

According to Berglund (2009), biogas effluent contains a higher proportion of plant-available nitrogen (ammonium) than the raw substrate as a result of the degradation of organically bound nitrogen by bacteria through de-amination. Also, Al Seadi *et al.* (2006); Schenkel (2009) and Svensson *et al.* (2004) reported that ammonium rate of Total Nitrogen (TN) increases more than half in biogas effluent compared to the influent.

Furthermore, NH_4-N usually increases with increase in pH and reduced solids content. This phenomenon poses risk of significantly increased emissions of NH_3 during post-

digestion storage and may require proper application procedures. Also, nitrogen in biogas effluent can be taken up directly and more rapidly as NO_3-N after conversion of NH_4-N to NO_3-N in the soil through nitrification. The rapid conversion may however affect ground water through NO_3-N leach. NO_3-N may also occur as a potent green house gas as N_2O following denitrification. For this reason, land spreading of biogas effluent is restricted to a limit of 170KgN/hectare/year (Lukehurst *et al.*, 2010).

2.5.2.2 Total P_2O_5

There is evidence to suggest changes in biogas effluent P_2O_5 availability (water soluble) compared to the influent. This is due to the release of P from organic forms during AD (Smith *et al.*, 2007). Nevertheless, biogas effluent (digestate) has low phosphorus content and may need to be complemented with superphosphate to avoid P deficit in soil (Schenkel, 2009). Excess P in digestate may however result in the fixation of iron and zinc, making them less available to plants. Phosphate overload can also lead to diffuse pollution and subsequent eutrophication of coastal and inland waters. For these reasons a limit of 110kgP/ha/year is set for land application (Lukehurst *et al.*, 2010).

2.5.2.3 pH

pH is the measure of the acidity or basicity of a medium. It is an important parameter in the process of anaerobic digestion and stability of the effluent NH_4-N. As methane level stabilizes, the ideal pH in the biogas effluent (digestate) ranges from 7.0 to 7.5 (Fry, 1973). According to Smith *et al.* (2007), pH of biogas effluent on the whole is higher than pH of the raw influent in order of 0.5 pH units. It is however significant to note that the range of pH of biogas effluent from 7.0-8.5 has no effects on the

environment (Schenkel, 2009). Further, the solubility of most nutrients is highest at pH 6.3-6.8, but below 5.5, plant nutrients (N, P, K, S, Mg and Ca) become insoluble and unavailable for plant uptake (McKenzie, 2003). Also, fish and other aquatic vertebrates are intolerant to pH extremes outside the range 5 to 9 (McKenzie, 2003). Nevertheless, the Ghana Environmental Protection Agency (GEPA) maximum acceptable level of pH is 6.0 – 9.0.

2.5.2.4 TS or Organic Dry Matter (ODM)

The process of AD in a biogas plant causes destruction of dry matter and a reduction in viscosity (Pfundtner, 2002). The reduction of TS in biogas effluent is largely due to the loss of organic carbon in the formation of CH_4 and CO_2 (Drouillon *et al.*, 1997). According to Vetter *et al.* (1987), biogas effluent has a lower ODM content than the raw influent in the range of 50-75%. This low ODM content improves surface infiltration, enhances water holding capacity, conserves slurry N and P_2O_5, increases soil cation exchange capacity (CEC) (Smith *et al.*, 2007), results in less risk of plant scorch, low odour and small NH_3 losses if immediately incorporated into the soil (Smith and Chambers, 1992; Pfundtner, 2002).

2.5.2.5 Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) is a measure of the amount of oxygen consumed in the biological processes that break down organic matter within 5 days at 20^0C. Biogas effluent normally has low BOD after the process; the lower the BOD, the lower the degree of pollution. The effluent resulting after the digestion process has between 60-80% BOD compared to the influent (Arthur, 2000). On the basis of environmental pollution, BOD of biogas effluent spread on land is not considered a

major issue, even when it is spread as liquid without dewatering (ISE, 2010). But for discharge into water bodies the GEPA maximum acceptable standard is 50-200mg/l.

2.5.2. 6 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is the amount of oxygen (measured in mg/L) that is consumed in the oxidation of organic and inorganic matter under test conditions. It is used to measure the total amount of organic and inorganic pollution in wastewater and to determine the biodegradability of organic waste. In a study to determine the biodegradability of human faecal matter, Nwaneri *et al. (*2008) found that, COD values for the faecal material present in the first layer were significantly lower than those measured for fresh faeces. Typically, COD of most waste water, range from 25 to 50 mg/L. Nevertheless, values below 150 mg/L pose no restriction to irrigation use. The GEPA maximum allowable level for discharge of waste water is set at COD 250 – 1000mg/l.

2.5.2.7 Electrical Conductivity (EC)

Electrical conductivity is a reliable indicator of the total dissolved solids (salts) content of a sample. It is measured in mS/cm (millisiemens/cm. The salt content of digestate depend on the source and type of the feedstock used (Schenkel, 2009). High salt levels cause bacteria cells to dehydrate due to osmotic pressure (de Baere, 1984). They are required for microbial growth in moderate concentrations but, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (de Baere, 1984). The maximum allowable level of EC set by GEPA is 1500µS/cm

2.5.2.8 C/N ratio of digestate

According to Sasse (1988), fermented slurry with a lower C/N ratio has better fertilizing characteristics. Similarly, Bermejo and Ellmer (2009) indicated that, organic fertilizers with lower C/N ratio (less than 20:1) have faster nitrogen availability than fertilizers with C/N ratio greater than 20:1.This is because at lower C/N ratio, microorganisms will obtain adequate nitrogen for their protein needs and will convert the excess organic nitrogen into ammonium (NH_4^+) via mineralization.

2.6 Heavy metals

Heavy metals in digestate usually come from anthropogenic sources (Al Seadi, 2001). For example, domestic wastewater effluent contains metals from metabolic wastes or corrosion of water pipes, industrial effluents and waste sludges may substantially contribute to metal loading. Heavy metals are elements having atomic weights between 63 and 200. Living organisms require trace amounts of some heavy metals such as Co, Cu, Fe, Mn, Mo, V, Sr and Zn. However, excess levels can be detrimental to the organisms. The heavy metals of particular concern for the application of waste products and digestate as soil fertilisers are: Cd, Cr, Hg, Pb, Cu, Ni and Zn (Al Seadi, 2001). Table 2.4 presents heavy metal limits of bio-waste for disposal onto the environment.

Table 2.4 Metal limits in biogas effluent

Parameter	Unit	BTW,2nd Draft (2001)	Ghana EPA(2011) limits in mg/l
Cd	mg/kg	0.7	<0.1
Cr	mg/kg	100	0.1
Cu	mg/kg	100	2.5
Hg	mg/k	0.5	0.005
Ni	mg/kg	50	
Pb	mg/kg	100	0.1
Zn	mg/kg	200	5

Source: Biological Treatment of Bio-waste (BTW), 2nd draft (2001)

2.7 Pathogenic Indicator Organisms

Recommended Guidelines of Pathogenic Indicator Organisms

According to the WHO (1989) guidelines for coliform bacteria, limit of ≤1000FC/100ml is recommended for unrestricted irrigation (that is crops likely to be eaten uncooked, sports fields, public parks, flood irrigation or when children are exposed) but for restricted irrigation (that is irrigation of cereal crops, industrial crops, fodder crops pasture and trees), ≤ 10^5/100ml is recommended when adult farm workers are exposed to spray irrigation.

Notwithstanding, the United States Environmental Protection Agency (USEPA) and the US Agency for International Development have recommended stricter guidelines for waste water use. For unrestricted irrigation, no detectable faecal coliform bacteria

are allowed in ≤100 ml and for commercially processed and fodder crops, the guideline limit is ≤200 faecal coliform bacteria/100 ml (Blumenthal *et al.,* 2000). Also, for biogas effluent to be sanitised, *salmonella* and *clostridium* should be absent in 50g and 1.0g digestate respectively (The Biological Treatment of Waste, 2001). Also, helminth eggs should be ≤ 1.0/litre (WHO, 1989; WHO, 2006). Table 2.5 shows minimum recommended level of *E.coli* in waste water use in agriculture and aquaculture.

Table 2.5: Recommended minimum level for monitoring microbial targets of wastewater and excreta use in agriculture and aquaculture

Exposure or Activity	***E.coli* per 100ml (Arithmetic Mean)**
Agriculture-*unrestricted irrigation*	
Leaf crops	10^3
Root crops	10^4
Drip irrigation-high growing crops	10^5
Restricted irrigation	
Labour intensive high contact agric.	10^4
Highly merchanised agriculture	10^5
Septic tank	10^6
Aquaculture	
Produce consumer	
Ponds	10^4
Waste water	10^5
Excreta	10^6
Workers, local communities	
Ponds	10^3
Waste water	10^4
Excreta	10^5

Source: WHO (2006)

2.8 Advantages and Disadvantages of AD

Advantages

- Production of renewable energy (heat, light, electricity)
- Reduction of greenhouse gas emissions through methane recovery
- Reduction of solids to be handled (e.g. less excess sludge)
- Transformation of organic wastes into high quality fertilizer
- Improvement of hygienic conditions through reduction of pathogens, worm eggs and flies
- Process stability (high-loads can be treated but anaerobic sludge can also be preserved for prolonged periods without any feeding)
- The space requirements of anaerobic treatment are lower than for aerobic wastewater treatment systems

Disadvantages

- Experts are required for the design and construction
- Temperature dependent
- Reuse of produced energy (e.g. transformation into heat and power) needs to be established
- Sludge may require further treatment (e.g. aerobic composting, humification using sludge drying beds, etc.)
- High sensitivity of methanogenic bacteria to a large number of chemical compounds
- Requires seeding (start-up can be long due to the low growth yield of anaerobic bacteria)

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Introduction

This section covers description of the study areas, materials used, methods (including pretreatment of sampling containers and sampling procedure, as well as analytical methods for the determination of physicochemical and sanitary parameters) and statistical techniques used in analysing results for subsequent discussion.

3.2 Study Area

The study was conducted on two biogas digesters at Ashaiman Municipal and the Food Research Institute (FRI) in the Accra Metropolis. Figure 3.1 shows the study areas.

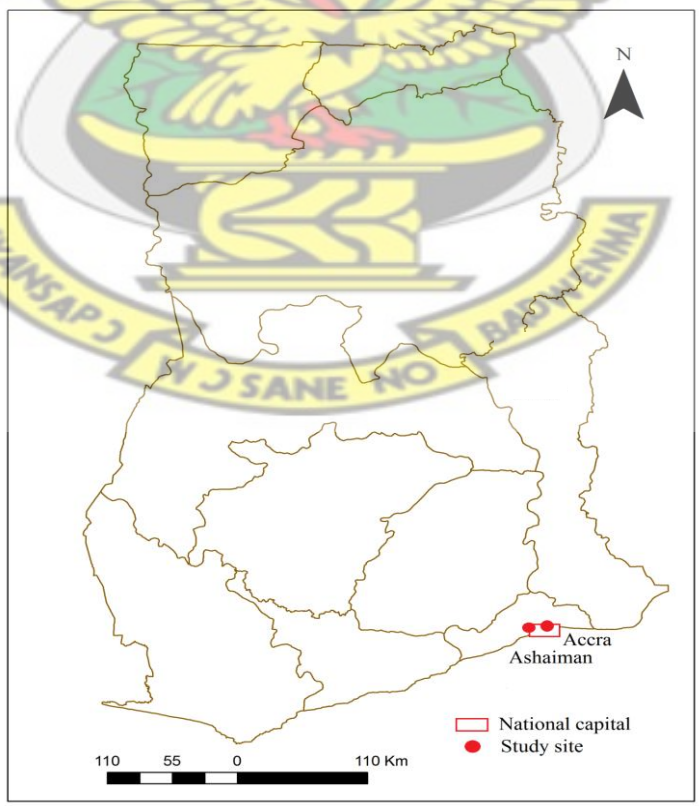

Figure 3.1: Map of Ghana showing Ashaiman Municipality and Accra Metro

3.2.1 Location, size and demography of the study areas

Accra Metropolis is located on latitude 05°35'N and longitude 00°06'W. It occupies a total land size of 200km^2 with total population of 1,752,093 and annual growth rate of 3.36 % (Ghanadistricts.com, 2010). It is made up of six sub-metros and the southern boundary is the Gulf of Guinea.

Ashaiman Municipality on the other hand is located on Latitude 5^0 42' 00"N and Longitude -2^0 00"W. It is bordered to the North by Madina-Abokobi District, East by Tema Metropolis, West by Prampram District and South by Accra Metropolis. It has a total population of 240,000 inhabitants (2010 National Population Census, Adopted from "Safisana" Ghana Limited).

3.2.2 The Environment: sanitation and waste management

Sanitation

According to ghanadistricts.com (2010), the state of sanitation in Accra is currently very unsatisfactory since it is characterised by choked drains, indiscriminate waste disposal and uncollected refuse in central waste containers. A survey of 960 Accra households by Boadi (2004) found that about 37.5% do not have toilet facility in their homes; of these, about 35% relied on public toilets and about 2.5% resorted to open defecation. Likewise, in Ashaiman municipality, sanitation is not encouraging. Only 15% of the people have direct access to sanitation facilities such as toiletries and waste collection containers (Safisana, 2011). Indiscriminate defecation and littering is very common. This state of unsanitary conditions normally results into water borne diseases such cholera.

Waste management

The people of Accra generate between 1,500–1,800 tonnes of wastes daily and an average of 1,200 tonnes collected per day. The remaining wastes find the drainage systems and other open spaces as their final destination resulting in choked drains and consequent flooding in most areas. Nonetheless, waste management is the sole responsibility of the Assemblies which engage the services of some private companies in waste collection. These companies place containers at designated points for households to dislodge their domestic waste for on-ward carriage to the final disposal or incineration sites. In addition, door-to-door collection is prominent in affluent areas where the companies charge fees monthly or fortnightly based on contractual arrangement between the companies and the clients (households) (Ghanadistricts.com,2010).

3.2.3 Vegetation and Climate

Greater Accra lies in the Savannah zone. The vegetation of the metropolis is characterised by natural growths such as shrubs, grasses and coastal trees. In addition to the natural vegetation, trees such as neems, mangoes, cassias, avocados and palms have been introduced. The area is relatively dry since it falls within the dry coastal equatorial climatic zone with temperatures ranging between 20° and 30° Celsius and annual rainfall ranging from 635 mm along the coast to 1,140 mm. It has two rainy seasons; the first starts in May and ends in mid-July and the second in mid-August and ends in October. Relative humidity is generally high, varying from 65% in the mid-afternoon to 95% at night. Wind speed normally ranges between 8 to 16 km/hr. The maximum wind speed recorded in Accra is 107.4 km/hr (58 knots) (ghanadistricts.com, 2010).

3.3 Materials

This section outlines the materials used during the research. Notable among these are fixed dome biogas digesters installed at Ashaiman and the Food Research Institute (FRI) in Accra. The Ashaiman biogas plant is an on-going project designed to evaluate energy from human excreta and slaughterhouse waste undertaken by Safisana Ghana Limited. Figure 3.2 illustrates the layout of the biogas plant at Ashaiman.

The digester at the Food Research Institute is a pilot project funded by the German Agency for Technical Cooperation (GTZ) GTZ to support "Natures Best" a Ghanaian entrepreneur in generating energy from fruit waste for drying fruits for the international market. Both digesters are loaded daily and operate with very short hydraulic retention time. Figure 3.3 illustrates a 50m^3 biogas digester at the FRI.

Figure 3.2: Digester I: A layout of a twin biogas plant treating human excreta, Ashaiman (Courtesy: Safisana Ghana Limited)

Figure 3.3: Digester II: A 50m^3 biogas plant treating Fruit Waste at the FRI, Accra (courtesy: FRI)

Other materials used during the study included: plastic containers, a thermometer, a pH meter, glassware, microscope and stock chemical solutions for analysis of physicochemical and sanitary parameters.

3.4 Methods

This section outlines the procedure for digester start-up, sampling procedures and analytical methods for the determination of macronutrients, pollution monitoring indicators, metals and pathogenic indicator organisms.

3.4.1 Digester start up and acclimatization

Prior to this research, the digesters studied were already operating. However, according to the digester managers, the digesters were started with about 1/3 digester volume with slaughter house waste and 2/3 water.

3.4.2. Preparation of sampling containers

The sampling containers were washed with non-ionic detergent, rinsed with clean tap water and later soaked in 10%HNO_3 for 24hours and finally rinsed with non-ionised water prior to usage (Akan *et al.*, 2008).

3.4.3 Sampling procedure and sample pre-treatment prior to analysis

In all, eight sterilised plastic containers of 1.5 litres volume were used for sampling (Four for each digester: Two for the influent and two for the effluent). Prior to usage, both substrates (influent and effluent) were thoroughly stirred to ensure even distribution of solids. Each container was filled and emptied with the respective substrate three times and finally dipped to about 10cm below the respective substrates, filled to the neck level and firmly capped. The content were stored in a cool box containing ice blocks to maintain the temperature at 4^0C to reduce warming up and change in any biochemical activity (Koopmann, 2008), and transported for analysis at the Soil Research Institute (SRI) and Water Research Institute (WRI) of the Council for Scientific and Industrial Research (CSIR) laboratories, Kumasi and Tamale respectively.

3.5 Analytical Methods

3.5.1 Analysis of macronutrients

The macronutrients (Total Nitrogen, ammonium-N, phosphorus and potassium were analysed following the schedule in Table 3.1.

Table 3.1: Periodicity for analysis of macronutrients

Parameter	Periodicity of Analysis
TKN	7 times at 14 days interval
NH_4-N	//
Total P_2O_5	//
Ortho(P_2O_5)	//
Total K_2O	//

3.5.1.1 Determination of TKN

TKN was determined following procedure outlined by Koopmann (2008). 1.0g sample was placed in a digestion flask and 10 ml H_2SO_4 added, allowed to cool and 2.5g potassium sulfate (catalyst) added and heated until the mixture was clear. The mixture was then boiled gently for 5hours, cooled and 20 ml of water added while shaking. The content was then transferred to a distillation apparatus and 20ml NaOH solution made to run through the distillation apparatus and 100ml of the distillate collected into a conical flask containing 5ml boric acid. Few drops of mixed indicator was finally added to the 100ml distillate and titrated with sulfuric acid to a violet end point.

Calculation:

$TN = (V_1 - V_2) \times cH^+ \times M_N / m \times m_t \times 100$…….3.5.

Where,

TKN=content of nitrogen, V_1= volume (ml) of sulfuric acid used in the titration of the sample, V_0= volume (ml) of sulfuric acid used in the titration of the blank test, c (H^+) = concentration of H^+ in the sulfuric acid in moles per litre, M_N is molar mass of nitrogen in grams per mole (=14), m= mass of test sample, mt = dry mass of test sample, g/100g

Reagents are shown in appendix 1.

3.5.1.2 Determination of ammonium nitrogen (NH_4-N)

NH_4-N was determined following procedure outlined by koopmann (2008) in the field of sludge, bio-waste and soil. 10ml mass to volume (m/V) potassium chloride was added to 3g homogenized sample in an extraction bottle and shaken for one hour at room temperature. The extract was filtered through a pore size of 8-12 μm and NH_4-N in the filtrate determined according to EN ISO 11732, continuous flow analysis (CFA).

Reagents are shown in appendix 2.

Organic Nitrogen (ON)

The organic nitrogen portion of the material was calculated by subtracting the ammonium N from TKN of the sample.

Organic Nitrogen = TKN – NH_4-N

3.5.1.3 Determination of total P_2O_5

This was determined following a procedure outlined by Koopmann (2008) in the field of sludge, bio-waste and soil. 21ml HCL was added to 3.0g sample in 250ml vessel and swirled. With a reflux condenser connected to the vessel and at room temperature, some boiling aids were added into the reaction vessel and transferred to a heating device for 2hours.The mixture was allowed to cool and the reflux condenser rinsed into the reaction vessel with 10 ml water. Phosphorus content of the analysed material in mg/l was calculated according to the following formula:

$W_N = C \times V / m \times m_t$ ……3.6

Where

W_N = content of phosphorus, C = concentration of phosphorus measured in the extraction solution, mg/l, V = Volume of the volumetric flask, ml, m = mass of the test sample, m_t = dry mass of the test sample, g/100g

Reagents are shown in Appendix 3.

3.5.1.4 Determination of orthophosphate (PO_4-P)

This was done following procedure outlined by Molina (2011). 2.5 gram sample and 50ml of 0.5 M sodium bicarbonate (pH 8.5) solution were shaken for 30 minutes. The mixture was then filtered through Whatman filter paper and the ortho-phosphate in the filtered extract determined colorimetrically (at 630 nm in Technicon Auto Analyzer II) by reacting it with ammonium molybdate using ascorbic acid as the reducing agent.

3.5.1.5 Determination of Potassium

Potassium was analysed following a method outlined by Holstege *et el*. (2010). A digestion block was pre-heated to 80^{o}C. 300 mg sample was transferred into a digestion tube and weighed. 6.0ml nitric acid was added and thoroughly mixed. The mixture was predigested at room temperature for 20 minutes. The predigested sample was then placed in the digestion block for 10 minutes, removed and cooled for 2 minutes. The temperature of the digestion block was raised again to 140^{o}C and 2 ml 30% H_2O_2 solution added and digested again for another 60minutes. The content was then removed finally, cooled for 30minutes and diluted to 100ml with de-ionised water and potassium quantitatively analysed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) at 404.7 nm wavelength.

Reagents are shown in appendix 4.

3.5.2 Pollution monitoring indicators

Pollution monitoring indicators (Total Solids-TS, Volatile Solids-VS, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), pH, Electrical Conductivity (EC) were analysed following the schedule on Table 3.2.

Table 3.2 Periodicity for analysis of pollution indicators

Parameter	Periodicity of Analysis
%TS	7 times at 14 days interval
%VS	//
COD(mg/l)	//
BOD(mg/l)	//
pH	Daily
EC(µS/cm)	7 times at 14 days interval

3.5.2.1 Total solids

This was analysed following procedure described by America Public Health Association - APHA (1999) method 2540G. 10g of fresh sample was weighed and dried in an oven at 105^0C. The sample remaining after drying at 105^0C was weighed and calculated for total solids using equation 3.1.

$TS = weight_{105^0C} / Weight_{fresh\ sample} \times 100$…….. Equation 3.1

3.5.2.2 Volatile solids

This was analysed following procedure described by APHA (1999). 10g of the sample dried at 105^0C was ignited at 550^0C for two hours in a muffle furnace (Heron 12-PR/200 Series 8B) and sample remaining after ignition at 550^0C weighed. Volatile Solids was calculated using equation 3.2.

$VS = (Weight_{105^0C}) - (Weight_{550^0C}) / Weight_{fresh\ sample} \times 100\%$.....equation 3.2

3.5.2.3 Chemical Oxygen Demand (COD)

COD was determined following a procedure outlined by APHA (1999). A known quantity of the sample was taken into a conical flask. 10ml of potassium dichromate

(0.25N) was pipetted into a conical flask and 20ml of sulphuric acid rapidly added to the conical flask and the mixture kept for half an hour for digestion. After half an hour, 4-5 drops of ferroin indicator was added and titrated against ferrous ammonium sulfate solution (0.25N). The change in colour from blue green to wine red indicated the end point.

Calculation: COD (mg/L) = (B-A) X 8000 / C ……..equation 3.3

Where, B = Volume of the titrant used against blank, A = Volume of the titrant used against sample, C = Volume of the sample taken and 8000 is milliequivalent weight of oxygen

3.5.2.4 Biochemical Oxygen Demand (BOD)

This was analysed following procedure 5210B outlined in the Standard Methods 1984.The sample to be analysed was measured to ensure that, the pH was between 6.5 and 7.5. The initial Dissolved Oxygen (DO) of the sample was then measured and recorded, and incubated at 20^0C for five days and the final DO measured.

Calculations: BOD mg/l = (Initial DO - DO_5) x Dilution Factor……3.4

Where dilution Factor = Bottle Volume / Sample volume

3.5.2.5 pH

This was done on the field using a pH meter. The pH-meter was first calibrated using buffer solution of 4.0 and 9.22. After the calibration**,** the pH-meter was dipped into the influent to a depth of about 4cm at three different points and the value read on the screen. The average value was taken and recorded. The pH meter was washed with

distilled water to clear any remnants of the influent and the same procedure repeated for the effluent. Figure 3.4 illustrates pH determination in the field.

Figure 3.4: Determination of pH on the field using a pH meter on the field

3.5.2.6 Conductivity

EC was determined following procedure outlined by Johnsson *et al.* (2005) in the field of soil, sewage sludge and bio-waste. 20g of the sample was weighed and transferred into a centrifuge bottle. 200ml water with specific electrical conductivity not higher than 0.2 mS/m at 20^0C was added. The bottle was centrifuged for 30minutes and content filtered using a filter paper with low ash content and high retentive properties. The electrical conductivity of the filtrate was measured using a conductivity meter.

3.6 Analytical method of heavy metals (Cd, Cu, Pb and Zn)

Heavy metals such as Cd, Pb and Zn were analysed following the schedule in Table 3.3.

Table 3.3: Periodicity for analysis of heavy metals

Parameter	Periodicity
Cd	7 times at 14 days interval
Pb	//
Zn	//

About 1.0g sample was weighed into a 100ml digestion bottle. 20ml nitric acid (1:1) was added and the mixture thoroughly stirred and heated in an autoclave at 120^0C (200kPa) for 30minutes. The mixture was allowed to cool, filtered and the solution transferred into a 100ml flask. The solution was then diluted to the mark and ready for metals determination using the Inductive Couple Plasma-Atomic Emission Spectrum (ICP-AES) at the following wavelengths: Cd at 226.502nm, Pb at 220.353nm and Zn at 213.856nm

3.7 Analytical procedures for sanitary parameters (pathogenic indicator organisms)

The schedule in Table 3.4 was followed in analysing pathogenic Indicator organisms.

Table 3.4 Periodicity for analysis of indicator organisms

Parameter	Periodicity
Total Coliform (TC)	5 times at 14 days interval
Fecal Coliform (FC)	//
Escherichia Coli	//
Salmonela species	//

3.7.1 Determination of TC and *E. coli*

This was done following procedure outlined by USEPA (2002), method 1604. About 10g slurry was diluted to 100 ml and filtered through a 47 mm, 0.45-µm pore size cellulose ester membrane filter to retain the bacteria present in the sample. The filter was then placed on a 5-ml absorbent pad saturated with 2-3 ml of MI broth and incubated at 35°C for 24 hours. All blue colonies on each MI plate under normal/ambient light, were counted and recorded. This represented E. coli. Also, each MI plate was exposed to long wave ultraviolet light (366 nm), and all fluorescent colonies [blue/green fluorescent; E. coli, blue/white fluorescent TC other than E. coli, and blue/green with fluorescent edges (also *E. coli*)] counted.

Calculation: E. coli/100ml = Number of blue colonies/volume of samples filtered (ml)] x100

TC/100ml = Number of fluorescent colonies + Number of blue, non-fluorescent colonies (if any)/volume of sample filtered] x 100

3.7.2 Determination of FC

This was done using a procedure described by USEPA (2002) Method 1680: Fecal Coliforms in bio-solids by Multiple-Tube Fermentation (also called Most Probable Number-MPN). In this method, a presumptive test was first performed. Five fermentation tubes containing 10ml/tube of Lauryl Tryptose Broth (LTB) were inoculated with the sample and incubated for 24 hours at 35 ° C. The tubes were observed for the production of gas. Any tube that showed gas production at the end of 24hours or 48hours indicated positive for fecal coliform. In the confirmation test, the positive presumptive cultures were transferred to EC broth and incubated at 44.5°C for 24hours. The tubes were then removed and inspected for gas production. Any tube that produced gas at the end of 24hours confirmed positive for fecal coliform. All positive fermentation tubes were recorded on a test data sheet and fecal coliforms estimated and reported as Most Probable Number (MPN)/100 ml.

3.7.3 Determination of *Salmonella species*

The principle of the method involves homogenising and serially diluting the sample. The diluted sludge was filtered through a membrane filter and incubated at 36°C on a sterile glass fibre filter saturated with tetrathionate broth resuscitation medium. After incubating for 24 hours, the membrane filter was further incubated at 36°C on chromogenic medium (Rambach agar). The membranes were examined after 24 and 48 hours and positive colonies enumerated. The presence of *Salmonella species* was indicated by the presence of bright red colonies resulting from the fermentation of propylene glycol (Thompson *et al*, 2004)

3.8 Statistical Analysis

Means, standard error (SE) for each parameter were computed for all the digesters using Microsoft Office Excel (2007).

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Macronutrients Level in Terms of TN, NH_4-N, P_2O_5, K_2O

Tables 4.1 and 4.2 show results of macronutrients of undigested and anaerobic digested faecal matter obtained from a 8 m^3 fixed dome twin biogas digester in Ashaiman and fruit waste from a 50 m^3 fixed dome digester at the Food Research Institute(FRI) in Accra respectively. In the discussion, Table 4.1 is referred to as digester I and Table 4.2 as digester II. Values for the separate domes of digester I are shown in the appendix.

Table 4.1 Level of Nutrients in digester I, Ashaiman (a twin biogas plant)

Parameters	Mean Infl. and SE.	Mean Effl. and SE;	% Change
TKN(mg/l)	14.15 ± 0.15	16.15 ± 0.05	
NH_4-N(mg/l)	10.67 ± 0.07	14.25 ± 0.25	25.1
NH_4-N (% of TN)	75.45	88.2	
P_2O_5(mg/l)	1.18 ± 0.01	1.53 ± 0.005	
Ortho P_2O_5(mg/l)	1.045 ± 0.005	1.26 ± 0.0015	17.06
% Ortho of P_2O_5	55.9	65.6	
Total K_2O(mg/l)	2.6 ± 0.26	3.47± 0.32	25.07

Table 4.2 Level of Nutrients in digester II, FRI

Parameter	Mean Infl. and SE.	Mean Effl. and SE.	% Change
TKN(mg/l)	4.14±0.081	5.14 ± 0.044	19.5
NH_4-N(mg/l)	2.23 ± 0.027	2.86 ± 0.017	22.0
NH_4-N (% of TN)	53.8	55.6	
P_2O_5(mg/l)	1.18 ±0.026	1.53 ± 0.036	
Ortho P_2O_5(mg/l)	0.4 ± 0.031	0.5 ± 0.0053	20
Ortho(% of P_2O_5)	33.9	32.7	
Total K_2O(mg/l)	2.74± 0.0265	3.545 ± 0.05	22.7

Infl – Influent, Effl- Effluent, SE- Standard Error

4.1.1 TN, NH_4-N

Results from the undigested human excreta in digester (I) showed that TN recorded a mean of 14.15 mg/l and SE of 0.15. After the process of anaerobic digestion, TN of the effluent increased from a mean of 14.15 to a mean value of 16.15 mg/l with SE of 0.05. The mean value of NH_4-N content of the raw human excreta was 10.67 mg/l with SE of 0.07. This increased to a mean value of 14.25 mg/l with SE of 0.25 in the effluent representing 25.1%, which agrees with similar finding by Monnet (2003) and Frost and Gilkinson (2010). The % NH_4-N rate of TN in the raw influent and digested effluent of digester I was found to be 75.45% and 88.2% respectively. This means that NH_4-N fraction of TN in the effluent of digester I was more than half of TN which agrees with Al Seadi *et al.* (2006), Schenkel (2009) and Smith *et al.* (2007).

In digester II, TN of the fruit waste was 4.14 mg/l and SE of 0.081. After the process of anaerobic digestion, TN of the effluent increased to 5.14 mg/l and SE of 0.044.

The NH_4-N in the raw fruit waste was 2.23 mg/l with SE of 0.027. This increased to 2.86 mg/l with SE of 0.017 in the effluent after the digestion process, representing 22.0%. This agrees with similar findings by Smith *et al.* (2007). Also, the NH_4-N fractions of TN were 53.8% and 55.6% in the influent and effluent of digester II respectively, which also agree with Al Seadi *et al.* (2006) and Schenkel (2009). The results of the two digesters showed that, effluents from digester I (human excreta) and digester II (fruit waste) contained more readily NH_4-N than their raw state. Nevertheless, NH_4-N in digester I recorded a significantly higher NH_4-H than digester II. The reason might be due to more organically bound protein in human excreta than in fruit waste as reported by Berglund (2009) and Smith *et al.* (2007). In terms of agricultural utilisation and environmental safety, the results showed that NH_4-N from digester I (14.25 mg/l) can be disposed on arable land but slightly higher than the GEPA acceptable limits of 1-10 mg/l for discharge into water bodies. However, the NH_4- N value of 2.28 mg/l in the effluent of the fruit waste was within the GEPA acceptable limits.

4.1.2 Total and Orthophosphate (P_2O_5)

The results of the study shown in digester I showed that, the raw human excreta (as input substrate) for anaerobic digestion was 1.87 mg/l and SE of 0.01 of total P_2O_5. After the digestion process, the content of total P_2O_5 increased to 1.92 mg/l and SE of 0.005 in the effluent. The orthophosphate fraction of total P_2O_5 was estimated at 1.045 mg/l and SE of 0.005 in the raw waste, representing 55.9% of total P_2O_5 and 1.26 mg/l and SE of 0.0015 in the digested effluent, representing 65.6% of total P_2O_5. The percentage orthophosphate readily available in the effluent of human waste was found to be 17.06% (that is, an increase from 1.045 mg/l in the influent to 1.26 mg/l of orthophosphate in the effluent). This finding agrees with Smith *et al.* (2007).

In digester II, the raw fruit waste and the anaerobic digested effluent were found to contain 1.18 mg/l with SE of 0.02 and 1.53 mg/l with SE of 0.036 of total P_2O_5 respectively. The water soluble (orthophosphate) portion in the raw fruit waste was 0.40 mg/l and SE of 0.031 representing 33.9% of total P_2O_5. Also, the effluent after the anaerobic digestion process was recorded at 0.50 mg/l and SE of 0.0053 representing about 32.7% of total P_2O_5. The water soluble (orthophosphate) portion of total P_2O_5 in digester I was almost twice that of digester II. The reason might be due to the release of organically bound phosphorus in human faecal matter than in fruit waste during the digestion process as stated by Smith *et al.* (2007). However, the mean effluent values of total P_2O_5 in digester I (1.92 mg/l) and digester II (1.53 mg/l) as well as the means of orthophosphate in digester I (1.26 mg/l) and digester II (0.50 mg/l) were within GEPA maximum acceptable level of 1.0-10 mg/l for discharge into water bodies or use to irrigate crops.

4.1.3 Total K_2O

The study revealed that total K_2O content of raw human excreta in digester I was 2.6 mg/l with SE of 0.26. After the digestion process, 3.37 mg/l with SE of 0.32 total K_2O was recorded in the effluent representing a percentage increase of 25.07. In digester II, the input fruit waste was found to contain 2.74 mg/l with SE of 0.0265 total K_2O. This increased significantly in the effluent to a value of 3.545 mg/l with SE of 0.0053, representing 22.7% with respect to the influent. The analysis showed that, the effluent from the fruit waste (digester II) contained more K_2O than the effluent from the human excreta (digester I).

In general, the study revealed that, macronutrients (NH_4-N, P_2O_5, and K_2O) in the influent of digester I and II remained almost the same in the effluent after the

digestion process. This finding is also reported by Smith *et al*. (2007), ISE (2010), Frost and Gilkinson (2010). The order of increase in macronutrients in both digesters (I) and (II) effluents was NH_4-N > K_2O> P_2O_5, which also agreed with studies done by Sasse (1988) and Al- Seadi (2006). The high content of NH_4-N in both digesters might be due to more nitrogen content of the input organic waste or a well balanced carbon to nitrogen ratio. Also, K_2O > P_2O_5 in the effluent of both digesters might be due to the concentration of potassium in plant cells, mixed diets and fruits than in phosphorus (Wikipedia, 2011) used as the input substrate.

4.2 Pollution Monitoring Indicators

%TS, %VS, COD and BOD of biogas effluent serve as good indicators for digester treatment efficiency. This means that, the lower the concentration in the effluent, the more efficient the performance of the biogas facility (Monnet, 2003). Tables 4.3 and 4.4 present results of means and SE of influent and effluent of pollution monitoring indicators (TS, VS, COD, BOD, pH and EC) obtained from digester I (faecal matter) and digester II (fruit waste) respectively. In the discussion, Table 4.3 is referred to as digester I and Table 4.4 as digester II and all numerical values reported represent means.

Table 4.3 Means and SE of pollution indicators, digester I, Ashaiman

Parameter	Mean Infl. and SE.	Mean Effl. and SE.	% Reduction
TS	13.75±0.05	5.25 ± 0.05	61.8
VS	76.2 ± 0.05	36.7 ± 1.3	51.8
BOD(mg/l)	12,460 ± 60	4,250 ± 10.0	65.9
COD(mg/l)	13,869 ± 1.0	5,794 ± 4.0	58.6
pH	5.75 ± 0.05	7.25 ± 0.05	
EC(µS/cm)	6.35± 0.05	3.95± .05	37.8
Turbidity	146.5 ± 3.2	98.7 ± 0.7	32.8

Table 4.4: Means and SE of pollution indicators at the FRI, Accra

Parameter	Mean Infl. and SE.	Mean Effl. and SE.	% Reduction
%TS	17.9± 0.29	7.4± 0.30	58.7
%VS	79.7 ± 1.21	35.07 ± 1.36	55.9
BOD(mg/l)	12,845.7 ± 141	6,594.3 ± 102.5	48.7
COD(mg/l)	13,541 ± 163.6	4,971.4 ± 259.8	63.3
pH	4.4 ± 0.25	6.74 ± 0.067	34.7
EC(µS/cm)	3.9 ± 0.096	1.87 ± 0.037	52.1
Turbidity(NTU)	136.4 ± 3.4	95.4 ± 1.9	28.7

4.2.1 Total Solids (TS)

The results of the study in digester (I), showed a reduction of %TS in the digested effluent from a mean of 13.75% and SE of 0.05 to a mean of 5.25% and SE of 0.05, representing 61.8%. In digester II, the study found that, the influent of the undigested

fruit waste was 58.7% with SE of 0.29. This decreased to 7.4% with SE of 0.30 in the effluent after anaerobic digestion representing a percentage change of 58.7. The above changes revealed that both digester (I) and (II) recorded reduction of TS or Dry Matter (OM) in their respective effluents. Nevertheless, reduction in digester (I) was higher (61.8%) than digester (II) (58.7%).

The reason for the greater reduction in (I) might be due to the low solid content associated with human excreta contrary to fruit waste. In practice, the results showed that biogas effluent has lower TS content than the undigested waste. In both digesters, the TS content in the effluents decreased more than half with respect to the influents. This agrees with similar finding by Vetter *et al.* (1987) and Drouillon *et al.* (1997) finding. Other reasons for the greater reduction in digester (I) might be due to temperature differences (32.5^0C) in digester (I) as against 30.5^0C in digester (II). This is because, high temperature increases degradation of organic waste during anaerobic digestion (Song *et al.*, 2004). Furthermore, the removal of organic carbon in the formation of CH_4 and CO_2 during the digestion process might have accounted for TS reduction in both digesters as reported by Smith *et al.* (2007).

4.2.2 Volatile Solids (VS) or Organic Dry Matter (ODM)

The study results showed that anaerobic digestion can substantially reduce volatile solids or organic matter of biogas effluent. Volatile solids of the raw human excreta in digester (I) (influent) were found at 76.2% and SE of 0.05. As a result of the digestion process, the content of VS in the effluent significantly decreased to 36.7% and SE of 1.3 representing about 51.8%.

In digester (II), the results of the study showed that, VS in the raw fruit waste as input substrate was recorded at 79.7% and SE of 1.21 and decreased from 79.7% to 35.05% with SE of 1.36 representing 55.9%. Both digesters demonstrated reduction of volatile solids in the effluent during the study, indicating that undigested wastes contain high organic matter or VS than anaerobic digested effluent. The reason for the reduction might be due to the conversion of the organic component of the raw waste into biogas during the process of anaerobic digestion as reported by Sasse (1988).

4.2.3 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is used to measure the total amount of organic and inorganic pollution in wastewater. The study results showed that anaerobic digestion can substantially reduce chemical oxygen demand. In digester (I), the research found COD of the raw human excreta at 13,869 mg/l and SE of 1.0. This decreased from 13,869 mg/l to 5,794 mg/l with SE of 0.4 in the effluent, representing a change of 58.6% which agrees with Nwaneri *et al.* (2008) study to determine the biodegradability of human faecal waste in a latrine.

In digester (II), COD of undigested fruit waste was recorded at a mean of 13,541.9 mg/l and SE of 163.6. This decreased from 13,541.9 mg/l to a mean of 4971.4 mg/l with SE of 259.8, representing 63.3% reduction of COD in the effluent with respect to the influent. The reduction in both digesters showed that, the effluent COD in digester II was greater (63.3%) than digester I (58.6%). The reason might be due to the acid content of the fruit waste that served as a catalyst for digester (II) content during the digestion process. It can be deduced from the two digesters that, undigested wastes contain higher COD than anaerobic digested effluent. In terms of effluent disposal on the environment with high or low COD, the reduction in digester (I) effluent to 5794

mg/l and 4971 mg/l in digester (II) exceeded the Ghana Environmental Protection Agency (GEPA) maximum permissible level of 250 mg/l – 1000 mg/l for discharge into water bodies or used for irrigation.

4.2.4 Biochemical Oxygen Demand (BOD)

The study results showed that anaerobic digestion similarly reduces the level of BOD in the effluent. In digester (I), BOD of the raw human excreta was 12,460 mg/l and SE of 60.0. The effluent BOD recorded a value of 4250 mg/l and SE of 10.0 indicating a reduction of 65.9% with respect to the influent. In digester (II), the study found BOD of the raw fruit waste at 12,845.7 mg/l and SE of 141. This decreased from 12,845.7 mg/l to 6594.3 mg/l with SE of 102.5 in the effluent representing 48.7%. It was observed that, both digesters recorded BOD reduction in their respective effluents. Nonetheless, digester (I) was found to undergo a higher reduction (65.9%) than digester II (48.7%). The above findings suggest that BOD of undigested waste is higher than anaerobic digested effluents.

Furthermore, the lower BOD reduction in digester (II) might be due to poor substrate agitation, high solid content of fruit wastes that are insoluble and short hydraulic retention time. This is because, fruit and vegetable wastewater contains soluble (dissolved) and insoluble (particles) BOD. The soluble BOD is readily degradable by healthy microbes in a medium while the insoluble BOD requires longer hydraulic retention time to degrade as reported by (Wensloff, 2011). However, the research found the reduction in the effluent of digester (I) from 12,460 mg/l to 4250 mg/l and digester (II) from 12,845.7 mg/l at 6594.3 mg/l above the GEPA maximum acceptable standard of 50-200 mg/l for discharge into water bodies or use to irrigate food crops.

Despite this, it has been reported that BOD of biogas effluent spread on land is not considered a major issue, even when spread as liquid without dewatering (ISE, 2010).

4.2.5 Turbidity

The research found that turbidity of the effluent in both digesters was reduced but not significant to meet GEPA standards of 75NTU for disposal into water bodies or use for irrigation. In digester (I), the influent was highly turbid with a mean of 146.5 NTU and SE of 3.2. In the effluent, turbidity decreased from a mean of 146.5 NTU to a mean of 98.5NTU with SE of 0.7, representing 32.76%.

In digester (II), turbidity decreased from a mean of 136.4NTU and SE of 3.4 in the influent to a mean of 95.4NTU in the effluent representing 30.05%. The reason for the reduction might be attributed to the reduction in viscosity as a result of the anaerobic digestion process as stated by (Pfundtner, 2002).

4.2.6 pH

pH is a measure of acid-base content of a medium. The data from the research revealed that, pH of the undigested human excreta in digester (I) increased from a mean of 5.75 with SE of 0.05 to 7.25 with SE of 0.05 in the effluent, representing about 20.7% (or 1.5 pH units). In digester (II), the input fruit waste recorded a mean pH value of 4.4 and SE of 0.25. As a result of the digestion process, pH of the effluent increased from a mean of 4.4 to 6.74, representing 34.7%. The pH of the effluents from both digesters showed that, pH of anaerobic digested waste is higher than pH of undigested waste, which agrees to similar findings by Smith *et al.,* (2007). Also, the increase in pH in both digesters might be due to bacteria activity in breaking down organically bound protein in the waste to produce more ammonia (alkaline) in the medium during the digestion process. For utilisation of the effluent, the pH values

found for digester I (7.25) and digester II (6.74) were within the GEPA maximum acceptable standard of 6.0 – 9.0 for discharge into water bodies or use for agriculture and aquaculture.

4.2.7 Conductivity

Electrical conductivity estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in a sample. The EC during the study recorded a mean value of 6.35 µS/cm and SE of 0.05 in the raw human excreta in digester (I). In the effluent, the EC decreased from 6.35 µS/cm with SE of 0.05 to 3.95 µS/cm with SE of 0.05, representing 37.8% reduction. In digester (II), the mean EC of the raw fruit waste (influent) was 3.9 µS/cm with SE of 0.096. After the digestion process, the EC of the effluent was 1.87 µS/cm and SE of 0.037 representing 52.05% reduction. The results of EC obtained from the two digesters showed greater reduction in digester (II) than in digester (I). This might be due to high salts in the influent (human excreta) of the people using the toilet facility as against the fruit waste. The reduction in EC in digester (I) to 3.95 µS/cm with SE of 0.05 and digester (II) to 1.87 µS/cm with SE of 0.037 exceeded the GEPA maximum acceptable limits of 1.50 µS/cm.

4.3 Heavy Metals (Cd, Pb and Zn).

The study compared the presence of heavy metals in undigested (influent) and anaerobic digested effluent of human excreta and fruit wastes. The results of the study are presented in Table 4.5 for digesters (I) and (II)

Table 4.5: Mean values of heavy metals of undigested and anaerobic digested human excreta and fruit wastes

Parameter	Unit	Means			
		Digester I		**Digester II**	
		Influent	Effluent	Influent	Effluent
Cd	mg/l	0.0247	0.0250	0.0266	0.0265
Pb	mg/l	0.00345	0.0035	0.146	0.447
Zn	mg/l	1.246	1.247	2.490	2.490

The data in Table 4.5 shows that mean Cd in digester (I) and (II) effluents were almost the same as their respective influents. Digester (II) however, contained slightly higher Cd concentration than digester (I). The reason might be due to the fruit waste coming from a fruit drying industry. In terms of environmental safety, the concentration of Cd in digester I (0.0250 mg/l) and digester II (0.0265mg/l) did not exceed GEPA maximum permissible level of < 0.1mg/l and 0.7 mg/kg set by the biological treatment of bio-wastes (2001) for discharge into water bodies or use in agriculture.

Lead (Pb) concentration in digester (I) and (II) effluents did not differ significantly from their respective influents. Nonetheless, digester II had higher lead concentration than digester (I). The mean effluent values of Pb in digester I (0.0035 mg/l) and digester II (0.447 mg/l) found during the study did not exceed GEPA maximum permissible level of 0.1 - 2.0 mg/l for discharge into water bodies or use for irrigation. Zinc (Zn) level in digesters I and II did not change after the process of anaerobic digestion. However, the level of Zn in the effluents of both digesters was higher than Cd and Zn. Despite this, the mean values of Zn in digester I (1.247mg/l) and 2.490

mg/l in digester II were within the GEPA maximum permissible level of 5.0 mg/l for discharge into water bodies or use for irrigation.

In practice, the study found that, digester (II) contained more metal (Cd, Cu, Pb) concentration than digester I. The reason might be due to the feed of digester II (fruit waste) coming from the fruit drying industry. This is because; metals are mostly associated with industrial organic and inorganic wastes products (Al-Seadi, 2001). The study also revealed that, Cd, Pb and Zn concentrations in the influents did not change significantly or vary in the effluents after anaerobic digestion. This agrees with Monnet (2003) who asserted that, heavy metals reduction in digestate/biogas effluent is not feasible during anaerobic digestion.

4.4 Pathogenic Indicator Organisms

Comparison of indicator/pathogenic organisms of input substrates (influent) and the corresponding digested product (effluent), revealed a decrease in indicator organisms for all the parameters analysed in digesters (I) and (II) after anaerobic digestion. Tables 4.6 and 4.7 present the means, standard errors and % reduction (%Red) of indicator organisms of digesters (I) and (II) during the study period. In the discussion Table 4.6 is referred to as digester I and Table 4.7 as digester II.

Table 4.6 Means, SE and % reduction of indicator organisms for Digester I-Human Excreta, Ashaiman.

Parameters	Unit	Influent	Effluent	% Red
TC	CFU/100ml	5,135,000 ±5000	1,909,000±1000	62.8
FC	CFU/100ml	3,635,000±15,000	1,295,000 ±5000	64.4
E. coli	CFU/100ml	2,350,000±50,000	925,000±5,000	60.6
Salmonella		6/25g sample	2/25g sample	66.7

Table 4.7 Means, Standard Errors and % Reduction of Indicator organisms of fruit waste, FRI.

Parameter	Unit	Influent	Effluent	%Red*
TC	CFU/100ml	37,980±265.3	18,380±330.8	51.6
FC	CFU/100ml	27,600±748.32	12,860± 492.56	53.4
E. coli	CFU/100ml	11,730±488.8	4,896±458.01	58.9
Salmonella		4/25g	1/25g	75%

%Red*- % Reduction

4.4.1 Total Coliforms (TC)

In digester I, mean TC in the raw human excreta was enumerated at 5,135,000 CFU/100ml with SE of 15,000 and 1,909,000CFU/100ml with SE of 1,000 in the anaerobic digested effluent, representing about 62.8% reduction in TC bacteria. In digester II, mean TC in the influent (pineapple and mango peels slightly mixed with slaughter house waste) were enumerated at 37,980CFU/100ml with SE of 265.3. After the process of anaerobic digestion, TC bacteria recorded a mean reduction of 18,380CFU/100ml with SE of 330.8 in the digested effluent representing a percentage

of 51.6. The presence of TC in digester II might be largely due to the slaughter house waste.

The results of digester I and II showed a significant reduction in TC bacteria in digester I (62.8%) than digester II (51.6%). Despite the reduction, TC in the effluents of digesters I (1,909,000cfu/100ml) and II (18,380cfu/100ml) were above GEPA maximum permissible level of 400 CFU/100ml for discharge into water bodies and WHO (2006) guideline of ≤ 1000 for unrestricted irrigation (that is crops likely to be eaten uncooked, sports fields or public parks) or ≤ 10^5/100ml for restricted irrigation (that is irrigation of cereal crops, industrial crops, fodder crops, pasture and trees).

4.4.2 Fecal Coliform (FC)

The mean FC enumerated in digester I recorded 3,635,000 CFU/100ml with SE of 15,000 in the raw influent (human excreta). After the anaerobic digestion process, this decreased from 3,635,000 CFU/100ml to a mean of 1,295,000 CFU/100ml with SE of 5,000) in the digested effluent, representing a percentage reduction of 64.4. In digester II, FC in the fruit waste was enumerated at a mean of 27,600 CFU/100ml with SE of 748.32. After the anaerobic digestion process, the effluent was found to contain 12,860 CFU/100ml with SE of 492.56 in the effluent, representing a percentage reduction of 53.4. The results of the study showed more than half reduction of FC in digester I (64.4%) and II (53.4%). Despite this reduction, the effluent quality in terms FC did not meet the required acceptable standards of 10^3- 10^6 CFU/100ml set by the WHO (2006) for use in agriculture and aquaculture.

4.4.3 Escherichia coli

The mean *E. coli* enumerated in the influent (raw human waste) of digester I was found at 2,350,000 CFU/100ml with SE of 50,000. After anaerobic digestion, the study recorded a reduction from 2,350,000 CFU/100ml to 925,000 CFU/100ml in the effluent after anaerobic digestion representing 60.6%. In digester II, *E. coli* in the raw fruit waste were about 11,730 CFU/100ml with SE of 488.8. After the process of anaerobic digestion, there was a reduction from 11,730 CFU/100ml to 4,896 CFU/100ml with SE of 458.01 in the effluent. Both digesters recorded more than half reduction of *E.coli*. Nonetheless, the mean reduction in digester I (925,000 CFU/100ml) and 4,896 CFU/100ml in digester II were above WHO (2002) guideline 10^3 *E. coli*/100ml for use in irrigation of leafy crops or aquaculture. Moreover, digester II effluent *E. coli* was within 10^5-10^6 /100ml suitable for drip irrigation, highly merchandised agriculture and root crops (WHO, 2006).

In practice, TC, FC and *E.coli* as indicator organisms were reduced after anaerobic digestion but not significant enough to meet maximum allowable standards. This might be due to both digesters being subjected to operate under mesophilic conditions and very short HRT to generate more gas. This finding has been buttressed by other researchers including Song *et al*. (2004) and (PF" Fluid, 2010) that short HRT together with mesophilic temperature does not inactivate pathogens completely in an anaerobic digester.

4.4.4 *Salmonella Species*

The absence of *Salmonella* in 25g of effluent or waste is considered the standard for its safe use as fertiliser as a guarantee of bacteria/pathogen absence (Biological Treatment of Biowaste, 2001). However, the results of the study found that *Salmonella* was detected in both influent and effluent of the five samples analysed. In digester (I), about 6.0/25g *salmonella* were enumerated in the influent. This decreased from 6.0/25g to 2/25g in the effluent, representing 66.7% reduction.

Also, in digester II, 4/25g *Salmonella species* were detected in the influent of the fruit waste. As a result of the digestion process, 1/25g *salmonella* was detected in the effluent, representing 75% reduction. The resistance of *Salmonella* to be inactivated completely in both digesters might have been due to the short hydraulic retention time as mentioned above adopted at the project sides to maximize energy production at a lower cost.

4.5 Estimated Volume of Effluent from digester I and II and Technical Design of Anaerobic Baffle Reactor

The estimated volume of digester I effluent in Ashaiman was 120 litres/day and assuming hydraulic retention time of 100days in an Anaerobic Baffle Reactor (ABR) as shown in Figure 4.1, a digester volume of 12 m^3 will be required to receive the required volume of effluent. At the FRI, 150 litres/day of effluent was estimated and assuming 100 days HRT, a digester volume of 15 m^3 is required.

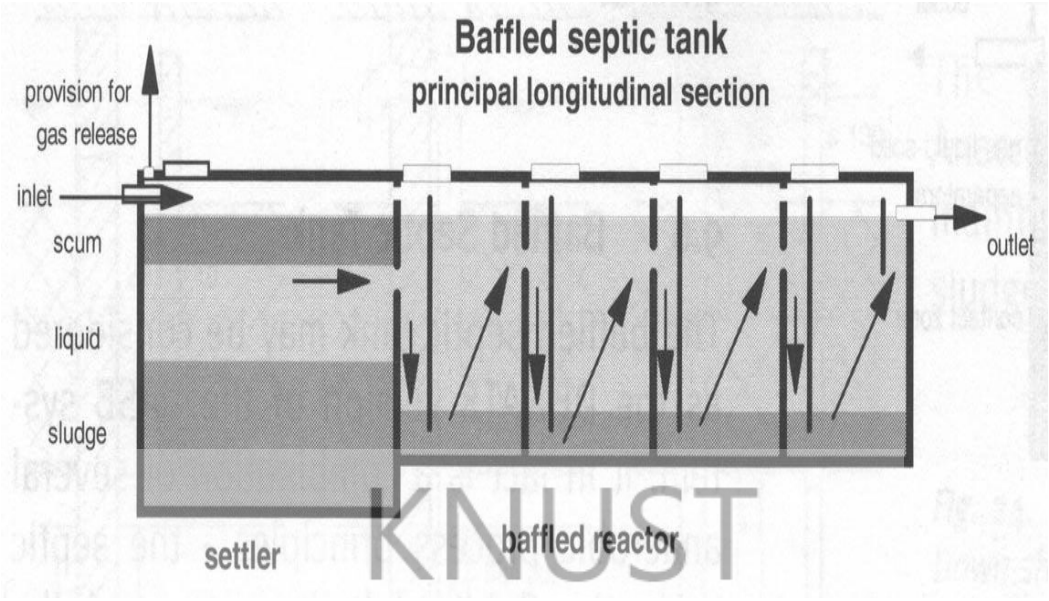

Figure 4.1 Design Principle of Anaerobic Baffle Reactor

In anaerobic baffle reactor, wastewater circulates in various chambers and is retained for further decomposition resulting in BOD, COD, inhibitory substances and pathogens up to about 90% (Wang *et al.*, 2004).

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Basically, the study revealed that the anaerobic digestion process with the adopted short HRT at the two project sites did not cause complete reduction of pathogenic indicator organisms (TC, FC, and *E.coli*) and pollution monitoring indicators (TS, VS, BOD and COD) but plant macronutrients(N,P,K) were substantially increased in both digesters. In digester I, reduction of TS in the effluent was 61.8% and 58.7% in digester II. Likewise, BOD in the digested effluent of the human excreta was higher (65.9%) in digester I than the digested fruit waste in digester II (48.7%). Reduction in COD was higher (65.9%) in the digested fruit waste in digester I than the effluent from the human excreta in digester II (58.6). In general, TS, VS, BOD and COD were all lower in the effluents of both digesters I and II with respect to the influents.

In terms of macronutrients, the study found NH_4-N (inorganic nitrogen up to 25.1%) in the effluent of the human excreta in digester I and 19.5% in digester II. An increased availability factor of phosphate content (water soluble fraction) was also realized. However, orthophosphate portion of total phosphorus was higher in the effluent of human excreta in digester I than in digester II. The study did not find heavy metals above the recommended maximum allowable limits set by the GEPA in both digesters. Nevertheless, metal concentration in the fruit waste effluent was higher than the effluent from the human excreta.

In sum, the study found that the reduction of BOD, COD and pathogenic indicators did not meet acceptable international standards hence further reduction of these

indicators is needed to make the effluent safe for reuse or disposal onto the environment. Usually a total of HRT of 100- 120 days is needed to reduce pathogens and pollution indicators to meet international acceptable standards (Werner *et al.*, 1989).

5.2 Recommendations

The following recommendations are made based on the findings above to reduce further BOD, COD and pathogens to the required level for safe utilisation or disposal.

1. The effluent compartment of both digesters should be extended by an Anaerobic Baffle Reactor (ABR) as shown in Figure 4.1 to allow the effluents to circulate in various chambers within 100 - 120 days for further decomposition and complete removal of BOD, COD and pathogens. It is therefore recommended that:

- The effluent receiving compartment of digester I should be extended by a 12 m^3 ABR.
- The effluent receiving compartment of digester II should be extended by a 15 m^3 ABR.

2. After further processing in the ABR for 100 or 120 days the effluent can be used by farmers close to the digester to

- Irrigate onions and leafy vegetables in the case of Ashaiman
- Fertilise fish ponds to improve fish yield.
- To irrigate lawns to maintain the landscape or pastures to feed animals

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APPENDIX

Appendix 1: Reagents for TKN analysis:

- Sulfuric acid of density = 1.84 kg /l
- Potassium sulfate catalyst mixture (200 g of potassium sulfate, 20 g of copper sulfate pentahydrate and 20 g of titanium dioxide was thoroughly ground and mixed with crystal structure of anatase).
- Sodium hydroxide, c (NaOH) = 10 mol/l
- Boric acid solution of density = 20g /l
- Mixed indicator(0.1 g of bromocresol green and 0.02 g of methyl red was dissolved in 100 ml ethanol)
- Sulfuric acid, c (H+) = 0.01 mol/l
- Ammonium sulfate NH_4SO_4

Appendix 2: Reagents for NH_4-N

- Potassium chloride c (KCl): 1 mol/litre (373 g of potassium chloride dried at 105°C was dissolves in approximately 3 litres of water and diluted to 5 litres with water)

Appendix 3: Reagents for Total Phosphorus

Hydrochloric acid, c (HCl) = 12 mol/l, ρ ≈ 1.18 g/ml, about 37 %

Nitric acid, c (HNO3) = 15.8 mol/l, ρ ≈ 1.42 g/ml, about 65 %

Nitric acid, about 1% (V/V):(10 ml of nitric acid was diluted to 1 litre in a flask with water)

Boiling aids: glass beads were used.

Appendix 4: Reagent for Potassium

- Deionized (DI) water < 0.056 of Conductivity at 25^{o}C/µS • cm-1 was used
- Nitric Acid (HNO_3), concentrated, trace metal grade.
- Hydrogen Peroxide (H_2O_2), 30% ACS Reagent
- Method blank solution. Digestion solution (6 mL concentrated HNO_3, 2 mL 30% H_2O_2, 92 mL deionized water)

Appendix5 Calibration of pH meter with buffer solution

- Potassium hydrogen phtalate ($C_8H_5O_4K$) at pH 4.00, and
- Disodium tetraborate decahydrate ($Na_2B_4O_7$**.** $10H_2O$) at pH 9.22.
- Distilled water with specific electric conductivity not greater than 0.2 mS/m at 25^{0}C and a pH not greater than 5.6.

Appendix 6: Means of the twin biogas plant at Ashaiman

Means and SE of influent and digested effluent of HE in a twin biogas plant at Ashaiman, Greater Accra

Parameters	Mean(s) and SE				% Change	
	Digester A		Digester B			
	Influent	Effluent	Influent	Effluent	A	B
TS%	13.7±0.24	5.3±1.28	13.8±0.28	5.2±0.19	61.3	62.3
VS%	76.2±0.34	35.5±1.68	76.1 ±0.39	38.1 ±2.07	52.9	50
COD%	13868±19.3	5790 ±18.7	13870±33.9	5798 ± 35.4	58.2	58.9
BOD%	12520±19.9	4240±50.9	12400±44.7	4260 ±50.9	66.3	65.6
pH	5.8 ±0.03	7.3±0.06	5.7±0.11	7.2±0.06	20	21.9
EC(ms)	6.4±0.057	4±0.098	6.3± 0.058	3.9±0.10	37.5	38.1
Turbidity(NTU)	149.7±2.45	98± 3.9	143.3±2.4	99.4±1.5	33.3	32.3
***TN(mg/L)**	14.4±0.29	16.1±0.37	13.9 ±0.25	16.2±0.39	10.5	14.2
***NH_4-N(mg/L)**	10.6 ± 0.16	14.0 ± 0.27	10.74 ±0.14	14.5 ± 0.33	24.3	25.9
NH_4-N(%TN)	73.6	86.9	77.3	89.5		
***P_2O_5 (mg/L)**	1.86± 0.017	1.91±0.011	1.88±0.009	1.92±0.002	2.08	2.6
Ortho (P_2O_5)	1.04±0.005	1.24±0.0037	1.05±0.006	1.27±0.005	17.3	16.1
Ortho P_2O_5(%t)	55.9	66.1	55.9	64.9		
***K_2O (mg/l)**	2.34±0.049	3.15 ±0.0048	2.86±0.048	3.79 ±0.068	42.9	44
Cu	-	-	-	-		
Cd	0.0245	0.0251	0.0249	0.0254		
Pb	0.0034	0.0035	0.0035	0.0035		
Zn	1.195	1.247	1.195	1.247		

* **Values are in multiple of 10^3**

Appendix 7: Effluent storage compartment of the twin digester in Ashaiman, covered with roofing sheets to prevent ammonia loss to the atmosphere