ANAEROBIC CO-DIGESTION OF FAECAL SLUDGE WITH PAPER OR FRUIT WASTE FOR BIOGAS PRODUCTION UNDER MESOPHILIC AND AMBIENT TEMPERATURES: A CASE IN KUMASI, GHANA.

By

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Science award and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment is made in the thesis

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DEDICATION

To my family and friends

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ABSTRACT

Increasing attention is being given to biogas generation through anaerobic digestion (AD) all over the world due to concerns over global warming, security of energy and the need for sustainable waste management. Very few areas in Ghana are sewered, the situation is no different in Kumasi where most of the people rely on onsite sanitation systems. Faecal sludge removed periodically from these sanitation systems is further treated in waste stabilization ponds at the Oti treatment facility. However, increasing population and thus increasing volume of sludge being brought to the facility has led to operational difficulties of the facility. This study therefore sought to assess the feasibility of using anaerobic co digestion (AcoD) as an alternative means of treating the faecal sludge brought to the treatment facility before discharging into the environment. By analysing the pH, TS, VS, COD, TP, TKN and helminth eggs according to standard protocols, the physicochemical and microbiological properties of the sludge were investigated. COD: TKN ratios were calculated to assess the suitability of the sludge for anaerobic digestion. Laboratory scale batch AD tests were performed both at mesophilic (35^{o}C) and ambient conditions (24-32^{o}C) to assess the biogas production, the impact of the two temperatures on the treatment, and the effect of sludge retention times on biogas production and sludge stabilization. The efficiencies of the treatment processes were then assessed by computing the percentage removal of TKN and COD from the sludge. Anaerobic co digestion with paper and fruit waste enhanced the faecal sludge characteristics and improved effluent characteristics. The faecal sludge brought to the treatment facility during the period of this study had the following characteristics: pH of 7.02 ±0.2, VS of 65.75 ± 10.98 as %TS, COD of 23050 ± 681.50 mg/L, MC of 98.57 ± 0.84%, TKN of 2842 ± 1094.53 mg/L, and COD: TKN of 8.11. Statistical analysis suggested difference (p-value of 0.003) in the biogas yields under the two temperature regimes however further analysis indicated that the difference was only in the first week. The digesters were filled with faecal sludge and paper or fruit waste in the ratio 1: 0.13. The results obtained in this study showed a total biogas production and weekly methane yield as percentage by volume for Inoculum (I) only, faecal sludge and inoculum (FI), faecal sludge plus inoculum and paper (FIP) and faecal sludge plus inoculum and fruit waste (FIFW) to be 635 (10 – 16.5%), 830 (18.9 – 23.7%), 1355 (16.5 – 47.05%) and 1760 (26.1 – 39.75%) ml respectively under mesophilic temperature and 1085 (0 – 15.5%), 1110 (3.8 – 17.7%), 1515 (14.3 – 41.1%) and 1875 (19.5 – 45.7%) ml respectively under ambient temperature. Effluent characteristics were better in the 4th week for all the digesters indicating that longer sludge retention times has a positive impact on anaerobic digestion.

TABLE OF CONTENS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACRONYMS AND ABBREVIATIONS

AD	-	Anaerobic digestion
C/N	-	Carbon to nitrogen ratio
COD	-	Chemical Oxygen Demand
EPA	-	Environmental Protection Agency
FI	-	Faecal sludge plus inoculum
FIP	-	Faecal sludge, inoculum and paper
FIFW	-	Faecal sludge, inoculum and fruit waste
FS	-	Faecal sludge
HE	-	Helminth eggs
HRT	-	Hydraulic retention time
I	-	Inoculum (cow dung)
JMP	-	Joint Monitoring Program
KMA	-	Kumasi Metropolitan Assembly
MC	-	Moisture content
OLR	-	Organic loading rate
SDG	-	Sustainable Development Goal
TKN	-	Total Kjeldahl nitrogen
TP	-	Total phosphorus
TS	-	Total solids
VFA	-	Volatile fatty acid
VS	-	Volatile solids
WHO	-	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Faecal sludge comprises of all contents (liquid and semi-liquid) of pits and vaults that accumulate in on-site sanitation systems, i.e. in public and private latrines or bathrooms, aqua privies and septic tanks not linked to sewers. Normally, these liquids are concentrated more than wastewater in suspended and dissolved solids (EAWAG and SANDEC-IWMI, 2003). Every year, huge amounts of faecal waste are being produced by humans globally; estimated at 127 kg per person per year (Asl and Hosseini, 2000). This quantity of waste poses an environmental threat with reference to greenhouse gas (GHG) emissions and pollution of water bodies if not properly managed (Miller et al., 2011).

Faecal sludge management is still a problem in most parts of Africa. In Ghana, only 18% of the total population of Ghana had access to basic sanitation facility as of 2017 (JMP 2000-2017 Report). Most of the urban Areas in Ghana including Kumasi are partly sewered, thus most parts of the country depend on onsite sanitation facilities. The sludge from these onsite facilities need to be further treated. The final treatment is mostly a problem as there are inadequate treatment facilities in the country (Oduro-Kwarteng et al, 2011).

Service delivery is not able to maintain pace with population growth and demand for services, as waste treatment is hardly provided any resources. Less than 5% of the houses in Accra and Kumasi are linked to piped sewer systems (water-flushed toilets joined to sewers) which are connected to waste treatment plants (Oduro-Kwarteng et al, 2011). Some urban residents discharge their faecal waste into septic tanks while greywater (kitchen and other wastewater) is channelled from home to neighbouring open drains. Most urban drains are open and therefore often serve as defecating channels for homes with insufficient sanitation

facilities. In 2006, 20% of all Ghanaian households practiced open defecation, reflecting the lack of toilet facilities in many homes (WHO/UNICEF, 2008).

The condition in the peri-urban regions is worse, often facing inadequate water supply and low access to sustainable fundamental sanitation. In these areas, the use of unimproved and shared sanitation facilities is common. In addition, there are cases where faecal sludge is disposed untreated straight into the environment (bush and water bodies). Such practices make faecal sludge management difficult (Oduro-Kwarteng et al, 2011).

With worries about these adverse environmental impacts, an effective use of faecal waste could be a useful alternative to existing treatment options. There is a serious need for an environmentally sound and sustainable energy source with the growing demand for energy supply. Previous studies have shown the potential of faecal waste in bioenergy production (Cantrell et al., 2008; Champagne, 2008; Kim et al., 2014; Kargbo, 2010). Biogas yields, however, vary from one faecal waste source to another depending on waste composition and microbial community structure (Wang et al., 2014; Farnworth et al., 1995; Li et al., 2015). Biogas produced from faecal waste containing methane could be accomplished through several procedures, including gasification, pyrolysis, and anaerobic digestion (Demirbaş, 2001).

Organic waste makes up a great portion of the waste stream of most societies. This can constitute a serious environmental issue if not properly managed (Cantrell et al., 2008). In many advanced countries, sustainable waste management is considered a top priority, waste prevention and reduction is encouraged. This contributes to efforts to reduce pollution and emissions of greenhouse gases as well as to alleviate global climate change. In most developing countries, including Ghana, sustainable waste management remains a problem (Appiah-Effah et al., 2014).

Anaerobic digestion is a natural biological process in which bacteria with little or no oxygen break down organic matter and release methane as one of the final products. The anaerobic process involves a diverse variety of micro-organisms and results in two main end products: biogas and digestate. Biogas is a flammable gas made up of methane, carbon dioxide, and small quantities of other gasses and trace elements while digestate is the decomposed substrate, enriched in macro and micro nutrients and therefore appropriate for use as a fertilizer for plants (Al-Seadi et al. 2008). Biogas production through anaerobic digestion (AD) converts organic substances into renewable energies and provides an organic fertilizer for agriculture. At the same time, the organic portion is removed from the total waste streams thus reducing the volume entering landfills (Al-Seadi et al. 2008). Anaerobic digestion significantly reduces the total amount of waste, produces solid or liquid fertilizer, and generates energy. However, low biogas (methane) generation of faecal sludge has been reported (Dohányos and Zábranská, 2001).

1.2 PROBLEM STATEMENT

Very few areas in Ghana are sewered, the situation is no different in Kumasi where most of the people depend on a shared toilet facility or rely on onsite sanitation systems including pit latrines, water closets with septic tanks, aqua privies among others. Faecal sludge removed periodically from these sanitation systems are sent for treatment in waste stabilization ponds at the Oti treatment facility.

Currently the high population coupled with high volumes of faecal sludge has led to operational difficulties of the facility. Therefore, the large amounts of faecal waste are not adequately treated before discharge into the environment and likely to cause human excreta-transmitted diseases like diarrheal, cholera, typhoid, hepatitis, polio, ascariasis, etc., that predominantly affect children and the poor (Thrift, 2007).

Faecal sludge has been known to have the potential of generating biogas, but due to differences in faecal sludge characteristics, lifestyle of users, choice of sanitation systems and variations in climatic conditions in different regions, one cannot assume the same results for faecal sludge from different areas (Arthur and Hammond. 2010). As a result of its reduced C / N proportion, according to Haq and Soedjono, (2010), the potential to produce biogas from faecal sludge alone is much smaller compared to other substrates. Therefore, the potential for co-digesting faecal sludge in Kumasi needs to be assessed. Although there are publications on anaerobic digestion of fruit waste. There is limited information for cases of such in Ghana. Similarly, no research documentation has been found for co digestion of faecal sludge with paper but paper has a higher C/N ratio compared to faecal sludge and this makes it suitable to enhance the characteristics of faecal sludge for anaerobic digestion. Therefore, knowing the effect of fruit waste and paper on faecal sludge in Kumasi will be of great importance.

1.3 JUSTIFICATION

The significance of excellent hygiene is undeniable because it is crucial to health and well-being. It provides a chance to save the lives of more than 1.5 million infants a year who would have diarrheal diseases and also enables many more to safeguard their health (WHO/UNICEF,2012). One of the ways to contribute to the realization of the of the sustainable development goal six is by looking for sustainable and environmentally friendly ways of faecal sludge treatment. There is therefore the need for a treatment mechanism to aid eliminating or reducing careless discarding of faecal sludge in the environment and promote reuse of untreated faecal sludge.

This research seeks to explore the use of Anaerobic co digestion as an alternative means of treatment and energy recovery from the faecal sludge brought to the Oti faecal sludge

treatment plant. Anaerobic co digestion improves the characteristics of faecal sludge for better biogas production (Esposito, et al., 2012).

Since the AcoD process has potential for resource recovery (Nghiem et al., 2017), findings from this research will provide data on the methane recoverability of the faecal sludge in Kumasi. The findings from this study will aid stakeholders in making informed decisions when deliberating on options to be adopted for the FS treatment.

Assessing the degradability and bio methane potential of the faecal sludge in Kumasi would help ascertain if it can be used to generate biogas. This if confirmed would improve the wellbeing of the people of Kumasi and the nation at large.

Research questions

The study results are aimed at answering the following research questions:

1. What are the characteristics of the faecal sludge for anaerobic digestion?
2. How does co digestion enhance the bio-methane yield?
3. What is the effect of retention time on sludge digestion and biogas production?
4. What is effect of mesophilic and ambient temperatures on stabilization of the substrate?

1.4 OBJECTIVES

The main objective of the research is to evaluate the degradability and bio methane production of faecal sludge discharged at the Oti faecal sludge treatment plant

1.4.1 Specific objectives:

1. To determine the characteristics of the faecal sludge.
2. To enhance the faecal sludge characteristics for anaerobic digestion using paper or fruit waste

3. To determine effects of retention time on sludge digestion and biogas production
4. To study the effect of mesophilic and ambient temperatures on stabilization of the Waste

1.5 SCOPE OF RESEARCH

This study was limited to anaerobic co digestion of faecal sludge in Kumasi with fruit waste (a combination of pineapple, mango and pawpaw waste or paper (newspaper). The faecal sludge used in this study was obtained from the Oti faecal sludge treatment plant while the fruit waste and paper were obtained from local vendors in Kumasi. The Faecal sludge characteristics were determined by analysing the pH, TS, VS, COD, TP, TKN and helminth eggs according to standard protocols. COD: TKN ratios were calculated to assess the suitability of the sludge for anaerobic digestion. The experiment was a Laboratory scale batch AD test performed both at mesophilic (35^{o}C) and ambient conditions (24-32^{o}C) to assess the biogas production, the impact of the two temperatures on the treatment, and the effect of sludge retention times on biogas production and sludge stabilization.

1.6 STRUCTURE OF THESIS

The thesis is divided into five (5) chapters. Chapter one (1) deals with the introduction presenting the study's background and the problem addressed, as well as highlighting the study's goals, hypothesis and justification. The second chapter deals with the study's exploration of appropriate literature. This included the waste management review, the method of anaerobic digestion, and the substrates used for co-digestion. Chapter three (3) also describes the strategy and methodology used to conduct the research. The findings acquired and all the research goals discussed are described in chapter four (4). This chapter presents the findings in the context of anaerobic co-digestion of faecal sludge and interprets them. In

the last chapter, chapter five (5), the conclusions and recommendations from the results and discussions as well as from the literature review are presented.

CHAPTER TWO: LITERATURE REVIEW

2.1 WASTE MANAGEMENT

As a result of rapid urbanization and economic development in many developing countries, liquid and solid waste management has become a serious environmental concern, and as such there is a growing concern to find ways to tackle waste management sustainably. Municipal waste management is a major problem in the world today as it deals with local municipal budget allocations, government recognition and negative environmental effects (Ramakrishna, 2013). Open dumping, composting, soil filling, incineration, direct and indirect recycling are prevalent ways of coping with / disposing waste in most developing countries. Common practice in the management of liquid waste includes disposal of untreated liquid waste in oceans or open drains. the use of treatment options that are economically feasible, environmentally friendly and socially acceptable are limited because of variables such as high price of operation and management. These bad techniques result in various environmental problems, such as surface and groundwater pollution, soil and air pollution (Sawyer et al., 2003).

The current waste management practices in most countries leads to the emission of significant amount of methane gas into the atmosphere. In 2015, landfills, animal waste treatment, and wastewater treatment were respectively the third, fourth, and seventh biggest sources of methane emissions in the United States (Eheliyagoda, 2015). Also most governments do not prioritize finding long-run solution schemes with respect to safe waste disposal especially for landfill planning and construction and this makes treatment of waste in most developing countries still a challenge (Eheliyagoda., 2015).

2.2 ANAEROBIC DIGESTION (AD)

2.2.1 HISTORY OF ANAEROBIC DIGESTION

Anaerobic treatment is a widely used technique for industrial waste and heavily loaded wastewater treatment plants, such as agro-industries (Arthur and Hammond, 2010). As early as the 10th century B.C.E (Before the Common Era, i.e., B.C.) (Lusk, 1998), historical evidence from Assyria and Persia show the use of biogas for heating bathing water. Jean Baptiste van Helmont (as cited in Zullo, 2016) perceived the production of fuel gas as a result of the decomposition of organic matter in lakes in the Middle Ages. The City of Exeter, UK, used biogas from sewage to power street lamps in 1895 (Zullo, 2016). Later, Alessandro Volta also performed a series of studies on fuel gas gathered from marsh sediments, observing a direct correlation between degraded organic matter and gas generation (Ferry, 1993). In 1808, a scientist named Humphry Davy found that methane was generated by anaerobically digested livestock manure, which suggested the likelihood of generating fuel gas from manure (Lusk, 1998).

Anaerobic digestion's industrial uses began in 1859 with India's first anaerobic digestion plant. In the 1930s, Buswell and others recognized anaerobic bacteria and circumstances promoting the production of methane. Anaerobic digestion method was common in the area of waste treatment because it has many benefits such as high effectiveness of treatment and capacity to generate methane gas (Zullo, 2016).

Onsite anaerobic digestion of animal manure is commonly practiced throughout Asia with or without the addition of Faecal Sludge (Koottatep et al., 2004). China and India have now embarked on a trend towards bigger, more sophisticated farm-based systems with better process control to generate biogas energy. The technology is now being used for both municipal and industrial waste treatment. Taiwan has reduced river pollution through the

adoption of conventional AD technologies due to the immediate release of waste generated from animal husbandry (Koottatep et al., 2004). Recently, for two important reasons, Europe has been under pressure to explore the AD market due to high energy prices and strict environmental regulations to regulate organic matter entering landfills (Zullo, 2016).

2.2.2 PROCESSES OF ANAEROBIC DIGESTION

Anaerobic digestion is a series of processes in which organic materials are degraded by micro-organisms in the absence of oxygen to produce renewable biogas containing (CH_4), carbon dioxide (CO_2), for industrial or domestic purposes. This gas can be used as a source of energy (Cheng, 2010).

Controlled anaerobic digestion has the advantage of decreasing the emission of greenhouse gases into the environment and also prevent the contamination of water bodies and land pollution by diverting organic waste from the open environment. It also offers a clean source of energy that can be used instead of fossil fuels. The liquid effluent and the solid digestate from the AD process is rich are nutrients and can replace chemical fertilizers and serve as soil amendments (Cheng, 2010).

The anaerobic digestion process involves a number of micro-organisms that include bacteria forming acetic acid (acetogens) and archaea forming methane (methanogens). These microorganisms feed on organic waste and then go through various procedures that convert it into intermediate molecules, including sugars, hydrogen, and acetic acid, and lastly convert it into biogas. In anaerobic digestion, methanogenic bacteria release the bulk of the chemical energy contained in the original material as methane (Fergusen and Mah, 2006).

Anaerobic microorganism populations generally take a considerable time to establish themselves to be fully efficient. Various bacteria species can survive at various temperature ranges. Those capable of living optimally at temperatures between 35 and 40 ° C are known

as mesophilic bacteria and those capable of persisting at hotter and more hostile circumstances (55–60 ° C) are known as thermophilic bacteria. Therefore, it is prevalent practice to introduce anaerobic microorganisms from materials with current populations, a method known as "seeding" digesters, typically by adding sewage sludge or cattle slurry (inoculum). Anaerobic digestion of organic matter happens in a controlled setting in four phases where the organic matter is broken down by the lack of oxygen into biogas. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis are the four phases of AD (Cheng, 2010; Drapcho et al., 2008).

2.2.2.1. HYDROLYSIS

At the hydrolysis phase, organic long-chain polymers comprising fats, proteins and carbohydrates are broken down into smaller parts, such as amino acids and simple sugars, making them accessible for further degradation by acetogenic bacteria. The duration for hydrolysis is reliant on the type and complexity of the substrate being degraded. The hydrolysis phase may span from a few hours to days based on the substrate being used. However, according to (Yang *et al.*, 2010), enzymes such as amylase and protease could be added to the degradation process in a controlled manner to improve the hydrolysis. These enzymes increase the substrate degradability, reduces the digestion duration and reduces the volume of digestate produced. Equation 2.1 represents the general hydrolysis reaction.

$(C_6H_{10}O_5)\ n + nH_2O \rightarrow n(C_6H_{12}O_6)$ Equation 2.1

2.2.2.2 ACIDOGENESIS

Bacteria break down the amino acids and sugars made accessible through hydrolysis into hydrogen (H_2), carbon dioxide (CO_2), ammonia (NH_3), volatile organic acids and alcohols in the next phase, which is the acidogenic phase. Methanogens can directly use the acetate and hydrogen produced in the first stages. However, it is necessary to convert other molecules

such as volatile fatty acids (VFAs) into compounds that can be used directly by methanogens. The biological acidogenesis process occurs with a further breakdown of remaining fractions by acidogenic (fermentative) bacteria. Here, volatile fatty acids are created along with ammonia (NH_3) carbon dioxide and hydrogen sulphide as well as other by-products (Boone et al., 2006) Equations 2.2 to 2.4 are examples of reactions that occur during acidogenesis.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad \text{Equation 2.2}$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad \text{Equation 2.3}$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad \text{Equation 2.4}$$

2.2.2.3 ACETOGENESIS

The by-products from acidogenic stage are further transformed by acetogens (bacteria) through carbohydrate fermentation into hydrogen, carbon dioxide and acetic acid, compounds that can be utilized by the methanogens in the next stage. When the conversion is incomplete however, the process can also lead to the production of valeric acid, butyric acid and propionic acids (Demirbas, 2016). The microorganisms responsible for the products formed during acetogenesis include butyrate decomposers such as *Sytrophomonas wolfei*, propionate decomposers such as *Syntrophobacter wolinii,* and other acid producers such as *Clostridium* spp., *Lactobacillus, Actinomyces* and *Peptococcus anerobus* (Verma, 2002). These microorganisms have slow growth rates and function optimally at pH ranges of 4.0-6.5. Fluctuating digester loading rates can affect these microorganisms (Zhang *et al.,* 2005). Equations 2.5 and 2.6 shows acetogenic reactions

$$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad \text{Equation 2.5}$$

$$CH_3CHOHCOOH + H_2O \rightarrow CH_3COOH + CO_2 + 2H_2 \quad \text{Equation 2.6}$$

2.2.2.4 METHANOGENESIS

In the fourth and final phase of the digestion process, methanogenic archaea converts the by-products produced during the acetogenic phase into biogas, which mainly consists of CH_4 and CO_2, although other trace gases such as nitrogen gas and hydrogen sulphide (H_2S) may also exist, depending on the feedstocks used and the circumstances under which the digestion took place (Drapcho et al., 2008). Methanogenesis is susceptible to pH below 6.5 and above 8 as it occurs between pH 6.5 and pH 8 (Furgusen and Mah, 2006). The left over, non-digestible material that the microbes are unable to feed on together with any remains of deceased bacteria is the digestate. Equations 2.7 to 2.9 shows examples of methanogenic reactions

$$4CH_3COOH \rightarrow 4CO_2 + 4CH_4 \qquad \text{Equation 2.7}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{Equation 2.8}$$

$$4CH_3OH + 6H_2 \rightarrow 3CH_4 + 2H_2O \qquad \text{Equation 2.9}$$

The anaerobic digestion processes are summarised in figure 2.1 below

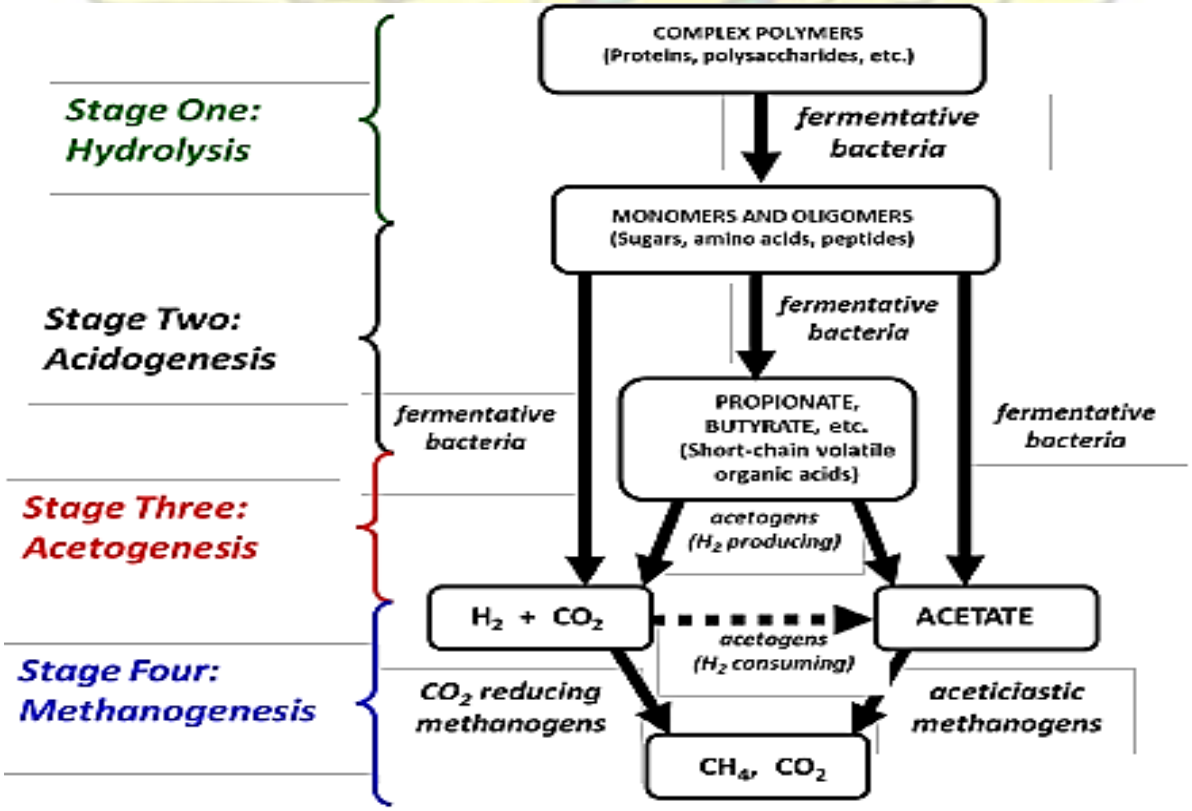

Figure 2.1: Anaerobic Digestion process (Hashsham, n.d.).

2.2.3 REACTOR SYSTEMS FOR ANAEROBIC DIGESTION

AD can generally occur under various types of reactor systems. Most of these systems can be divided into two groups: continuously fed and batch-fed. The primary distinction between these two is the digester's load rate. The digester is filled all at once in batch-fed systems with the waste to be degraded. The waste continues in the scheme until the end of the pre-specified digestion period during which the waste is removed and the reactor is refilled while the waste is added to the digester at pre-specified times in the continuously-fed units and older pre-treated waste is removed as waste is added. Most large-scale industrial digesters work in a continuously fed mode as it allows the digester to generate biogas continuously (Cheng, 2010).

2.2.4 CONDITIONS THAT AFFECT THE ANAEROBIC DIGESTION PROCESS

Various conditions are important to enable a stable anaerobic process. These conditions can affect the progression of the AD process, some of which include: pH, temperature, moisture content, organic loading rate, and retention time.

2.2.4.1 pH

There are distinct optimum pH ranges for distinct microorganisms. In addition to affecting the development rate of microorganisms, pH may influence other variables such as compound dissociation (ammonia, sulphide, organic acids, etc.) that are very essential to the anaerobic digestion system. The pH of the digester should be at a comparatively neutral level Drapcho et al. (2008). Methanogenic bacteria are extremely susceptible to differences in pH and require a variety of 6.7-7.4 to keep digester stability. Al-Seadi et al (2008) quotes a slurry pH between 5.5 and 8.5 as suitable for formation of methane. If the pH deviates from the ideal range, the activities of bacteria in the medium is highly impaired, resulting in low gas yields, less quality gas composition (more CO_2 content) and obnoxious odour (from the

production of H_2S) (Werner et al., 1989). pH buffers may be essential in keeping a neutral pH within the digester. Lime, ammonia, sodium hydroxide, soda ash, ammonium bicarbonate, and sodium bicarbonate are commonly used pH buffers (Liu et al., 2010).

2.2.4.2 Temperature.

AD system temperature is one of the most important parameters that can hamper anaerobic digestion success. In a digestion system, the temperature determines whether a certain type of microorganism in the reactor can survive or work well. Microbial groups are different at different temperature ranges The three basic temperature ranges within which anaerobic digestion can occur are psychrophilic (10-20 ° C), mesophilic (20-40 ° C) and thermophilic (40-60 ° C) ranges (Cheng, 2010).

A steady temperature is a significant consideration for a microbial consortium's survival because it can only withstand very small changes in temperature once it has adjusted to a certain temperature. A temperature shift just above or below the optimum can lead to a dramatic reduction in the growth rate of microbes (Madigan et al., 2003).

Wei et al., (2010) observed that the thermophilic temperature (55 ^oC) production of biogas was more than twice the psychrophilic temperature (15 ^oC) output. In addition, organic breakdown of nitrogen and phosphorus uptake increased as the temperature rates also increased (Sánchez et al., 2001). Thermodynamics indicates that greater temperatures are useful to endergonic responses (e.g., propionate breakdown into acetate, CO_2, H_2) (Appels et al., 2011). Temperature may also influence passive solid division, which is shown to be better under thermophilic than under psychrophilic circumstances (Kaparaju et al., 2008). When anaerobic digestion is performed at higher temperatures, many benefits are noted, such as greater conversion rate, thus better yield of methane, better impact of pathogen decrease and shorter retention time than when performed at lower temperatures (Ahring et al., 2001).

2.2.4.3 Moisture content

The digester's moisture content is a measure of the influent's solids content. In wet fermentation schemes, the complete solids of the influent are generally retained at 2%-10% (Drapcho et al., 2008). On the other hand, dry fermentation systems can operate with a content of slurry solids as high as 30% -40% (Liu et al., 2010). House (2010) and Leckie et al (1981) recommend that to facilitate digestate mixing and pumping, the total solids of the influent should be between 7%-9%.

Qu et al (2009) researched the impact of anaerobic methanation on moisture content. It was found that as moisture content increases, the methane production rate also increases. For a moisture content of 80%, the cumulative methane production increased by 60%. This increase was explained by the fact that cellulosic waste with high moisture content has an increased area of contact between the microbes, enzymes, and the substrates. An increase in attachment area enhances waste methanation and hydrolysis process.

2.2.4.4 Agitation or Stirring

Stirring is the process of causing turbulence to the slurry in a digester. There are basically two methods of stirring namely passive and active agitation. The passive agitation occurs when fresh feedstock is fed into the digester as well as by the up-flow of gas bubbles while active agitation is causing turbulence in the digester by using manual, mechanical, hydraulic stirring equipment (Al-Seadi et al., 2008).

Stirring is very important to ensure optimum operation of anaerobic digestion systems. When effectively done, stirring increases the rate of biogas production by 10 – 15% (House, 2010) and 50% in some cases (Al-Seadi et al., 2008). Stirring also tends to: prevent scum layers from forming, bring the micro-organisms into contact with the fresh particles of feedstock,

promote the up flow of gas bubbles and homogenize the distribution of heat and nutrients throughout the entire substrate mass (House, 2010).

2.2.4.5 Organic loading rate (OLR)

The organic load rate (OLR) is a measure of the daily amount of digestible materials entering the digester. The OLR can drastically affect the steadiness and the pH of the reactor. As new substrates are added to the digester, the acid-forming bacteria in the system breaks it down into volatile acids, which the methanogenic bacteria will further transform into biogas. There can be build-up of volatile acids in the system if the OLR is too high and not being able to be converted as quickly into biogas by the methanogens. When this happens, the pH of the system will be lowered, “souring” it and possibly killing the methanogens and stopping the reaction (House, 2010).

2.2.4.6 Retention time.

Retention time is the time it takes for the feedstock to remain in the digester before leaving the effluent system. Digester temperature generally influences the retention time. It is normally necessary to retain 40-100 days for psychrophilic digesters, 25-40 days for mesophilic digesters, and 15-25 days for thermophilic digesters (Drapcho et al., 2008). Higher retention time needs bigger installations for continuously-fed devices, as the digestate must stay for longer periods of time in the digester. It may therefore be economically advisable to operate bioreactors at higher temperatures, so as to limit the size of the digester to be constructed. The disadvantage though is the high energy input required to run the systems at higher temperatures (Drapcho et al., 2008).

2.2.4.7 Toxicity

Toxic substances like antibiotics, disinfectants, pesticides, detergents and chlorinated hydrocarbons such as chloroforms and other organic solvents can also inhibit the anaerobic

digestion process by affecting the metabolic activities of the bacteria thus resulting in lower gas yields (Fulford, 2006).

The sources of toxic substances include: substrates prepared from vegetables and fruits sprayed with pesticides and insecticides, cow dung of cattle that has been given or injected with antibiotics and detergents used for cleaning toilets from which substrates are taken (House, 2010). Some inhibitors may also be the by-products of the stages of the anaerobic digestion process, such as ammonia, which is a prevalent inhibitor of anaerobic digestion, and its slurry concentration can possibly rise with increased system temperature (House, 2010). Another by-product which can inhibit anaerobic digestion is volatile fatty acids. A high volatile-acid concentration at a lower pH value below 6.2 becomes harmful to methanogenic bacteria (Hoerz et al., 1999).

2.2.5 Characteristics of Feedstock or substrates

Most organic waste can undergo anaerobic digestion. These organic waste (feedstock) may include faeces from humans and animals, wastewater, garden / yard waste, food waste, oils, fats, etc. Some industrial waste or wastewater, such as wastewater from breweries and paper mills, can also be digested anaerobically (House, 2010; Leckie et al., 1981). The composition of biogas generated varies depending on the type of feedstock used. Feedstocks that has high carbohydrate tend to produce biogas with high CO_2 concentration.

Any organic material containing food substances such as carbohydrates, fats or proteins can be anaerobically degraded to produce biogas. The rate and efficiency of the digestion is however dependent on its physical and chemical structure (Fulford, 2006). Consequently, the rate of production and quality of the biogas generated does not depend only on the conditions of the process but also on the nature or type of the feedstock used for the anaerobic digestion (Bagudo *et al.*, 2011).

The frequently used parameters for characterization of Feedstocks for AD include: percentage total solids (%TS), percentage volatile solids (%VS), pH and the carbon-to-nitrogen (C/N) ratio (Leckie et al., 1981; Drapcho et al., 2008).

2.2.5.1. Total Solids (Dry Matter)

Total solids (TS) is the quantity of a substrate's dry matter (DM) left after removal of moisture by heating it to 105 ° C for 24 hours. TS is one of the ways in which the substrate concentration being fed into a digester can be measured (Lohri, 2009) and it is also used as one of the standard units of measuring the biogas generation potential of a substrate (Clemens, 2010). According to Nizami and Murphy, (2010), there are three primary substrates depending on their total solid content: wet or small solids (LS) substrates containing less than 12% TS; medium solids (MS) substrates containing 15-20% TS and dry or high solids (HS) substrates containing 22-40% TS.

2.2.5.2 Volatile Solids (Organic Dry Matter) and Fixed Solids

Volatile solids (VS) or organic dry matter (ODM) is the measure of the fraction of dry matter lost when the dry matter is burned at 500 ° C or 600 ° C while the fixed solids (FS) are the remaining ash after burning. FS includes mostly soil particles, inert vegetable parts, and some solid carbon left from food decomposition. Consequently, Monnet (2003) described volatile solids as organic matter in a sample measured by deducting the ash content from the total solids content obtained through the complete combustion of the feedstock.

For the most part, volatile solid is expressed as a percentage of total solids. It is an estimate of the total solid part or a digestible substrate (House, 2010). VS helps to determine the slurry concentration placed in a digester and the amount of biogas generated from the slurry weight per unit placed in a digester. Cow dung's volatile solids content for example is generally 80% of its total solids (Fulford, 2006).

Substrates with ODM content below 20% are used in wet digestion, whereas those with ODMs above 35% are used in dry digestion (Al-Seadi et al., 2008). Some substrates having ODM below 20 percent include animal slurries and manures and moist organic waste from food industries, while those with ODM that are greater than 35 percent include energy crops and silages.

2.2.4.3 Carbon to Nitrogen (C/N) Ratio

A substrate's C / N ratio is a test of the amount of carbon atoms in a substance divided by the number of atoms of nitrogen (House, 2010). The C/N ratio of a substrate fed into a digester affects its biogas production potential and methane content (Sasse, 1988). The carbon component of the substrate is the portion that is converted to methane in the process while the nitrogen component provides energy for the bacteria to carry out the process efficiently (Amenorfe, 2013). If the C/N is very low, the anaerobic digestion process slows down or stops due to the formation of ammonia which is toxic. High C/N ratio on the other hand slows the rate of methane formation and increases the content of organic acids resulting in increased pH of the process (Al-Seadi et al., 2008). Most researchers give C/N range of 25:1 to 40:1 as the best range within which anaerobic bacteria thrive well (Amenorfe, 2013) with the optimum value varying based on the substrate (Hoerz *et al.*, 1999).

The C/N ratio differs from substrate to substrate. Organic materials rich in carbohydrates (rice husks) have high carbon content but low nitrogen while those rich in protein are rich in nitrogen. Combinations of nitrogen-rich material (e.g. manure from poultry, faecal sludge) and carbon-rich material (e.g. rice husks, paper) produce elevated levels of biogas (Sasse, 1988).

The total solids, volatile solids content and C/N ratio of some organic materials are presented in table 2.1 below.

Table 2.1: Total solids (TS), volatile solids (VS) content and C/N ratio of some organic materials

SUBSTRATE	TS (%)	VS in TS (%)	C/N Ratio	REFERENCE
Cattle dung	25 – 30	75 – 85	20 – 35	Zupanic and Grilc, 2012, Fulford, 2006
Human Faces	14 – 22	79 – 84; 93	6 – 10	Chaggu, 2004, Nwaneri et al, 2008, Fulford, 2006
Pig manure	20 – 25	75 – 80	14	Zupanic and Grilc, 2012, *House*, 2010
Vegetable waste	5 – 20	76 – 90	11 – 19	Zupanic and Grilc, 2012, *House, 2010*
Fruit slurry	4 – 10	92 – 98	8	Zupanic and Grilc, 2012
Chicken manure	10 – 29	67 – 77		Zupanic and Grilc, 2012, Fulford, 2006

2.2.4.4 Physical Nature of the substrate

The physical nature refers to the sizes of particle in the substrate. Although the physical nature of the substrate does not affect its ultimate biogas production potential, it affects the rate at which the biogas is produced. Sasse et al. (1988) says that a substrate's gas yield is high when the content of organic matter is high and the ratio of C / N ranges from 20: 1 to 40: 1. The rate of gas production, however, depends on the substrate's physical properties and temperature (optimum at 35 ° C). It requires longer to digest dry and fibrous material than

fine-structured and moist substrates. Consequently, the physical nature of the substrate also affects its hydraulic retention time (Wellinger, 1999). In instances where fruit wastes and other municipal solid waste are used, size reduction is mostly needed to speed up the decomposition process (Monnet, 2003)

2.2.4.5 Percentage of Water added to substrate

The quantity of water added to a substrate to form slurry is essential in biogas production especially in the operation of simple continuous-fed plants. According to House, (2010) adding water to the substrate makes it less difficult for the methanogenic bacteria to interact with the feed material thus hastening the digestion process and increasing the rate of biogas production. In addition, adding water to the substrate makes it easier for stirring and it facilitates the uniform distribution of bacteria in the digester.

However, adding too much water to the substrate reduces the effective volume of the digester and encourages the formation of scum (House, 2010). It is therefore important to add the right quantity of water to the substrate in order to maintain the right amount of solids in the system. The recommended solids content in slurries (especially in the case of simple continuous-fed plants) is 5 to 10% (House, 2010), making the water content of the slurry to be 90 to 95%. Fresh livestock manure, for instance, consists of 16% solids and 84% water. The prepared fermentation slurry had a solid content of 8% and a water content of 92% (Sasse, 1988) when the livestock dung was mixed with water in the proportion of 1:1.

2.2.6 ANAEROBIC CO-DIGESTION

Anaerobic co-digestion (AcoD), is the combined anaerobic digestion of two or more substrates, AcoD helps to overcome the limitations of digesting only one substrate such as low biogas production, and improves the economic viability of anaerobic digestion plants

because it promotes higher biogas production compared to that from a single substrate (Esposito, et al., 2012).

Different writers researched the co-digestion of various organic substrates in the past and the findings suggested a synergistic impact of the mixed treatment as the biodegradability of the resulting blend was greater than the biodegradability of the individual substrates when investigated individually. In particular, the mixing of different substrates with appropriate percentages of each fraction may result in the production of a mixture with a ratio of carbon: nitrogen (C / N) falling within the optimal 20:1-30:1 range (Hawkes et al., 1980). Benefits of the co-digestion are greater biogas and energy production and the decreased amount of solid waste to be disposed since a higher percentage of the substrate is biodegraded to produce biogas (Tchobanoglous et al., 1993).

It is important to a co- substrate that blends ratios of the substrates to promote positive interactions, minimize inhibitory and/or toxic compounds, optimize methane production and preserve stability of the resulting digestate (Astals et al., 2011). Mata-Alvarez et al., (2011), observed that fruit waste is an ideal co substrates for sewage sludge because of the high amounts of easily degradable organic matter in the fruit waste, since the sewage sludge substrate is characterized by relatively low carbon-to-nitrogen ratio.

Many kinds of organic waste such as sewage sludge, industrial waste, slaughterhouse waste, fruit and vegetable waste, manure and agricultural biomass have been successfully digested either individually or in processes of co-digestion (Murto., et al 2004). Substrate with greater C / N ratios (> 50), such as rice and wheat straws, cornstalks, seaweeds, paper and algae, can be co-digested with substrates with lower C / N ratios, such as pig manure, poultry manure and food and kitchen waste, to attain nutrient equilibrium and to prevent inhibition and thus system instability and decreased biogas output (Hagos et al., 2017).

Although co-digestion of substrates, such as poultry manure and kitchen waste with low C / N ratio with those of higher C / N ratio, such as paper and agricultural waste including rice and wheat straw, is a solution to adjust its ratio to the optimum level, the existence of lignocellulosic material can lead to low biodegradability and prolonged retention time (Kim et al., 2006).

Such materials require pre-treatment techniques in order to speed up the hydrolysis, which is the rate-limiting step in the anaerobic digestion process. The main purpose of the pre-treatment is to increase the solubilisation of substrates by breaking down complex substrates, such as lignin in lignocellulosic substrates such as paper, to accelerate the rate of hydrolysis (Esposito et al., 2012).

The impact of using waste paper (WP) as co-substrate for microbial biomass (MB) on methane production was researched by Rodriguez et al (2017). Their research was designed to explore the effect on methane production of the mixing ratio of substrates (WP / MB) as well as the ratio of substrates to inoculum (S / I). At the S / I and WP / MB ratios of 0.2 and 50:50, respectively, they achieved the highest methane yield of 608 $mLCH_4/g$ VS. The methane yield observed at these substrates mixing ratio was more than that of the mono-digestion of the substrates. The highest rise in methane output was 49.58 percent at the same 50:50 co-digestion ratio and 0.4 S / I ratio. Their study confirmed the synergetic effect created by anaerobic co digestion.

Municipal solid waste (MSW) comprises about 25-30% by mass of paper and cardboard in the United Kingdom. Industry and companies are the largest source of waste paper, with 52% of total waste being paper (Burnley et al., 2007). As part of MSW's anaerobic digestion, anaerobic digestion of waste paper is generally researched. In some cases, the study was conducted on the various fractions of the MSW resulting in yields of methane from 58 to 100

L kg^{-1} VS for newsprint paper (Owens and Chynoweth, 1993), 208-369 L kg^{-1} VS for office paper (Owens and Chynoweth, 1993, Jokela et al., 2005) and 96 and 217 L kg^{-1} VS for cardboard (Yuan et al., 2012). At C / N ratios between 20 and 25, Zhong et al (2013) obtained peak methane output in algae and maize straw co-digestion. In another case, *Scenedesmus spp* co-digested waste paper and *Chlorella spp*. obtained peak yield of methane at a C / N ratio of 18 was accomplished (Yen and Brune. 2007).

2.2.7 COMPARISON BETWEEN ANAEROBIC DIGESTION AND OTHER TREATMENT OPTIONS

Human excreta is made up of about 65-85% of water with about 15-30% being particulate organic and inorganic matter (Buckley et al., 2008). The high content of organic matter in human excrement makes beneficial for reuse as soil amender or a fertilizer. However, the high presence of microorganisms creates the need for the excreta be treated before use in order to prevent contamination and the transfer of diseases (Winker et al., 2009). Various methods and approaches are available for treating faecal sludge and converting the organic content into a valuable resource. These include composting: a biological process that includes micro-organisms under controlled aerobic circumstances that decompose organic matter. Aerobic digestion, vermicomposting; where earthworms are used to reduce the volume of organic matter, deep row entrenchment includes digging profound trenches, filling them with sludge, then covering them with soil, then the trees are planted on top, benefiting from the organic matter and nutrients slowly produced from the FS (Singh et al., 2017). Comparing the above faecal sludge treatment techniques, anaerobic digestion is of interest because this particular treatment process has the advantage of biogas generation, stabilizing FS, reducing sludge volume and odour. Also the sludge produced has the advantage of being used as a fertilizer or for soil amendment. According to Mata-Alvarez (2003), due to the elevated energy recovery associated with the process and its restricted environmental impact,

anaerobic digestion (biomethanization) is often the most cost-effective among biological treatments.

Biomass waste anaerobic digestion is now a known and commercially demonstrated strategy to waste therapy and recycling (Vogt et al., 2002). MSW anaerobic digestion is the preferred method and reliable technology for energy supply and greenhouse gas emission reduction in comparison with combustion or incineration, pyrolysis, aerobic composting and landfilling.

2.2.8 FAECAL SLUDGE METHANE POTENTIAL ENHANCEMENT

Compared to other substrates, the potential to produce biogas from faecal sludge alone is very small, amounting to 0.009 to 0.028 m^3/kg VS. This may be due to the low C / N ratio of faecal sludge (about 7.9) (Haq and Soedjono, 2010), so it is necessary to add other substrates to the optimum, i.e. 20-30 (Dioha et al., 2013) by using anaerobic co-digestion process. Anaerobic co-digestion treatment can enhance the stability of the digestion system (Gokcekus 2011), minimize main substrate inhibitions, enhance the equilibrium of nutrients and enhance biogas output (Braun and Wellinger, 2002). The C: N faecal sludge ratio is usually small because of its elevated concentration of nitrogen. This often leads to the restriction of the faecal material's anaerobic digestion mechanism, resulting in low manufacturing of biogas. Furthermore, it has been noted that there is elevated production of hydrogen sulphide (H_2S) during anaerobic digestion of faecal sludge, which is detrimental to the development of bacteria, limits the production of biogas and is also liable for the manufacturing of an unwanted sickening odour. However, it is known that offering an anaerobic reactor electron donor can enhance the process of digestion and the yield of biogas (Haq and Soedjono, 2010).

Anaerobic digestion has become a global study focus for biogas manufacturing because it generates renewable and environmentally friendly energy. Starting in the 1970s, emphasis

was put on anaerobic digestion of municipal solid waste for bioenergy manufacturing (Kiely et al., 1997).

Many academic works on the efficiency of various anaerobic technologies available for the digestion of solid waste has been performed. Most of them concentrate on the idea of the organic part of municipal solid waste being digested anaerobically. A desirable feedstock for biogas production is the organic fraction of municipal solid waste, as these wastes are characterized by a large proportion of moisture and VS with elevated biodegradability above 90 percent. Rao et al (2000) referred to these wastes as municipal trash, the primary component of MSW (40–45 wt.%) is from various sources such as homes, canteens, fruit and vegetable markets, restaurants, etc. They are high in organic matter and can be used by anaerobic digestion to generate biogas. Currently, anaerobic digestion of organic waste continues to be studied, with efforts being made to create techniques that offer resource recovery accompanying waste stability.

Rao et al. (2000) conducted a batch anaerobic digestion of municipal garbage on a laboratory scale at temperatures of 25 ° C and 29 ° C with a total solids concentration range of 45 to 135 g TS / L. They discovered that the methane content of the biogas produced ranged from 62% to 72%, with a conversion effectiveness of around 85%. In a similar study, Rao and Singh, (2004) investigated municipal garbage digestion at room temperature (26 ± 4 ° C) to estimate its potential for bioenergy production and conversion efficiencies at 15-day HRT. They recorded a large output of 0.56 m3 of biogas kg $^{-1}$ VS with a methane content of 70% and a volatile solids decrease of 76.3%. These findings showed a strong potential for municipal waste to be a source of bioenergy (Lopez and Espinosa, 2008)

Macias-Coral et al. (2008) investigated the applicability of a two-phase pilot-scale anaerobic co-digestion system for the treatment of organic fractions of municipal solid waste

(OFMSW), cotton gin waste (CGW) and dairy cow manure (CM). The results obtained showed that 0.03 and 0.08 $m^3CH_4kg^{-1}$ VS added were produced respectively by the individual digestion of OFMSW and CM. However, a yield of 0.1 m^3kg^{-1} VS added was produced by the co-digestion of OFMSW and CM. The largest yield of 0.19 m^3kg^{-1} VS was achieved by co-digesting CGW and CM. Consequently, they found that the co-digestion of waste resulted in an elevated output of methane compared to the individual digestion of waste. Zhang et al. (2007) performed a batch anaerobic digestion experiment to study fruit waste (FW) biodegradability at 10-28-day HRT. The research achieved the largest methane output of 0.435 m^3kg^{-1} VS at the end of the 28-day digestion with 81% VS removal, followed by 0.348 m^3kg^{-1} VS at the end of 10-day digestion. Their findings stated that due to its elevated degradability and biogas output, FW was a good substrate for anaerobic co-digestion.

CHAPTER THREE: MATERIALS AND METHODS

3.1 STUDY AREA

The research was carried out at the Oti faecal sludge treatment plant which is located at Kaase in the Kumasi metropolis. Ghana. Kumasi is Ghana's second largest town with a population of 1,730,249 inhabitants (GSS, 2014). The landfill covers a land area of 40 hectares. Both solid waste and liquid waste from all over Kumasi metropolis is expected to be treated at the landfill site.

Plate 3.1: Pictorial View of Oti Faecal Sludge Treatment Plant.

A means to manage liquid waste generated in the Metropolis is through the use of waste stabilization ponds at the faecal sludge treatment plant at the Oti landfill, the treatment plant comprises of six anaerobic ponds, one facultative pond and two maturation ponds for treating faecal sludge and landfill leachate. The FS treatment plant began operation in January 2004 (Abuenyi, 2010; Strauss and Montangero, 2002). The design capacity of treatment plant was 300 m^3/day for faecal sludge and 300 m^3/day for leachate from the landfill site. The plant had a hydraulic retention time of 3 days and receives a daily discharge of about 52 trips of

cesspool emptiers, which represent approximately 350 m^3 FS. The faecal sludge that is brought there includes faecal sludge from septic tanks, public toilets, pit latrines, and KVIPS (KMA, 2015).

3.2 SAMPLING

The faecal sludge used in this study was sampled in composites at the treatment plant from the cesspool emptiers bringing faecal sludge from all over Kumasi to the treatment plant. A three points method was adopted. Once the trucks start off loading the sludge, samples are collected at the start, middle and near end of the offloading with the aid of a container mounted on a rod. These form the composite from each truck. At the end of the sampling time of 12 hours (6 am – 6 pm), a homogenized sample is created from the truck composites by thoroughly mixing the samples. This was done to obtain a representative sludge mixture that reflected the combined faecal material being discharged for treatment. The sampling method used was adopted from techniques used by Strande et al. (2016) and Klingel et al. (2002). The sampling was done on ten different days over the period of one month (February). A final sample was taken from the homogenized sample collected during each sampling period and transported in 5 L containers on ice to Kwame Nkrumah University of Science and Technology (KNUST) environmental quality laboratory for analysis of physicochemical and microbial parameters.

Fruit waste samples (pineapple, pawpaw and mango) were obtained from vendors around Kwame Nkrumah University of Science and Technology (KNUST) and the paper sample used were old newspapers. The temperature and pH of the faecal sludge were measured at the sampling site (immediately after sampling from the desludging trucks into the sampling containers).

3.3 EXPERIMENTAL SETUP

A completely mixed batch reactor (CMBR) system was adopted. This was because CMBRs are simple but ideal systems which offer a constant environment (temperature) and their contents are also easily homogenized. Process conditions in CMBRs are also easily controlled. The anaerobic digestion was performed both at mesophilic temperature (35 ^{o}C) following the protocol according to Holliger et al. (2016) and at ambient temperatures to compare the process performance at the prevailing ambient temperature and a regulated temperature. The experimental setup consisted of 900 mL glass bottles used as the anaerobic digesters. Gas collecting bags were connected to the digesters with flexible tubes that had regulators on them for controlling gas flow.

The setup was made up of 20 digesters setup under two different temperatures, mesophilic (35 ^{o}C) and ambient (26.1 ^{o}C to 32.6 ^{o}C) making 40 digesters in total. Four different substrate mixtures were studied in this experiment: inoculum only (I), faecal sludge and inoculum (FI) (in the ratio 1:2), faecal sludge, inoculum and paper (FIP) and faecal sludge, inoculum, and fruit waste (FIFW). Each digester had faecal sludge to co digestate (paper or fruit waste) ratio of 1: 0.13 and total substrate to inoculum ratio of 1:2 (Angelidaki *et al.*, 2009). Each of the substrate mixtures were made in quintets (5 each). The digesters were agitated on regular basis by shaking

Inoculum (cow dung) was added to each of the digesters in an inoculum to substrates ratio of 2:1 to obtain a highly diversified load of anaerobes for easy start-up of the substrate digestion process. With the aid of a funnel, the labelled digesters were gently fed respectively with the feedstocks in the chosen ratios. The digesters were then tightly closed with the airtight screwcaps through which a flexible gas tube extended from the digester headspace into gas bags for collection of the gas. The flexible tubes had two flow regulators for controlling gas

flow. The reactors were then placed in the incubator (35 ^{o}C) and the incubator was closed to start the regulated digestion process.

Another set of digesters were placed under ambient conditions where a temperature data logger was placed to monitor the ambient temperatures on hourly basis.

To monitor the rate of gas production, the gas bags were periodically detached (on weekly basis) and were connected to the gas composition analyser (Geotech-biogas 5000) to measure the composition of the accumulated biogas. The gas outlet of the analyser was passed into an inverted graduated cylinder in a bowl containing water to quantify the volume of biogas produced using the liquid displacement technique (plate 3.1). For this technique, the inverted measuring cylinder was placed in a bowl of water, the gas was then carefully bubbled into the inverted cylinder, by which process the gas displaces its volume by pushing the water out of the inverted cylinder. The difference in water level was then read off the graduated cylinder as the volume of gas produced.

Plate 3.2: Liquid displacement method

One digester was isolated in the second, third and fourth weeks of the experiment to aid the assessment of the effect of retention time on sludge digestion. The gas generated was analyzed for its composition and volume. The digestate (content of the digester after the digestion) was also analysed for their physico-chemical and bacteriological characteristics.

The experiment was run continuously for 30 days. plates 3-2 and 3-3 show the experimental setup under mesophilic (35 ^{o}C) and ambient temperatures respectively.

Plate 3.3: Experimental Setup Under Mesophilic Temperature

Plate 3.4: Experimental Setup under Ambient Conditions

3.4 PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SUBSTRATES

pH, temperature, moisture content, total solids, volatile solids, Chemical Oxygen Demand (COD), Total Kjeldhal Nitrogen (TKN), Total Phosphorus (TP) and Helminth eggs were the physico-chemical parameters of the substrates analysed. All physico-chemical analyses were conducted in accordance with the 20th edition Standard Water and Wastewater Examination Methods (APHA, 1998). Helminth eggs were analysed by the Ethyl acetate method (WHO, 1994; Schwartzbrod et al., 2006).

Table 3-1 gives a summary of the physicochemical and microbiological study performed on the various substrates.

Table 3.1: Physicochemical and microbiological analysis performed

SAMPLE ID	TEMP	pH	%TS	%MC	VS	COD	TKN	TP	HE
	GENERAL CHARACTERIZATION OF FAECAL SLUDGE								
faecal sludge	√	√	√	√	√	√	√		
		CHARACTERIZATION OF EXPERIMENTAL FEEDSTOCK AND DIGESTATE							
I	√	√	√	√	√	√	√	√	√
F I	√	√	√	√	√	√	√	√	√
FIP	√	√	√	√	√	√	√	√	√
FIFW	√	√	√	√	√	√	√	√	√

I – Inoculum (cow dung)

FI – Faecal sludge + inoculum

FIP – Faecal sludge + inoculum +Paper

FIFW - Faecal sludge + inoculum + fruit waste

HE – helminth eggs

VS – total volatile solids

MC – moisture content

TS – Total Solids

3.4.1 DETERMINATION OF PHYSICO- CHEMICAL PARAMETERS

3.4.1.2 pH

The faecal sludge pH was evaluated using the PCSTESTR35 pH meter of the EUTECH device. The pH meter electrode was rinsed with distilled water and cleaned with tissue paper. The delicate portion of the meter was submerged in the sample and pH values recorded when the display was stable on the meter.

3.4.1.3 TOTAL SOLIDS AND MOISTURE CONTENT

Clean ceramic crucibles were dried in the oven at 105 ° C for 1 hour. The dried crucibles were removed and cooled in the desiccator and then the weight of the empty crucible (W_E) was noted. 25 mL each of homogenized samples were measured into labelled crucibles and weighed. The weight of the crucibles with the samples (Wet weight) was recorded ($W_{W)}$. The samples were evaporated using a water bath and further dried for 12 hours in the oven at 105 ° C after which they were removed and placed in the desiccator for cooling. The dried crucibles with samples were then weighed one after the other and the dry weight (W_D) recorded. The drying and cooling process was repeated at 1hr heating intervals until a constant weight was obtained. Equations 3-1 and 3-2 were then used to calculate the percentage of total solids and moisture content.

%Total solids = $\frac{W_D - W_E}{W_W - W_E} \times 100$……...Equation 3-1

% Moisture content = $\frac{W_W - W_D}{W_W - W_E} \times 100$ ……. Equation 3-2

Where: W_W = wet weight (sample plus crucible before drying), (mg)

W_D = dry weight (residue plus crucible after drying), (mg)

W_E = weight of crucible only, (mg)

3.4.1.4 VOLATILE SOLIDS

The muffle furnace was preheated to a constant temperature of 550 ^{O}C. The oven-dried crucibles with the samples were ignited in the furnace for 4 hours after which they were allowed to cool down to about 110 ^{O}C in the furnace and then taken out to cool down to room temperature in the desiccator. Then, using the analytical balance, the ash weight of the samples was determined and recorded as WB and the ignition process was repeated at 30 min ignition intervals until a constant weight was achieved. The stable reading was documented as the ash weight for each sample and then the percentage of volatile solids calculated as shown in equation 3-3.

Volatile solids (as % of total solids) = $\frac{W_D - W_B}{W_D - W_E} \times 100$ Equation 3- 3

Where: W_D = dry weight (residue plus crucible after drying), (mg)

W_E = weight of crucible only, (mg)

W_B = ash weight (ash plus crucible after ignition), (mg)

3.4.1.5 CHEMICAL OXYGEN DEMAND

The DRB200 COD reactor was powered and preheated to 150 ^{o}C. 2 mL of the homogenized sample was pipetted into the HR (20 -1500 mg/L) COD digestion reagent vial. The vials were tightly closed and then the content of the vial was mixed by inverting it severally. The vials were cleaned and then put into the preheated reactor and heated for 2 hours. 2 mL of distilled water was also pipetted into another vial to serve as a blank. After the two-hour digestion time, the reactor was turned off and the vials were transferred to a rack and allowed to cool to room temperature. The vials were taken to the DR 3900 Hach spectrophotometer for measurement The DR 3900 Hach spectrophotometer was powered and the program for COD HR was selected. By inserting the cleaned blank vial into the cell holder, the instrument was initially zeroed. After which cleaned sample vials were inserted one after the other into the cell holder and their values recorded.

3.4.1.6 TOTAL PHOSPHORUS

The method adopted for the total phosphorus determination comprised of the acid persulfate digestion method followed by the PhosVer 3 (ascorbic acid) method (Wah et al., 1997). A graduated cylinder was used to measure 25 mL of the homogenized sample in the Erlenmeyer flask and potassium persulfate powder was added. Then the flask was swirled to stir its material after adding 30 mL of 5 N H_2SO_4. The sample was then boiled softly for 30 minutes, during which time distilled water was added to the concentrate in the flask of Erlenmeyer to maintain the sample volume near 25 mL. After the 30 minutes had elapsed, the sample was removed from the hot plate, cooled to room temperature and 30 mL of 5N NaOH was added. The flask was swirled to mix again, then poured into a 25 mL graduated cylinder and the volume adjusted by adding distilled water to the 25 mL mark. A sample cell was then filled with 10 mL of the digested sample and a PhosVer 3 phosphate reagent powder pillow was added and shaken to mix for about 30 seconds–the formation of a blue colour suggested the existence of phosphorus in the sample. A reaction time of 10 minutes was allowed. a blank was made by filling a cell with 10 mL of the sample. The blank cell was washed and inserted into the DR 3900 Hach spectrophotometer's cell holder to null the instrument after the 10-minute reaction time had expired. Then the sample cell was wiped, inserted into the cell holder and the complete phosphorus content was read for the sample.

3.4.1.7 TOTAL KJELDAHL NITROGEN (TKN)

The Kjeldahl method was used and includes digestion, distillation and titration. 10 mL of the sample was measured into a 500 mL long-necked Kjeldahl flask and a spatula full of Kjeldahl catalyst (that is a mixture of 1part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4) was added. Followed by adding 20 mL conc. H_2SO_4 and digested until it appeared clear and colourless. The flask was left to cool after which the fluid was transferred into a 100 mL volumetric flask and topped up to the mark with distilled water.

A 10 mL aliquot was pipetted into the Kjeldahl distillation apparatus The distillate was then collected over 10 mL of 4% Boric acid and 3 drops of mixed indicator were added in a 200 mL conical flask (the presence of nitrogen gave a green coloration). 100 mL of the collected distillate was then titrated with 0.1 N HCl till the green colour changed to pink.

TKN (mg/L) = $\frac{(V-B)\times N\times 14\times 1000}{Volume\ of\ sample}$

Where: N = normality of HCl = 0.1 N

V = volume of HCl titrated against the sample

B = volume of HCl titrated against the blank

3.4.2 DETERMINATION OF MICRO BIOLOGICAL PARAMETERS

3.4.2.1 HELMINTHS EGGS.

Ethyl acetate technique was used to identify helminth eggs in the samples (WHO, 1994; Schwartzbrod et al., 2006). In 15 mL acid-alcohol buffer solution (5.16 mL 0.1 N H_2SO_4 in 350 mL ethanol) the faecal sludge sample was suspended and approximately 5 mL ethyl acetate was added. The mixture was shaken and occasionally the centrifuge tube opened to let out gas for 3 minutes at 2200 rpm before centrifuging. A diphasic solution (aqueous and lipophilic phase representing acid / alcohol and ethyl acetate, respectively) was produced after centrifugation. As much of the supernatant as possible (beginning from the lipophilic and then the aqueous phase) was sucked out with a micropipette, leaving roughly 1 ml of deposit that was examined under the microscope. Based on their form and size, helminths eggs were recognized and compared to the bench aids for intestinal parasite diagnosis (World Health Organization, 1994). The counting was performed at X40 magnification under a light microscope in both chambers of a hemocytometer.

3.5 DATA ANALYSIS

Data acquired from this study were analysed statistically by means of Microsoft Excel 2016 and Graph pad

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 CHARACTERISTICS OF SUBSTRATES USED

4.1.1 CHARACTERISTICS OF FAECAL SLUDGE DISCHARGED AT OTI LANDFILL

4.1.1.1 pH

The pH values obtained over the period of sampling (February) showed little variation from each other. The values ranged from 7.4 to 8.06 (Figure4.1) with an average of 7.7 and standard deviation of 0.19. Comparing these values to literature values 6.4 – 8.5 (Torondel ,2010; Kuffour et al.,2009; Appiah-Effah et al., 2014) indicated that the faecal sample could undergo the anaerobic process without an attempt to optimize the pH or pre-treat the sample (House, 2010).

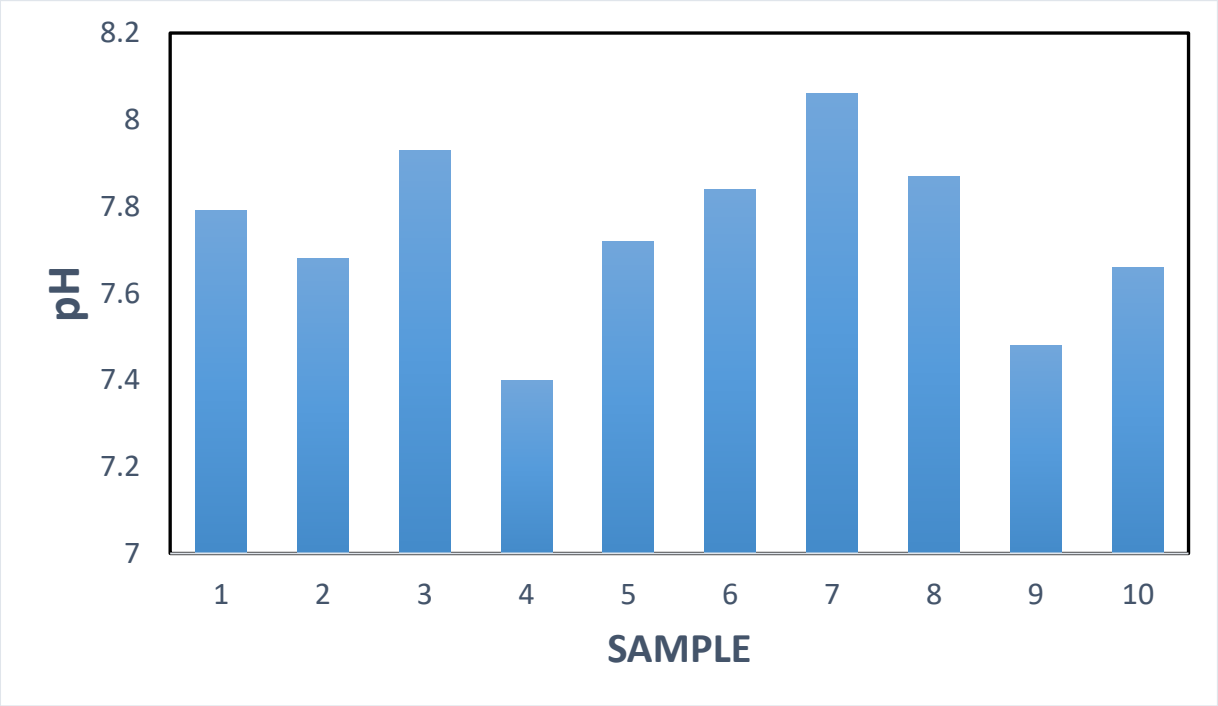

Figure 4.1: pH of faecal sludge

4.1.1.2 TEMPERATURE

The measured temperatures of the individual samples showed little variation from each other. The values ranged from 28 ^{o}C - 31 ^{o}C with an average of 29.6 ^{O}C ± 0.97 (figure 4.2).

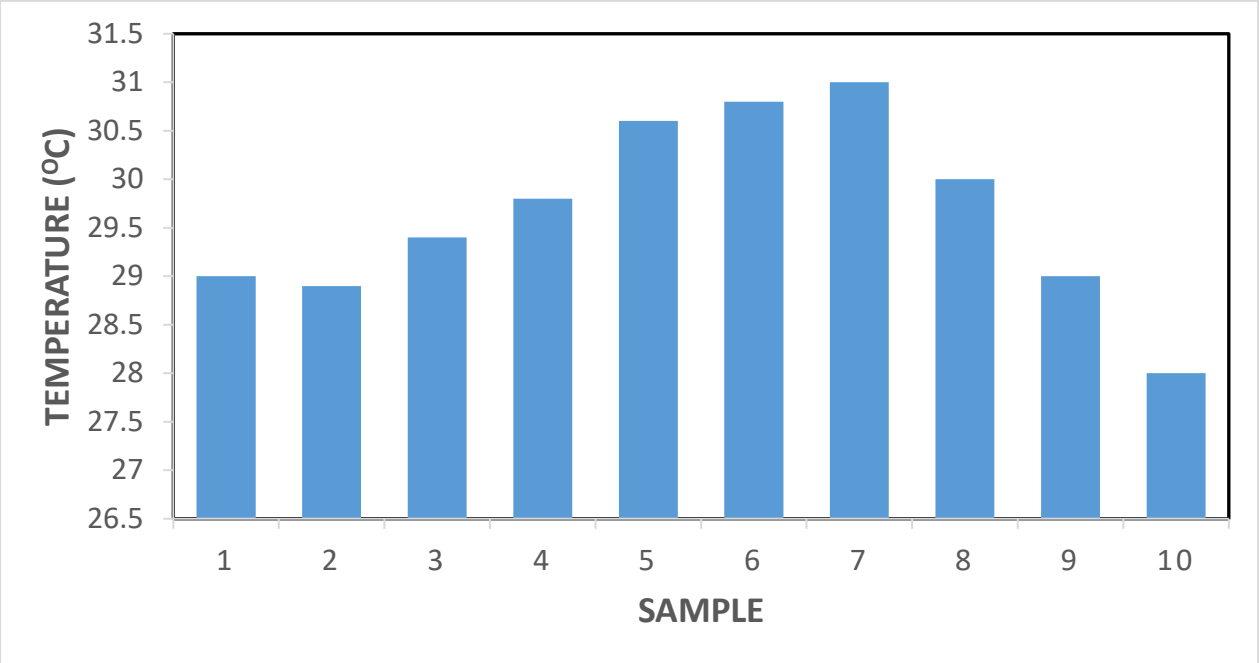

Figure 4.2: Temperature of faecal sludge

4.1.1.3 TOTAL SOLIDS (TS)

The total solids content of the faecal sludge brought to the Oti landfill during the study period ranged from 0.33 – 3.25 % (1.43 ±0.84) similar to the value < 3 stated by Kone and Strauss, (2004). The faecal sludge has less solids and more moisture content because most of Kumasi's faecal sludge comes from water closets, where a significant amount of water is used to flush the faecal matter (Agyei et al., 2011; Kuffour et al., 2013; Cofie et al., 2006). According to Drapcho et al. (2008), substrates with total solids content between 2%-10% are suitable for anaerobic digestion.

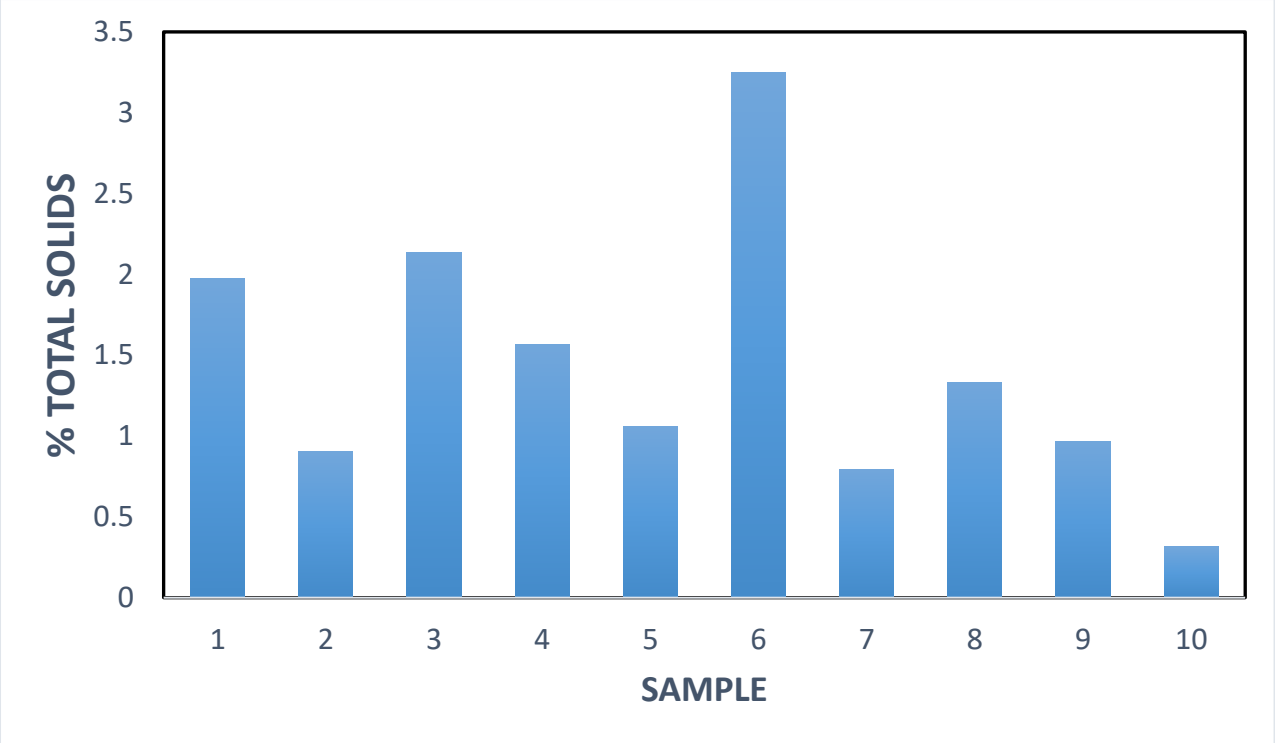

Figure 4.3: Total solids percentage of faecal sludge

4.1.1.4 MOISTURE CONTENT (MC)

The moisture content ranged between 96.75 – 99.69 % (98.57 % ± 0.84) (Figure 4.4). Samaras et al. (2008) reported 85.2 % MC. This shows that the faecal sludge brought to the Oti landfill has a considerable high moisture content probably due to the increasing number of water closet usage. Qu et al. (2009) stated that waste with high moisture content has an increased area of contact between the microbes, enzymes, and the substrates. An increase in attachment area enhances waste methanation and hydrolysis process.

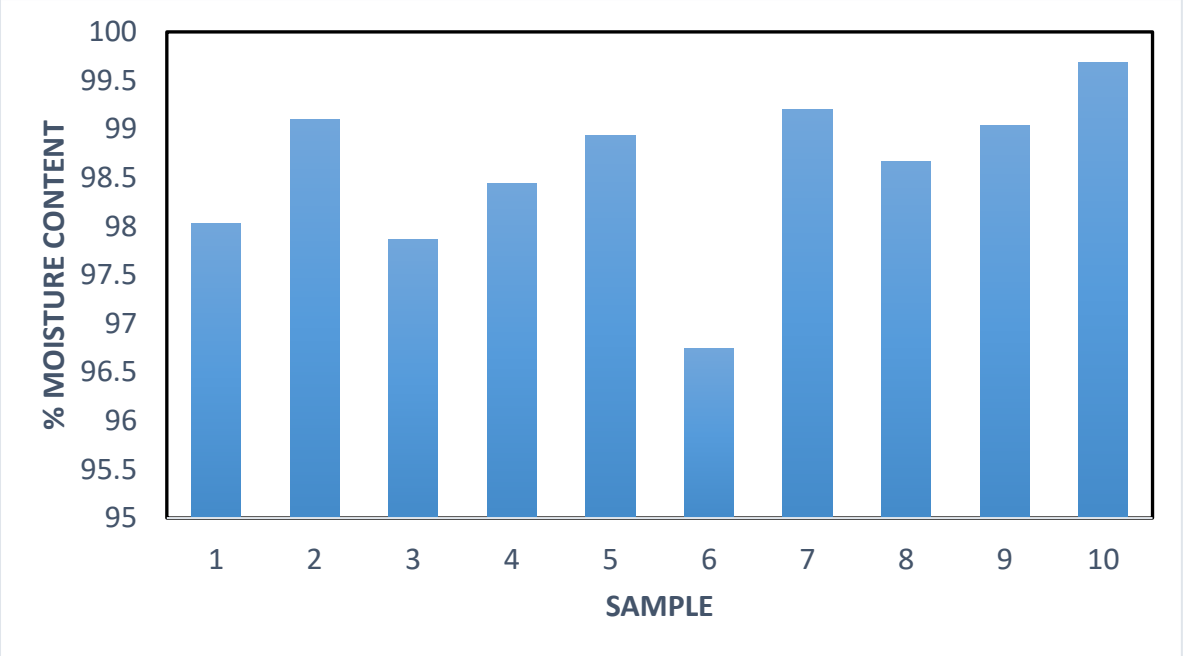

Figure 4.4: Moisture content of faecal sludge

4.1.1.5 VOLATILE SOLIDS (VS)

Volatile solids percentage shows the portion of solids that can be degraded and thus the stabilization of the waste (Al-Seadi et al., 2008). The volatile solids content obtained during the period of characterization ranged from 44.4 – 82.6 % (65.75 ± 10.98) (Figure 4.5), an indication of substantial stabilization of the faecal sludge. These values differed a bit from the values stated in most works done by other researchers: 50 – 84 % as reported by Koné and Strauss (2004), (Chaggu, 2004) and (Nwaneri et al., 2008). This may be due to the high variability of organic content of substrates. Tsunatu et al. (2014) recommended that the VS (%TS) content be within the 80-90% range in order to generate the optimum biogas. Thus the faecal sludge used in this study may not be able to produce substantial biogas on its own.

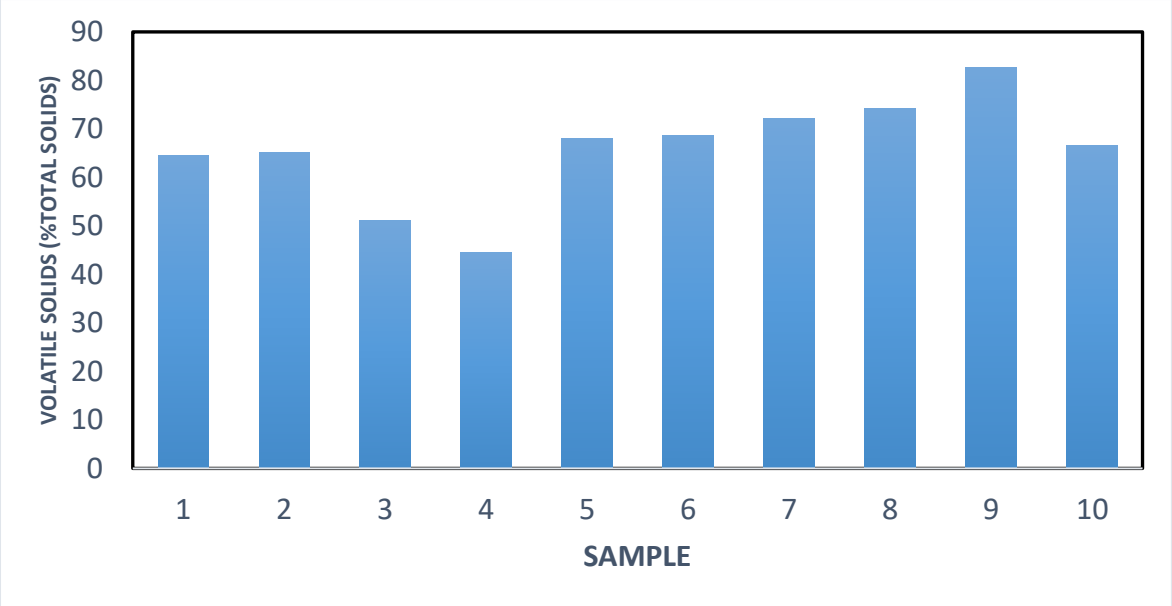

Figure4.5: Volatile solids of faecal sludge

4.1.1.6 CHEMICAL OXYGEN DEMAND (COD)

The chemical oxygen demand values obtained in figure 4.6 (23050 ± 681.50 mg/l) falls within values quoted by Kone and Strauss, (2004), (20,000 to 50,000 mg COD/L). COD indicates degree of reduction of the organic material in wastewater. A higher COD measure indicates the presence of high organic matter content and hence a less stabilized sludge (Hagos et al., 2017).

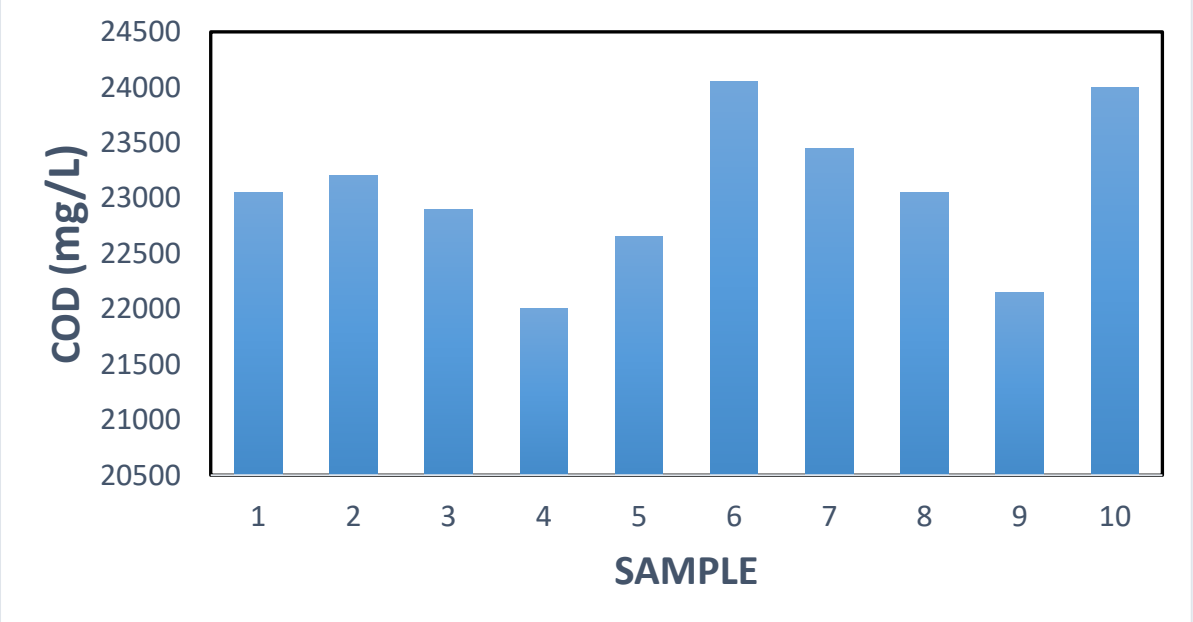

Figure 4.6: COD of faecal sludge

4.1.1.7 TOTAL KJELDHAL NITROGEN (TKN)

Figure 4.7 shows the TKN values obtained for the faecal sludge. The values recorded ranged between 1400 mg/L and 5040mg/L (2842 ± 1094.53 mg/L). Some of the values were above the values quoted in literature (200 mg/L – 4500 mg/L), (Kone and Strauss, 2004; NWSC, 2008).

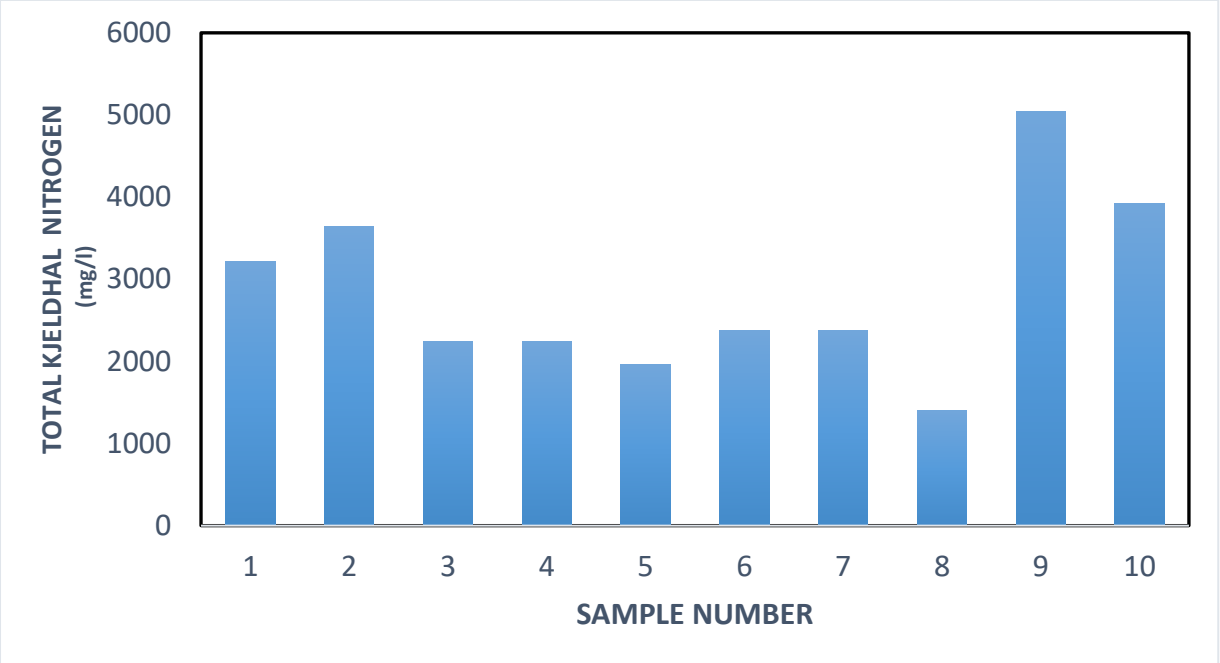

Figure 4.7: TKN of faecal sludge

The mean value of the faecal sludge characterized over the one-month period compared with values from literature are summarized in table 4.1 below

Table 4.1: Faecal sludge characteristics compared with literature values

PARA-METER	UNIT	MIN. VALUE	MAX. VALUE	AVERAGE	STDEV	LITER-ATURE	REFERENCE
pH		7.4	8.06	7.7	0.2	6.7 - 9	Torondel (2010), Kuffour et al (2009), Appiah-Effah et al (2014)
TEMP	^{o}C	28	31	29.6	0.97	30 - 45	de Bertoldi et al., 1983
TS	%	0.31	3.25	1.43	0.84	<3	Kone et al (2004)
VS	%TS	44.44	82.61	65.75	10.98	50 – 73	Kone et al (2004)
MC	%	96.75	99.69	98.57	0.84	>97	Kone et al (2004)
COD	mg/l	22000	24050	23050	681.50	20,000 - 50,000	Strauss et al. 2000, Kuffour et al (2009)
TKN	mg/l	1400	5040	2842	1094.53	1000 - 3400	Kuffour et al (2009), Fanyin-Martin et al (2017)
COD: TKN		4.34	16.46	8.11	3.25	6 – 10	Chaggu, 2004, Nwaneri et al, 2008, Fulford, 2006

Most of the values obtained during the study fell within the values found in literature. The high standard deviations observed during the study confirmed the wide variability of faecal

sludge in terms of its organic and nitrogen contents (Chandran, 2014; Strande et al., 2014). The values obtained shows that the faecal sludge although partially decomposed still needs to be properly treated before being released into the environment. The high levels of nitrogen can lead to eutrophication if released into the environment (Chislock et al., 2013).

COD: TKN was calculated to check the suitability of the faecal sludge to be used for anaerobic digestion. The value obtained (8.11) indicated that on its own, faecal sludge will not be suitable for anaerobic digestion with the aim of biogas production as the recommended ratio is between 20 and 30. This confirmed the need for adding other substrates with higher carbon content in order to raise the carbon to nitrogen ratio to achieve optimum C/N ratio (Hagos et al., 2017).

Based on the characteristics of the raw faecal sludge brought to the Oti treatment plant during the period of this study, there was the need to enhance its characteristics by co-digesting with other substrates in order to increase biogas production.

Newspaper and fruit waste were chosen as the substrates for co-digestion in this study. The effects that they had on biogas production and sludge digestion were observed.

4.2 CHARACTERISTICS OF SUBSTRATES USED

Before digestion, the faecal sludge, inoculum and each of the co substrates (paper and fruit waste) were blended using a blender. Parameters such as pH, moisture content (MC), total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total kjeldahl nitrogen (TKN), total phosphorus (TP) and helminth eggs were investigated for the various substrate combinations: inoculum only (I), faecal sludge and inoculum (FI), faecal sludge, inoculum and paper (FIP) and faecal sludge, inoculum and fruit waste (FIFW). The outcomes obtained are presented in the figures below.

4.2.1 COD: TKN

The substrates mixtures used in this experiment were chosen after a few trials to choose mixtures which fell within the recommended range for anaerobic digestion between 20 and 30 as shown in figure 4.8 (Vandevivere et al., 2000).

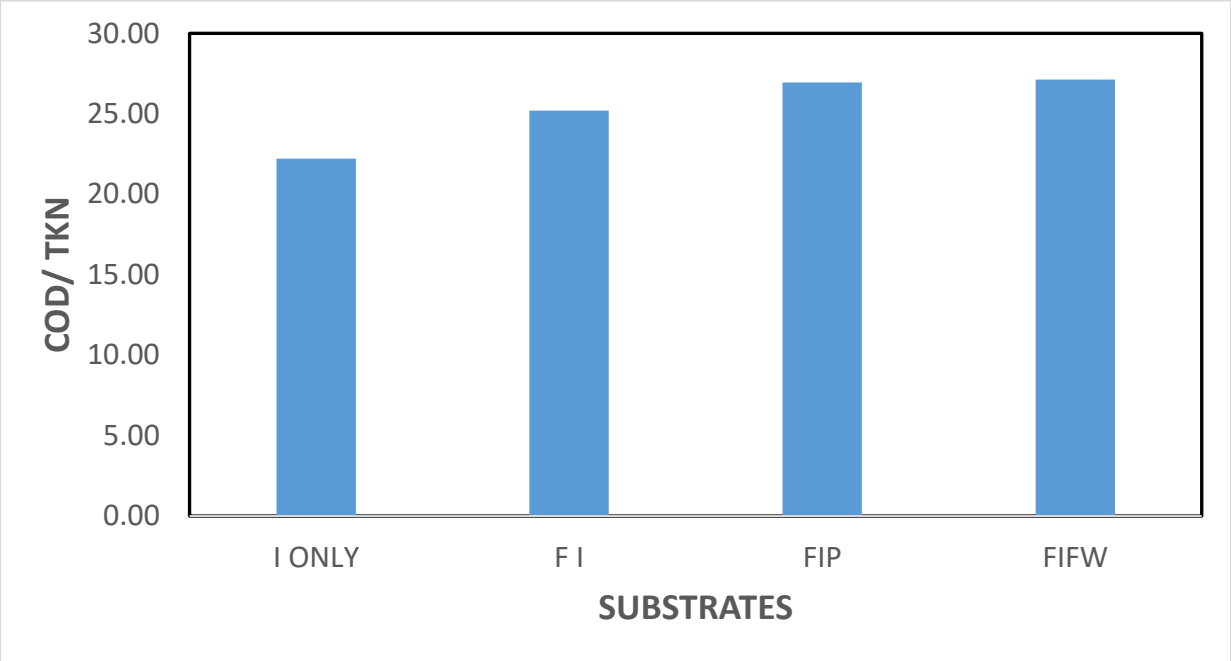

Figure 4.8: COD to TKN ratio of substrates

Faecal sludge was co-digested with paper or fruit waste in the ratio 1: 0.13. The fruit waste mixture (FIFW) had the highest C: N ratio of 27.14 followed by the paper mixture (FIP) of 26.95. the values obtained were far higher than that obtained for the raw faecal sludge (8.11). Similarly, Esposito et al (2012) confirmed during their study that co digestion improved C: N ratio of substrates. This indicated that co digestion of faecal sludge can aid the production of biogas. In anaerobic digestion, the proportion of carbon and nitrogen plays a significant role. The carbon functions as a source of energy, and microbial development is enhanced by nitrogen (Ren, 2010).

4.2.2 pH OF SUBSTRATES

The mean pH values (7.4 – 8.2) (figure 4.9) obtained for all the substrates used for the experiment were near neutral and fell with the optimum range (6.7 – 8) suggested by Kigozi et al (2014) as suitable for anaerobic digestion.

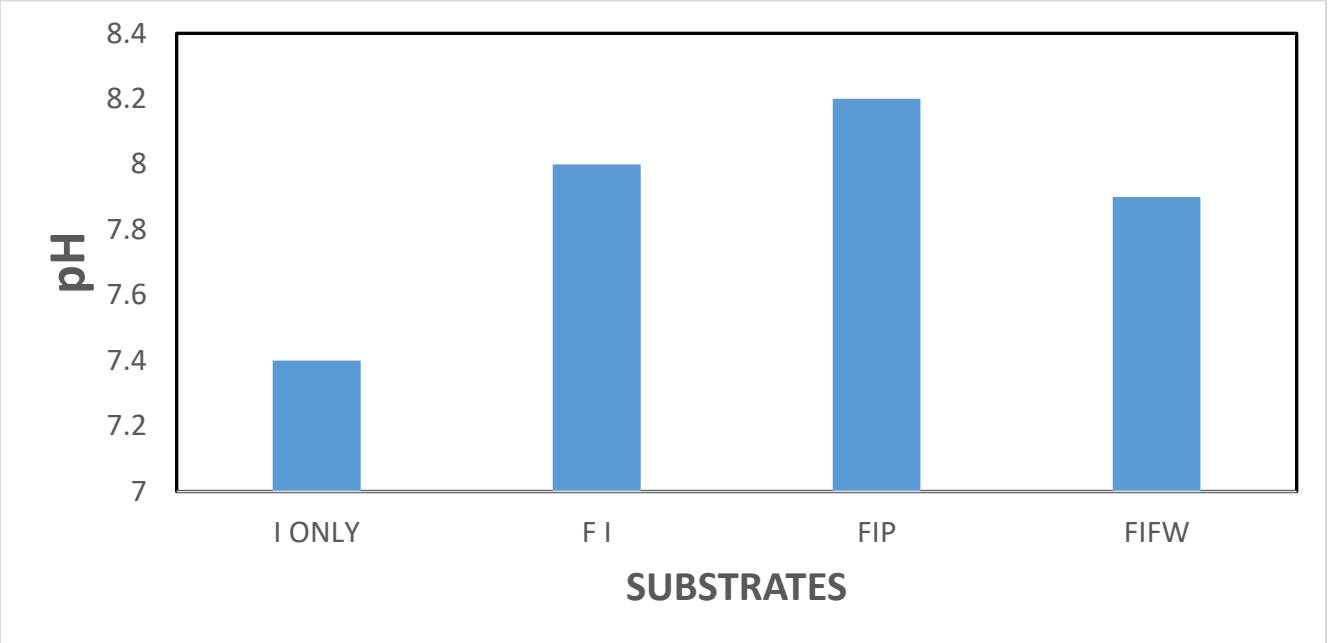

Figure 4.9: pH of substrates

pH outside this range could inhibit the growth of the methanogens and hence the biogas production (Kigozi et al., 2014). The paper mixture (FIP) recorded the highest pH of 8.2 while inoculum had a pH of 7.4.

4.2.2.1 TOTAL SOLIDS OF SUBSTRATES

The %TS obtained was in the range 0.50 to 5.23 (figure 4.10) as against the optimum range of 2 – 12% quoted by (Deublein and Steinhauser, 2008). The paper mixture had the highest dry matter content of 5.23 % while inoculum had the least (0.50 %).

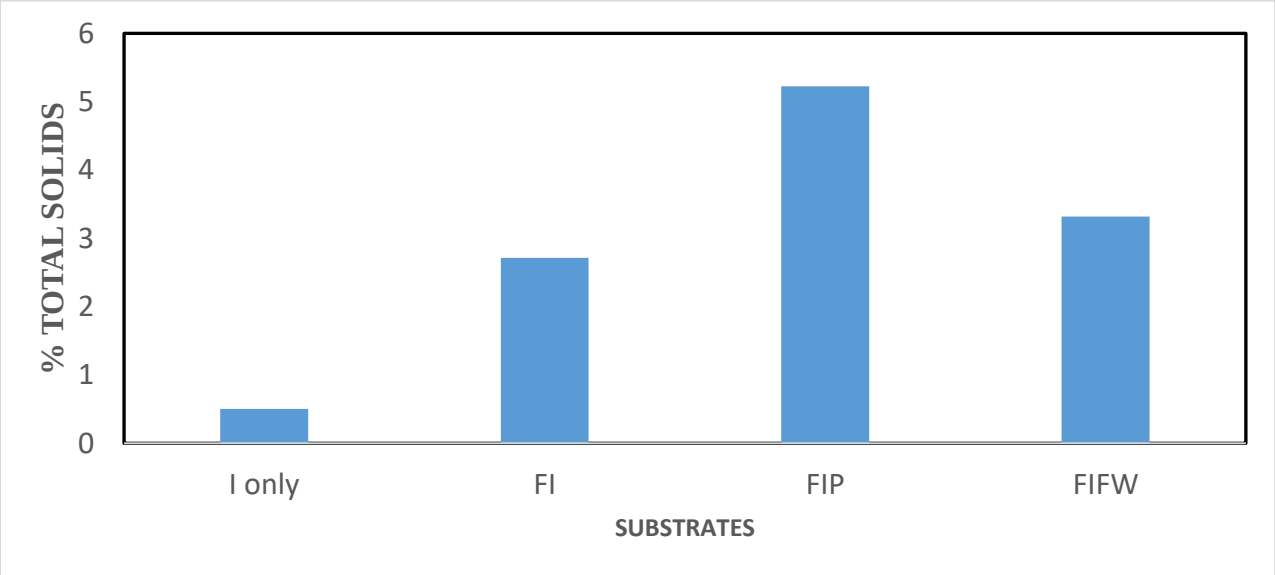

Figure 4.10: Total solids content of substrates

The substrates were ideal for anaerobic digestion as they were not above the recommended range (≤12 %)which could have led to non-functionality of the system (Nizami and Murphy, 2010). From these values it was expected that the microorganisms' mobility was not hindered. Hindered mobility of the microorganisms would mean a longer retention time (Kossmann and Ponitz 1999)

4.2.2.2 VOLATILE SOLIDS OF SUBSTRATES

The volatile solids percentage obtained from the different substrates combination fed into the digesters showed that the paper mixture (FIP) had a higher percentage (86.54 %) of its total solids being organic while inoculum only had the least amount of total solids being organic (80%).

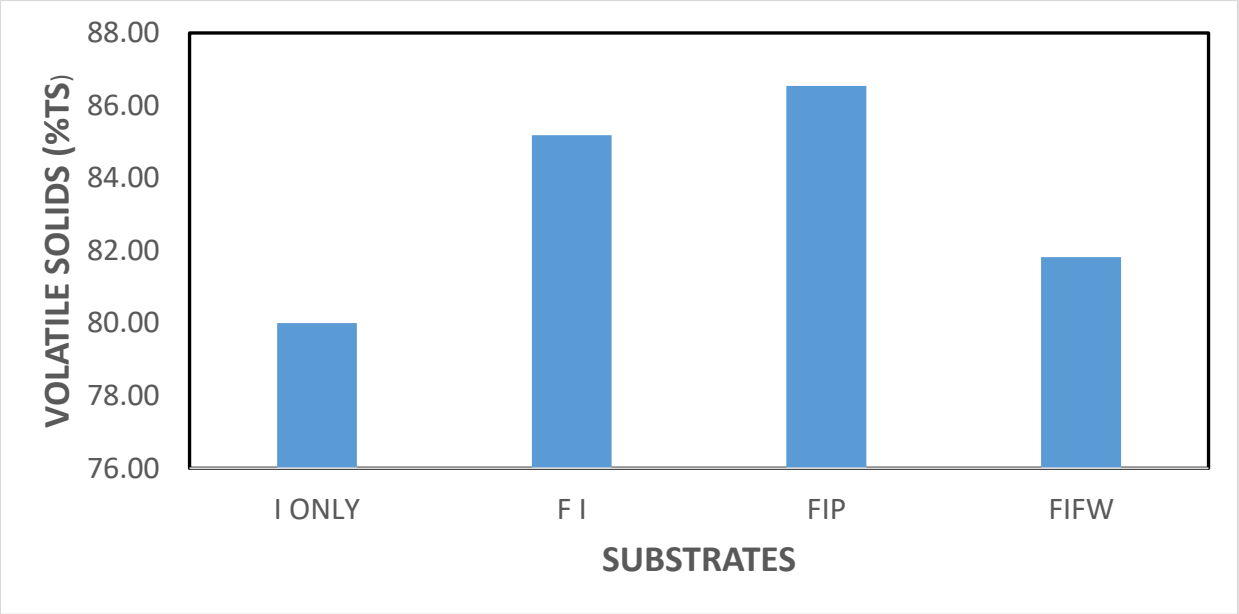

Figure 4.11: VS percentage of substrates

The volatile solids values obtained however conformed to the values stated by Fulford, (2006) as being suitable for anaerobic digestion. These values were also higher than the mean value obtained for the raw faecal sludge (67.75 %). This shows that adding the substrates for co digestion increased the organic solids percentage and had higher potential for conversion into biogas.

4.3 EFFECT OF PAPER AND FRUIT WASTE ON BIOGAS PRODUCTION

4.3.1 BIOGAS VOLUME UNDER MESOPHILIC TEMPERATURE

The biogas volumes recorded for digesters set under mesophilic temperature (figure 4.12) showed that biogas production peaked during the third week for almost all the substrates and decreased in the 4th week except for the digester containing the paper mixture which continued increasing even in the 4th week. FIP produced the highest volume (1760 mL) while I only and FI produced the least (635 mL and 830 mL respectively). The continual increase in the volume of biogas generated by the FIP indicates that the organic content of the mixture was not fully exhausted during the study period.

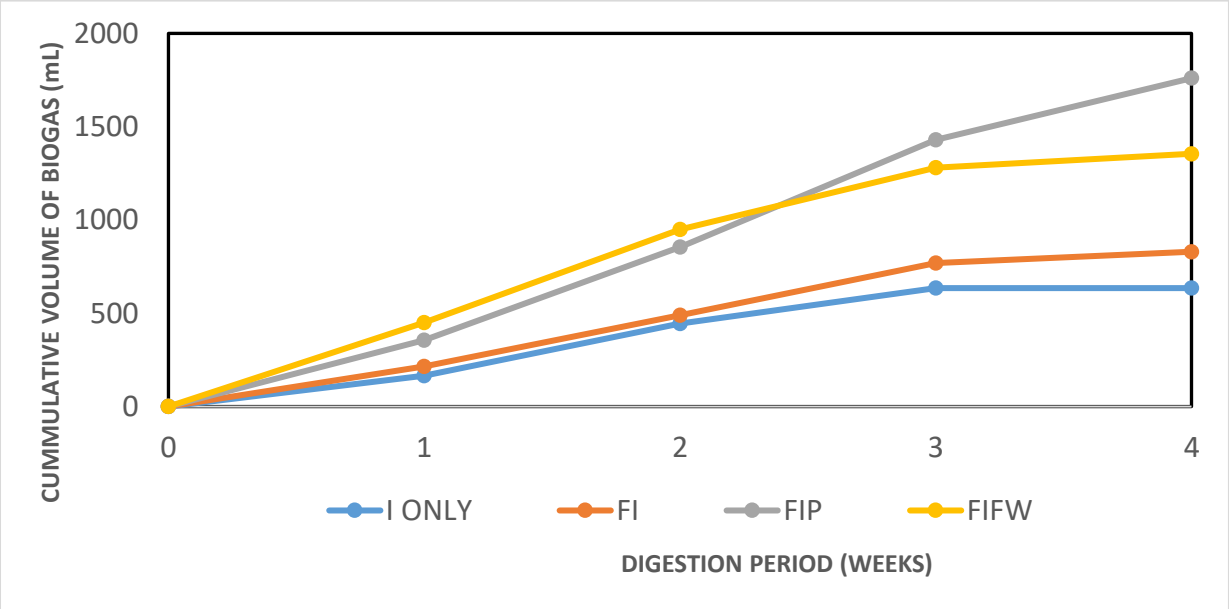

Figure 4.12: Biogas volume for mesophilic temperature

The FI digester had lower biogas generation rates compared to the FIP and FIFW digesters throughout the digestion period. When algal sludge was co-digested with waste paper in similar works conducted by Yen and Brune, (2007), they saw a rise in biogas production. Wendland et al (2006) also reported better biogas production when black water (toilet water) was co digested with kitchen waste. This confirms that anaerobic co digestion enhances the production of biogas.

Therefore, paper and fruit waste had a positive effect on biogas production by increasing gas production of FIP and FIFW by 112.05% and 63.25% respectively.

4.3.1.1 METHANE YIELD UNDER MESOPHILIC TEMPERATURE

Biogas comprises mainly of CH_4 and CO_2 according to Drapcho et al (2008); (Cheng, 2010). CH_4 generally accounts for 60-70% of the total quantity and 30-40% for CO_2 (Cantrell et al., 2008). However, the results obtained from this study deviated from the range given although the pH recorded at the end of the experiment (7.57 – 7.91) eliminated the possibility of ammonia or volatile fatty acids inhibitions and as such the methanogens were assumed to have functioned properly because Chen et al. (2008) stated that pH lower than 6.5 leads to production of organic acids which is lethal to methanogens while ammonia or VFA inhibitions occurs at pH above 8 (Angelidaki and Ahring, 1993).

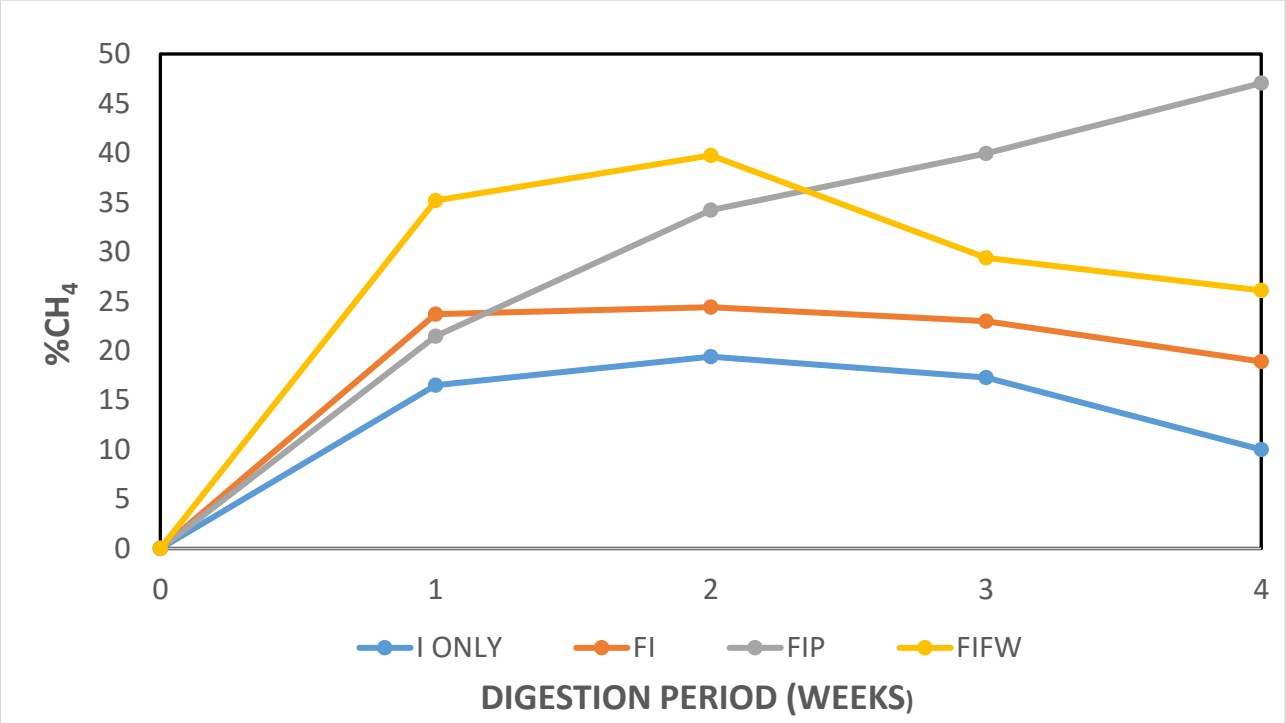

Figure 4.13: Methane yield for mesophilic temperature

The digester containing FIP produced the highest amount of methane (47.05 %) in the 4th week while FIFW produced its highest of 39.75 % in the second week and began to decrease in the 3rd week. The methane levels of I only and FI had lower methane levels compared to the co digested substrates in all the weeks. The higher levels of methane observed for FIP may be due to increased activity of cellulase. Cellulase is an inducible enzyme that is mostly secreted by microorganisms in the environment during its development on cellulose-containing products (Busto et al.,1996).

The lower methane levels at the start of the digestion period can be attributed to the inactivity of methanogenic bacteria as the digestion process the wastes undergo three stages; hydrolysis, acidogenesis and acetogenesis (Al-Seadi et al., 2008) before the methanogenesis stage. Therefore, at the start, acid-forming bacteria are the prominent microbial communities and result in the production of primarily CO_2 at the initial stages of the digestion.

4.3.2 BIOGAS VOLUME UNDER AMBIENT TEMPERATURE

FIFW produced the highest volume of biogas of 1875 ml while FIP produced 1515 ml biogas for the entire period (figure 4.14). And similar to results obtained under mesophilic temperature, biogas production from FIP kept on increasing to the 4th week although there was a decrease in week 2. This varying trend could be due to the fluctuating temperatures unlike a stable temperature for the mesophilic digestion. The continual increase in the volume of biogas generated by FIP mixture indicates that the organic content of the mixture was not fully exhausted during the study period.

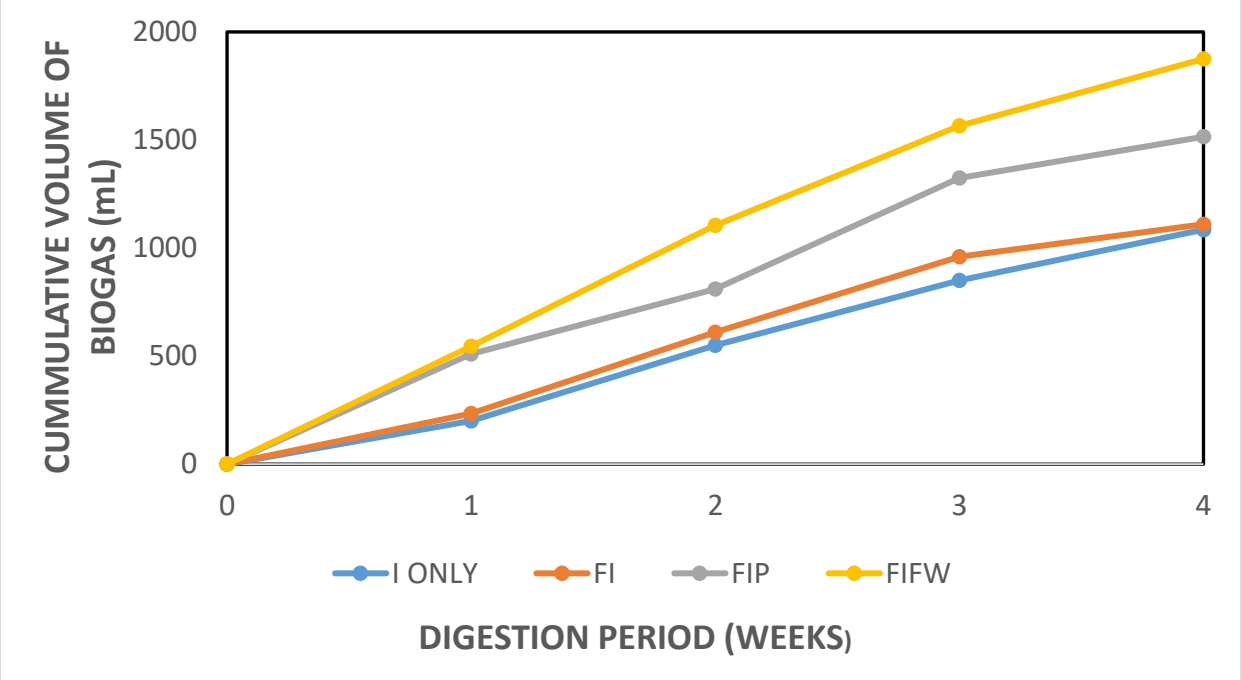

Figure 4.14: Biogas volume for ambient temperature

The slow biogas generation rate at the beginning of the digestion could be due to the microorganisms getting used to the environment in the digesters and also characterization of the substrates before the start of digestion showed that conditions were ideal for biogas production. Also, as was the case for mesophilic digesters the FI digester had lower biogas productions compared to the FIP and FIFW digesters throughout the digestion period.

4.3.2.1 METHANE YIELD OF CODIGESTION UNDER AMBIENT TEMPERATURE

Under Ambient temperatures, FIFW produced the highest methane percentage 45.7 % in the 2nd. FIP recorded an increase in methane values till it dropped in the 3rd week (from 29.8% to 23.8%) and then shot up in the 4th week (41.1%).

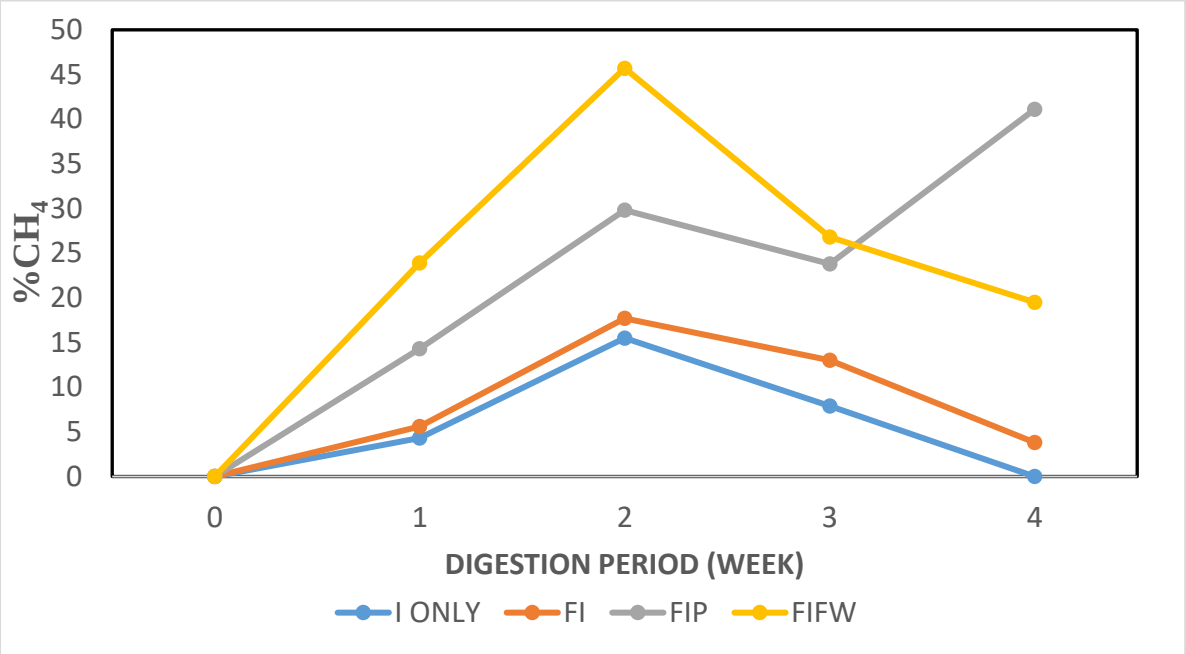

Figure 4.15: Methane yield for ambient temperature

The methane levels of I and FI only were lower from the onset and recorded its highest in 2nd week and then reduced from the 3rd week and FI had lower methane levels compared to the co digested substrates. The higher methane levels observed for FIFW could be due to ease of breakdown of the organic matter in fruit waste compared to the paper as paper contains lignin.

4.4 EFFECTS OF TEMPERATURE ON BIOGAS PRODUCTION

4.4.1 BIOGAS PRODUCTION FROM CO DIGESTING WITH PAPER

A constant temperature (mesophilic 35 ^{o}C) was maintained for one set of digesters while the other set was placed under ambient conditions which had fluctuating temperatures (24-32 ^{o}C) over the 30 days' period.

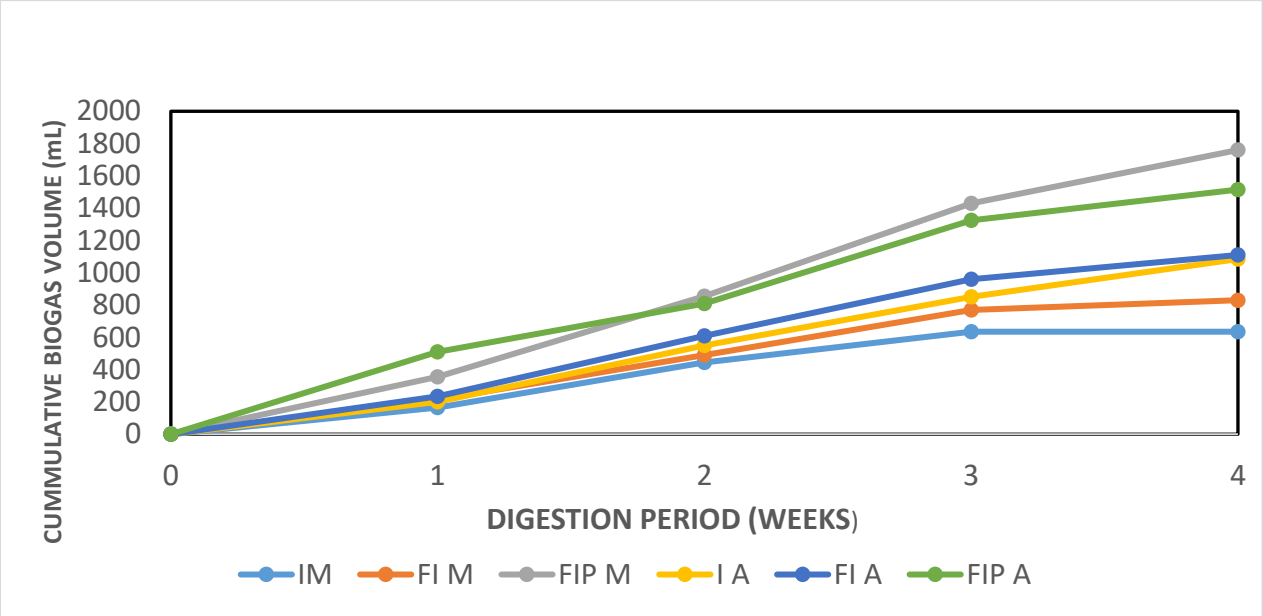

Figure 4.16: Biogas volume under mesophilic and ambient temperature

In the figure 4.16 above, the biogas production for FIP under the two different temperatures were compared with the production from the I only and FI. From the graph it can be observed that the substrates did better under the mesophilic temperature compared to performance under ambient temperatures. Although FIP had higher biogas generation under ambient temperature during the early days of the digestion period, it was lower in the third and fourth week. FIP had a biogas production of 1760 mL and 1515 mL under mesophilic temperature and ambient temperatures respectively

Statistical analysis of the methane yield under both temperatures yielded p=0.1395 which is greater than 0.05. therefore, it shows that there was no significant different in the methane yield under mesophilic and ambient temperatures.

4.4.2 BIOGAS PRODUCTION FROM CO-DIGESTING WITH FRUIT WASTE

Unlike the FIP, FIFW took a different trend, the biogas production was rather higher for digesters under ambient temperature. This trend deviates from the usual trend found in literature. However, it can be observed that the biogas production under both temperatures were lower in the first week of the digestion process compared to the rest of the digestion period.

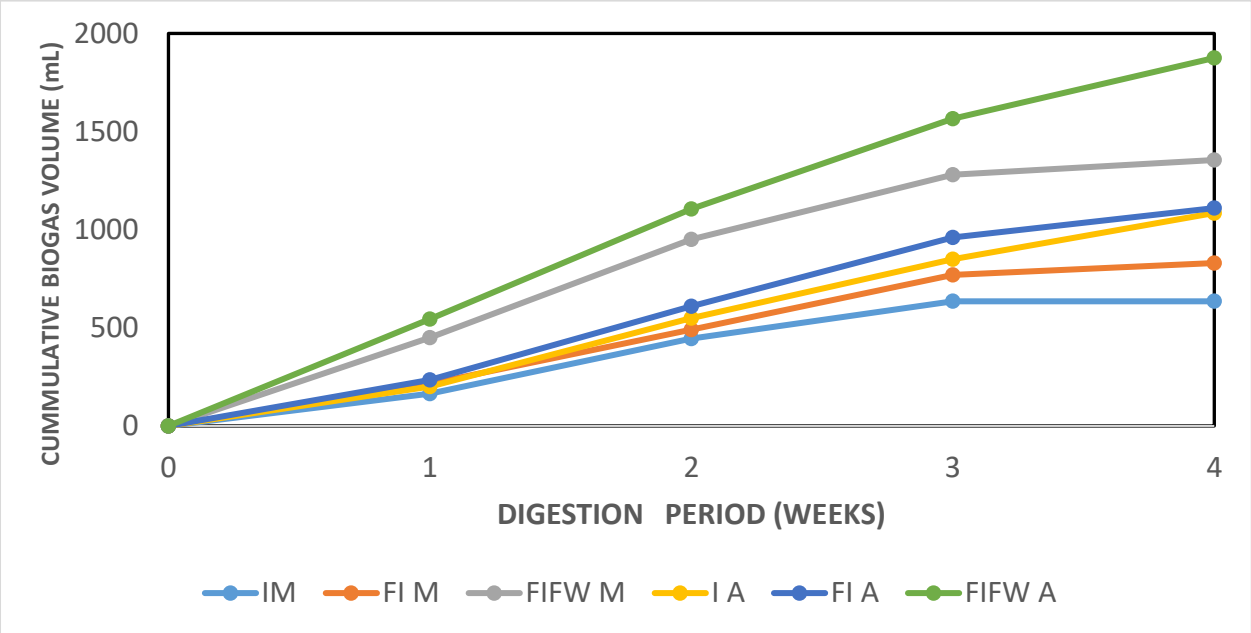

Figure 4.17: Biogas volume under mesophilic and ambient temperature

Statistical analysis of the methane yield for FIFW under both temperatures yielded p=0.003 which is less than 0.05. therefore, it shows that there was significant different in the methane yields under mesophilic and ambient temperatures. Further analysis (table 4.2) showed that the significant difference was from the yields in the first week (the *p* value of week 1 was less

than 0.05 while those of week 2 to 4 were greater than 0.05). This could be due to time taken for the microorganisms to get adapted to the environment within the digesters.

Table 4.2: Statistical analysis of effect of temperature on methane yield of fruit waste within weeks

Row Factor	Difference	t	P value	Summary
Week1	-11.30	5.201	**P<0.01****	
Week2	5.950	2.739	P > 0.05	ns
Week3	-2.600	1.197	P > 0.05	ns
Week4	-6.600	3.038	P > 0.05	ns

4.4.3 EFFECT OF TEMPERATURE ON REMOVAL EFFICIENCY

The initial and final COD and TKN contents of the substrates used were determined in order to ascertain the level of removal using anaerobic digestion under both mesophilic and ambient temperature. This provides insight into where the final effluent from the digestion process will be safe to be discharged into the environment. The results obtained are presented in figure 4.18 and 4.19 below

4.4.3.1 COD REMOVAL IN SUBSTRATES USED

The efficiencies of COD removal acquired in this research were similar to those reported in literature ranging from 55 to 75% for anaerobic co-digestion process (Claudia 2008). The percentage removal for COD were high under both temperatures. COD removal percentages were better in the co digested samples. However, FIP had the least COD removal percentages for co digested substrates under both temperatures. This could be attributed to the organic content not being fully exhausted during the 30 days' period (figure 4.12 and 4.14). Removal

efficiency for FIFW were 74.36 % and 73.50 % for mesophilic and ambient temperatures respectively. Wendland et al., (2006) also reported that the co digestion of kitchen waste with black water improved the COD removal efficiency (61%).

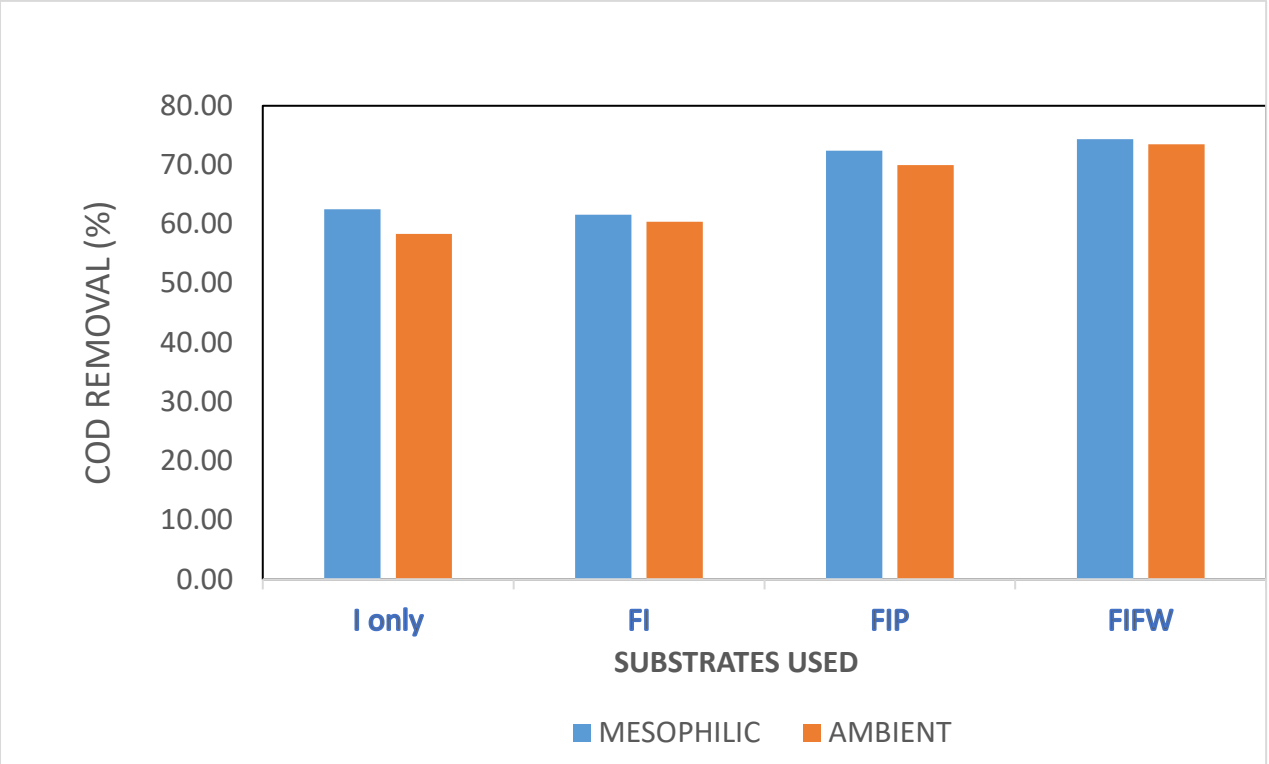

Figure 4.18: %COD removal for substrates

Although percentage removal was high, the final effluent values obtained (5000 mg/L – 15500 mg/L) (Appendix 1) were above the maximum permissible levels (250 mg/L) by Ghana Environmental Protection Agency. Hence further treatment will be necessary if the effluent will be discharged into the environment.

4.4.3.2 TKN REMOVAL OF SUBSTRATES

Nitrogen removal was generally poor for effluents under both temperatures (figure 4.19). The percentage removals ranged from 20.75 - 62.22 % for digesters at mesophilic temperature and 19.85 – 66.67 % for digesters at ambient temperature.

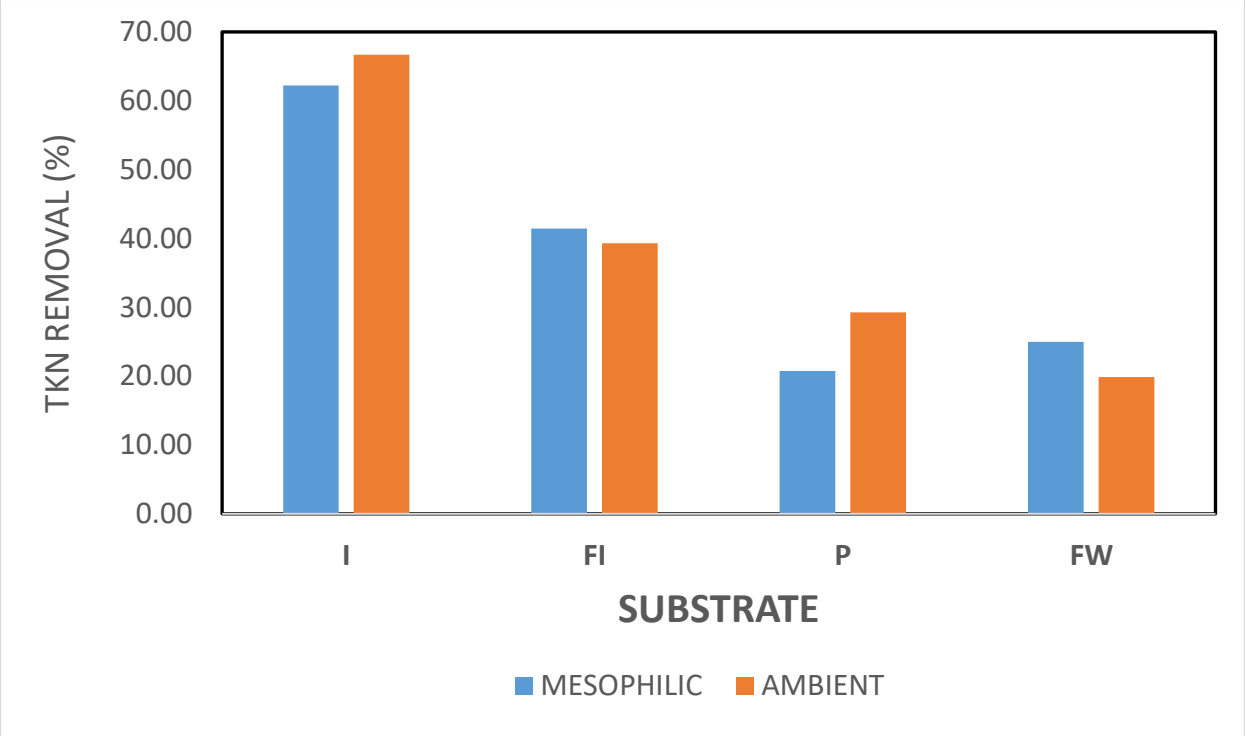

Figure 4.19: TKN removal for substrates

Due to the anaerobic conditions created in the digesters, there was no oxygen present as the digestion proceeded to enhance the nitrification – denitrification processes in the digesters and hence the high TKN concentrations obtained in the effluents. Also the final effluent TKN values (Appendix 2) were above the permissible levels (50 mg/L) of Ghana Environmental Protection Agency. House, (2007) reported that human waste through anaerobic digestion is a credible ethical sanitation technology and removes chemical oxygen demand from sewage but conserves nutrients (especially nitrogen compounds) this is evident in the results obtained. This indicated that further treatment will be needed for the effluent from anaerobic treatment before it is released into the environment especially water bodies because high nutrient levels (phosphorus and nitrogen) can be dangerous to aquatic life (Xu et al., 2010).

4.5 EFFECT OF SLUDGE RETENTION TIME (SRT) ON SLUDGE DIGESTION

Retention time here refers to how long the substrates are allowed to be digested. It is a significant variable in anaerobic digestion because the quantity of biogas produced is influenced by the retention time of sludge. Drapcho et al. (2008) indicated that for mesophilic temperatures, the AD process will require a retention time within 25-40 days. In general, a longer SRT will allow more degradation and pathogen inactivation of the substrate under the same operating conditions compared to a shorter SRT (Dohányos and Zábranská, 2001).

4.5.1 COD

Retention time had positive impact on the chemical oxygen demand of the samples in that COD decreased under both temperatures as the number of days increased (figure 4.20).

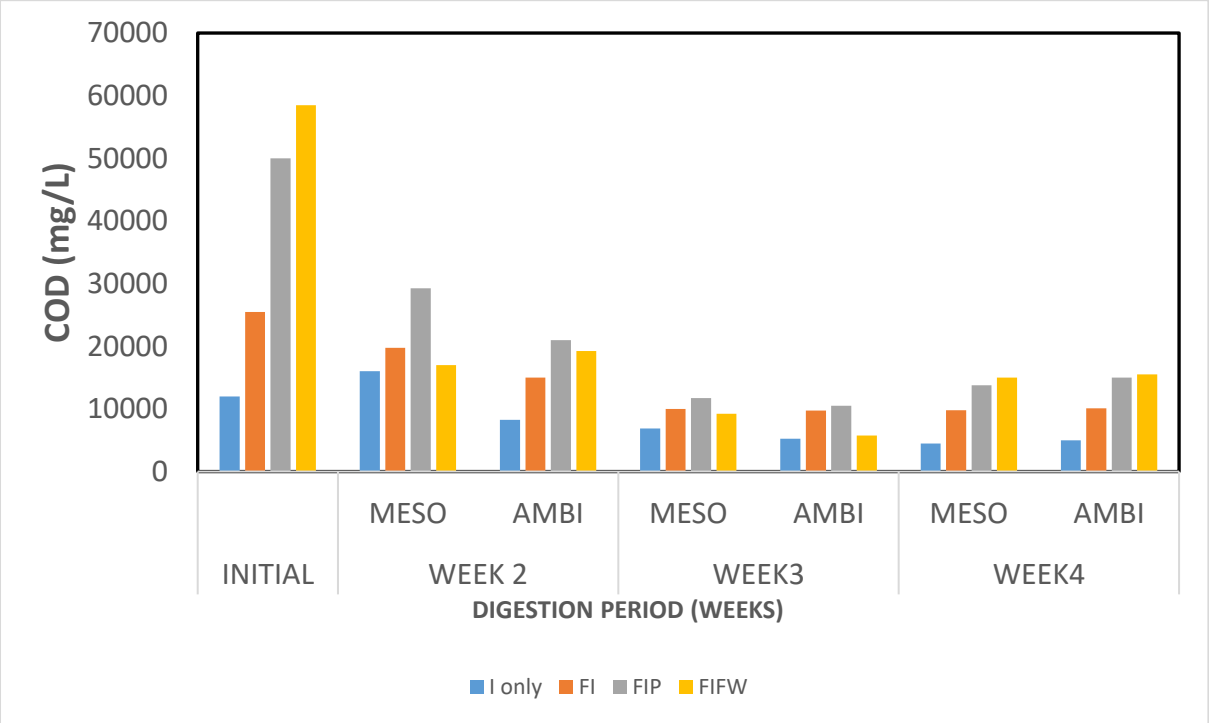

Figure 4.20: COD of substrates over digestion period

COD removal is an indication of the anaerobic digestion process (Bahtiyar, 2012). This shows that longer retention times is key to stabilizing sludge in anaerobic digestion as the results in figure 4.20 indicates a better COD removal over longer retention times.

4.5.2 TKN

The total kjeldahl nitrogen content for the substrates used over the digestion period is presented in figure 4.21 below

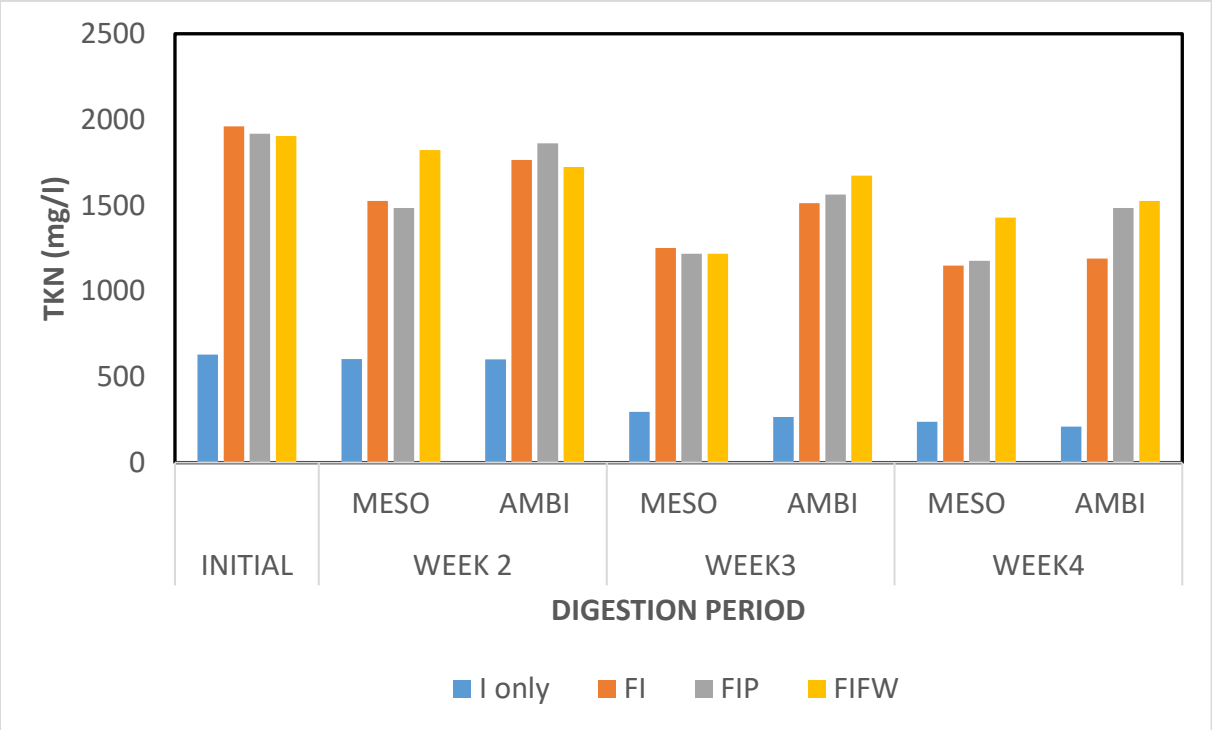

Figure 4.21: TKN of substrates over digestion period

The TKN values (figure 4.21) for all the substrates decreased along the weeks under both temperatures. FIP decreased from 1918 mg/L to 1176 mg/L and 1484 mg/L under mesophilic and ambient temperatures respectively while FIFW decreased from 1904 mg/L to 1428 mg/L and 1326 mg/L under mesophilic and ambient temperatures respectively. Hobson et al. (1974) also reported a decrease in nutrient concentration after anaerobic digestion.

4.5.3 TOTAL PHOSPHORUS

The total phosphorus content for the substrates used over the digestion period is presented in figure 4.22 below

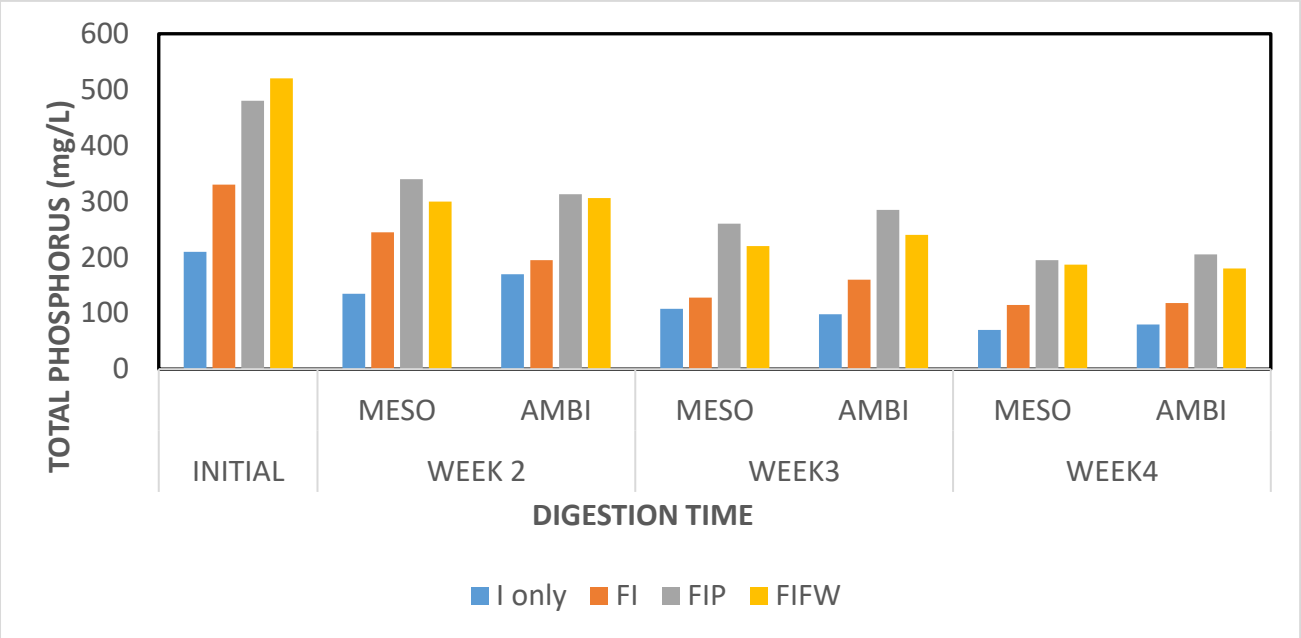

Figure 4.22: Total phosphorus of substrates over digestion period

The total phosphorus concentration also took a decreasing trend over the digestion period. FIP decreased from 480 mg/L at the start of experiment to 195 mg/L and 205 mg/L under mesophilic and ambient temperatures respectively while FIFW decreased from 520 mg/L to 187 mg/L and 180 mg/L under mesophilic and ambient temperatures respectively at the end of the experiment.

4.5.4 HELMINTH EGGS

The helminth eggs concentration for the substrates used over the digestion period is presented in figure 4.23 below

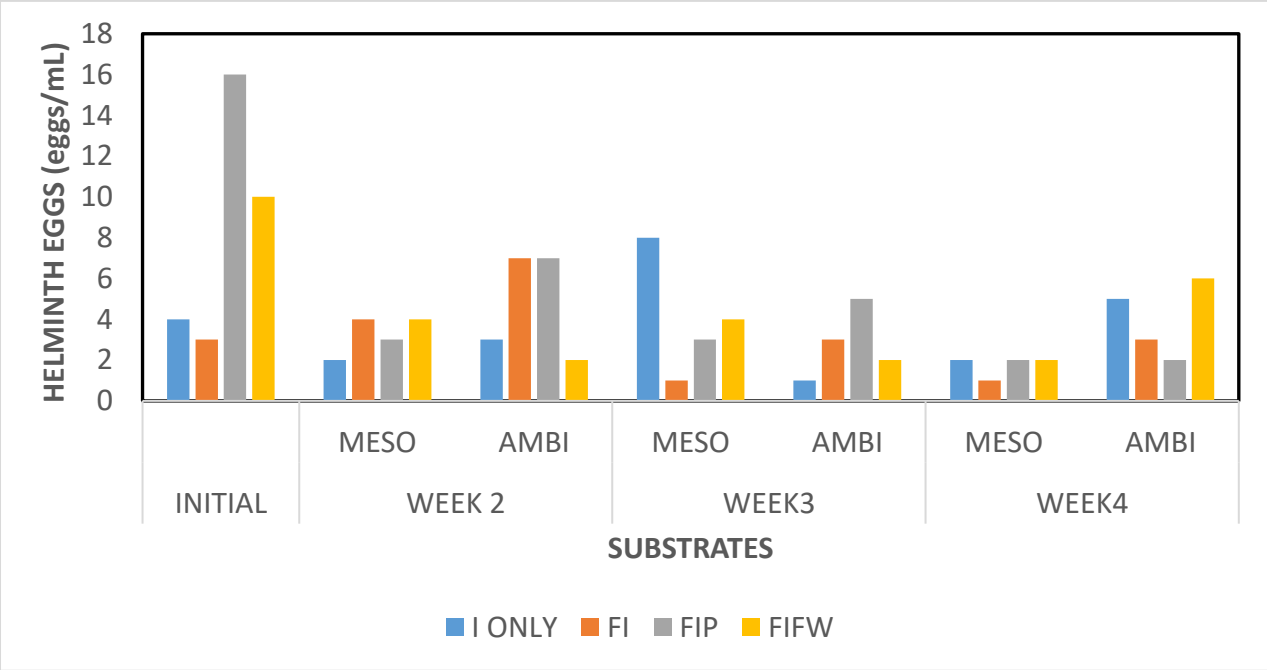

Figure 4.23: Helminth eggs concentration of substrates over digestion period

The main helminth eggs found in the substrates were *Ascaris lubricoides, Hookworm, Trichuris trichiura, Strongyloides stercoralis* and *Schistosoma (mansoni, haematobium).* Helminths eggs are relevant because they are able to withstand many mechanisms of disinfection, including inactivation of anaerobic digestion-related physical or chemical treatments (Ghiglietti et al., 1997). *Ascaris lumbricoides*, the intestinal roundworm, infects almost 800 million individuals worldwide, particularly in tropical and subtropical areas. To spread from host to host, it depends on ova deposition on moist soils (Pullan et al., 2014).

Although the number of helminth eggs per millilitre of most of substrates recorded decreased with in the 4th week compared to that which was recorded at the start of the digestion period, except for FIP which had a decreasing pattern, the rest did not follow a particular pattern. FIFW had an initial Helminth eggs concentration of 10 eggs/ml and decreased to 2 eggs/ml in

the 3rd week and then increased to 6 eggs/ml under ambient temperature had an initial concentration of 3 eggs/ml but increased to 4 and 7 under mesophilic and ambient temperatures respectively.

According to Popat et al. (2010) and Manser et al. (2015), there is minimal effect on inactivating *Ascaris* at mesophilic temperatures. Ghiglietti et al. (1997) stated that due to the nature of helminth eggs, they are mostly removed by settling in anaerobic digesters. This may have accounted for the irregular pattern of the concentrations recorded. Pecson et al (2007) also discovered that the time required to fully inactivate helminth eggs ranged from 5 days to 180 days for temperatures between 30°C and 40°C. This shows that at an appropriate temperature, retention time will contribute to the removal of the eggs.

Based on the findings of this research, longer retention times will be advised or additional treatment will be necessary before the digestate is discharged into the environment for shorter retention times.

4.5.5 SUMMARY OF DIGESTATE / EFFLUENT CHARACTERISTICS

The final characteristics of the substrates used in this experiment are presented in table 4.2 below

Table 4.2: Final effluent characteristics of substrates used

	I ONLY		FI		FIP		FIFW	
	MESO	**AMBI**	**MESO**	**AMBI**	**MESO**	**AMBI**	**MESO**	**AMBI**
pH	7.91	7.03	7.97	7.66	7.52	7.2	7.57	7.2
TS (%)	0.46	1.57	0.78	0.68	5.04	5.28	0.4	1.02
VS (%)	80	64.7	66.67	63.63	83.82	77.32	78.57	64.52
COD (mg/l)	4500	1700	3700	2600	13800	19600	3700	4000
TKN (mg/l)	238	210	1148	1190	1176	1484	1428	1526
TP (mg/l)	70	80	115	118	195	205	187	180
HE (eggs/ml)	2	5	1	3	2	2	2	6

Although there has been some level of removal of these parameters, comparing the effluent quality with the Ghana EPA discharge standards and WHO effluent guidelines of 250 mg COD/L, 75 mg N/L, 2 mg P/L, and <1 helminth eggs /L, then post treatment steps such as sedimentation, coagulation and flocculation must be applied to further reduce these concentrations.

Nutrient removal is very important to any system used in wastewater treatment depending on the final use or disposal of the effluent. In cases where the final effluent is discharged into the environment (typically a water body), organics, nitrogen and phosphorus removals are of great importance as the effluents usually contain higher concentrations than permissible discharge concentrations. Phosphorus for instance, is a limiting factor to algae proliferation

and must be reduced to control eutrophication in the receiving waster body (Xu et al., 2010). High concentrations of inorganic nitrogenous compounds, especially ammonia, are poisonous to aquatic life (Camargo et al., 2005).

In the U.S., most anaerobic digestion effluent is utilized for on-site agricultural applications. The liquid portion is used as fertilizer, while the solid portion is composted and used for agriculture or as animal bedding (Alexander, 2012). In such cases, effective measures need to be taken to ensure that the effluents do not contain any contaminants (Frischmann, 2012). Also, the digestate can generate energy through combustion, and the ash can be utilized in building materials (Li et al., 2013).

The results from this experiment confirms that post treatment of anaerobic digestion unit effluents is very necessary in order to produce effluent of high quality.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

The characterization of faecal sludge used in this study confirmed it was inadequate to be used alone for anaerobic digestion as the carbon to nitrogen ratio (8.11) was below the recommended range (20 -30).

The study also showed that anaerobic co-digestion improves the characteristics of faecal sludge for anaerobic digestion. The C/N of the co digested samples faecal sludge, inoculum and paper (FIP) (26.95) and faecal sludge, inoculum and fruit waste (FIFW) (27.14) fell within the recommended range and therefore had higher methane yield (FIP: 16.5 – 47.05%, FIFW: 26.1 – 39.75% for mesophilic temperature and FIP: 14.3 – 41.1%, FIFW:19.5 –45.7% for ambient temperature and better effluent characteristics compared to faecal sludge and inoculum (FI) with methane yield of 18.9 – 23.7% and 3.8 – 17.7% for mesophilic and ambient temperatures.

Although there were some variations in the performances of the substrates used under mesophilic (35 ^{o}C) and ambient temperatures (24 – 32 ^{o}C), the analysis of variance 0.003 (less than the alpha value of 0.05) indicated that there was significant difference between the outputs under the two temperature regimes however further analysis showed that the difference is only with performance in the first week (the *p* value of week 1 was less than 0.05 while those of week 2 to 4 were greater than 0.05).

Comparing the characteristics of the digesters as the experiment proceeded revealed that longer sludge retention time produces a better stabilized sludge and improved effluent characteristics. The anaerobic co-digestion process was able to reduce the pollutant loads in the substrates to levels stipulated in literature (55 to 75%) for anaerobic digestion. The

temperatures in this experiment were inadequate for complete sterilization of the faecal sludge as Helminth eggs removal was not successful.

COD removal efficiency for FIP were 72.40% and 72% and 74.36 % and 73.50 % for FIFW under mesophilic and ambient temperatures respectively

Nitrogen percentage removal ranged from 20.75 - 62.22 % for digesters at mesophilic temperature and 19.85 – 66.67 % for digesters at ambient temperature.

Although the treatment process was able to reduce the pollutant loads, the final effluent characteristics were above levels permissible for discharge into the environment by the Ghana Environmental Protection Agency and thus, further treatment will be required before it can be safely discharged into the environment.

5.2 RECOMMENDATIONS

The following suggestions were made on the basis of the results of this research:

1. The experiment should be repeated with similar substrates under temperatures with a wide variability and then compare the outcome to the findings of this experiment.
2. The experiment should be repeated under similar temperature ranges but considering longer sludge retention times than the one used in this study.
3. Future experiments using paper as a co digestate should consider pre-treatment techniques to enhance its characteristics for anaerobic digestion.
4. A pilot field test should be performed to evaluate the real-time feasibility in order to be well informed towards the design of large scale AD systems
5. Post treatment options particularly, the use of facultative and maturation ponds are highly recommended to further stabilize the anaerobic digestion effluent before end use or discharge into the environment.

6. The effluent from substrates used in this experiment should be studied into to confirm its safety for direct used on plants and for soil enhancement.

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APPENDIX

Table 1: pH of Substrates Over the Digestion Period

	INITIAL	WEEK 2		WEEK3		WEEK4	
		MESO	AMBI	MESO	AMBI	MESO	AMBI
I only	7.4	6.95	6.02	6.79	5.74	7.91	7.03
FI	8	7.86	7.91	8.25	7.59	7.97	7.66
FIP	8.2	7.47	7.69	7.75	7.4	7.52	7.2
FIFW	7.9	7.96	7.63	7.6	7.4	7.57	7.33

Table 2: COD of Substrates Over the Digestion Period

	INITIAL	WEEK 2		WEEK3		WEEK4	
		MESO	AMBI	MESO	AMBI	MESO	AMBI
I only	12000	16000	8250	6875	5250	4500	5000
FI	25500	19750	15000	10000	9750	9800	10100
FIP	50000	29250	21000	11750	10500	13800	15000
FIFW	58500	17000	19250	9250	5750	15000	15500

Table 2:TKN of Substrates Over the Digestion Period

	INITIAL	WEEK 2		WEEK3		WEEK4	
		MESO	AMBI	MESO	AMBI	MESO	AMBI
I only	630	224	602	266	266	238	210
FI	1960	1526	1764	252	1512	1148	1190
FIP	1918	42	1134	1484	1862	1176	1484
FIFW	1904	1722	1624	1218	1372	1428	1526

Table 3:TP of Substrates Over the Digestion Period

	INITIAL	**WEEK 2**		**WEEK3**		**WEEK4**	
		MESO	**AMBI**	**MESO**	**AMBI**	**MESO**	**AMBI**
I only	45	135	80	410	270	260	180
FI	127.5	115	65	245	295	330	260
FIP	157.5	195	65	360	435	780	1225
FIFW	187.5	45	130	280	315	300	315