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ANALYSIS OF ABATTOIR WASTE FOR BIOGAS PRODUCTION IN KUMASI GHANA

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DECLARATION

I, Amos Chenamani, hereby declare that this thesis represents my personal work, realized to the best of my knowledge. I also declare that all information, material and results from other works presented here, have been fully cited and referenced in accordance with the academic rules and ethics.

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ABSTRACT

Animal rumen content and blood waste have been found to increase the economic feasibility of biogas plants. However, there are problems that may occur in a full-scale biogas plant using abattoir waste which can have significant impacts on anaerobic process efficiency. Therefore, the purpose of this study was to analyse abattoir waste characteristics for anaerobic biogas energy production in Kumasi Ghana. Cattle Rumen Content and Blood waste were collected for Laboratory Analysis. The Rumen content were found to be having 14.47±0.89% Total Solids, 84.63±1.30% Volatile Solids, 38.43±11.32% Carbon and 4.90±4.17% Nitrogen. The Blood waste was found to have 17.36±0.82% Total Solids, 95.02±1.25% Volatile Solids, 40.80±4.44% Carbon and 9.90±0.09% Nitrogen. Due to the different backgrounds of the cattle slaughtered at the abattoir, statistical analysis proved that there is variation in the Rumen content and Blood waste characteristics. Biogas produced from the anaerobic digestion of Rumen Content, Blood wastes and mixture of Blood and Rumen Content at 37 °C found methane content of 46%, 5.5% and 39.9% respectively. Findings from this study will assist in anaerobic digestion process design, monitoring and operation. It is recommended that further research is conducted into ways to improve biogas production from abattoir waste.

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LIST OF ABBREVIATIONS

AC …….Ash Content
C/N Ratio……..Carbon to Nitrogen Ratio
DM……...Dry Matter
EU……European Union
HRT…….Hydraulic Retention Time
KNUST……Kwame Nkrumah University of Science and Technology
LCFA…….....Long chain fatty acids and glycerol
MC…….Moisture Content
Mc…….....Mass of Crucible
OLR……Organic Loading Rate
pH……Hydrogen Ion Concentration
TS……...Total Solids
VFA…….Volatile fatty acids
VS……...Volatile solids
FS……...Fixed Solids
DS…….. Dry Sample
WS……Mass of Wet Sample

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1 INTRODUCTION

1.1 Background of the study

Human beings' basic needs of water, food and shelter cannot be met without the need for energy. Therefore, Energy is considered as the backbone of economies and societies (Reza *et al.*, 2016; Smith, 2008). It is needed for mobility, heating, cooling and lighting (Ezekiel *et al.*, 2015). However, it is one of the most difficult challenges faced by mankind in the 21st century, as the world is growing the population is increasingly becoming dependent on fossil fuels (Reza *et al.*, 2016; Rajvanshi, 2010). The use of fossil fuel energy has led to the environmental problems and changes in climates (Frederica, 2018).

The need to ensure access to affordable reliable, sustainable, modern energy to developing countries continues, while taking urgent action to combat climate change and its impact has become a trending concern for most countries (AUC, 2015). Given the positive correlation between energy access and human development, access to modern energy services is vital for sustainable development. Most populations in African countries have less access to electricity and rely on wood, charcoal or Biomaterials for their energy needs (Mensah and Adu, 2013). Ghana's population is not different from the rest of the South of Saharan Countries, in many instances the electricity demand in Ghana is often too high to be met (Samuel *et al.*, 2015). Although, Ghana has one of the highest rates of access to electricity (IEA, 2014).

The Electricity consumption in the residential, commercial and industrial sectors has been growing at a rate well above 10% annually over the last decade, which has put considerable pressure on generation (Samuel *et al.*, 2015; PSEC, 2010). The unsuppressed peak demand and energy growth rates were 44% and 100% from 2000 to 2009 respectively (Samuel *et al.*, 2017; GEC, 2013). Among the three demand sectors, the residential and commercial sectors have been growing at the highest rate and have seen a total of 21.8% increment from 2000 to 2013 (GEC, 2013).

The existing power plants were unable to achieve full power generation capacity due to fuel supply constraints (Maame and Joe, 2016). Non-perennial rivers together with inadequate and unreliable rainfall arising due to climate changes particularly in northern

Ghana have significantly reduced inflows into hydroelectric power plants (Ebenezer, 2017). Currently thermal power dominates Ghana´s energy generation portfolio (Samuel *et al.,* 2017). About 51% of Ghana's electricity is generated from imported fossil fuels (Kwesi *et al.,* 2017; EC, 2015). Ghana's energy system is likely to suffer additional strain from rising energy demand fuelled by economic development, rapid urbanization and population growth (Marriette *et al.,* 2017).

The rapid increase in population witnessed by Ghana puts a strong declining thrust on the national resources and generating a burgeoning amount of waste (Samuel *et al.,* 2017). Thus, it is expected that the nation works towards maximum usage of resources as well as recovering whatever one could out of the available resources (Ebenezer, 2017). This will create a path towards attaining sustainability in terms of Reduce, Reuse and Recycle which should be kept in mind while working towards resource utilization. If optimum resource utilization is not supervised upon, it can lead to an increase in waste, pollution and a downfall in the economy.

The uncontrolled urbanization in Ghana has not allowed the towns and cities to cope up (George, 2010). They lack basic amenities like a proper sewage system, drainage system, agricultural waste management systems, which has led to a huge change in the amount of waste generated. This has led to an increased burden on the government, local authorities and the urban local bodies to manage the collection, processing and disposal of waste (George, 2010). The most common practice of managing waste today is landfilling, which poses a huge threat to the environment in the form of greenhouse gases emissions in the form of CO_2 and CH_4 and leachate production (Motasem *et al.,* 2016 and Simone *et al.,* 2009). Thus, there is an urgent need to come up with an environmentally, economically and socially sustainable solid waste management process (Kunwar *et al.,* 2017).

Waste to energy is one such processes that has not been taken serious in Ghana (Mohammed, 2017), despite the strong potential to derive energy from the unused waste resources as shown from different studies (Kodwo *et al.,* 2015; Kemausuor *et al.,* 2014; Mohammed *et al.,* 2013; Ofori-Boateng *et al.,* 2013), as many countries have started taken a step forward in recovering energy from waste. Therefore, there is a need for

Ghana to integrate a broad range of non-conventional energy technologies into its generation portfolio in order to improve energy security and provide insulation against external shocks such as price spikes of fossil fuels. Waste Energy as Sustainable energy systems can provide such an opportunity. Biogas produced from anaerobic digestion of organic wastes is a renewable methane content energy source for future and it provides sustainable energy supply (Akash *et al.,* 2017). In this regard, agriculture is a sector with many activities and necessities that generate a huge amount of biodegradable organic waste, which can be utilized for biogas production (Almomani, 2016). Consequently, Agriculture has a unique position to satisfy both food demand and biogas supply in farms, rural communities, cities, as well as can be applied as fuel within the transport and industrial sector.

In Ghana, the city of Kumasi host one of the big abattoirs in the country, with a slaughtering capacity of 7000 or more cattle per month (Daniel *et al.*, 2014). Each day, the abattoir generates a large amount of cattle rumen content and blood waste and the Kumasi Abattoir Company is intending to turn some of its waste to Biogas energy. Animal rumen content and Blood waste from the abattoir have high content of carbohydrates, proteins, fats, cellulose, and hemicelluloses as the main component of the waste (Mats *et al.,* 2003). Therefore, the Methane potential will be high on the abattoir waste, making it a highly demanded substrates for biogas production on competitive market basis especially in developed countries.

1.2 Statement of the Problem

The Animal rumen and blood waste will increase the economic feasibility of biogas plants. However, there are problems that may occur in a full-scale biogas plant using abattoir waste which can have significant impacts on anaerobic process efficiency, operational costs, and economic profitability (Ek *et al.,* 2011). Apart from the digester design problems and maintenance, operational problems such as formation of foam can be mentioned, which is usually caused by the high content of lipids and proteins present in these residues usually inhibit anaerobic digestion process performance (Boe *et al.,* 2012). The potential inhibition of the microbial activity may occur, subsequently leading to low methane production, process instability, and finally, failure of the biogas

digestion process (Bayr *et al.,* 2012). The slow degradation rate of the abattoir waste will lead to the accumulation of intermediary compounds such as long chain fatty acids, volatile fatty acids, ammonia, and ammonium nitrogen, causing inhibition in the system (Palatsi *et al.,* 2010).

1.3 Justification of the Study

Since proper development of the Anaerobic Digestion process is highly dependent on the type and the composition of the materials to be treated. The composition of animal residues differs from one another, depending on several factors such as weather conditions, animal breed, age, and the quantity/quality of the food. Therefore, there is a need to establish the baseline characteristics of the Kumasi abattoir waste resources as the cattle's slaughtered there, have different feeding background and are from different Ghana's regions. In addition, most literature on Kumasi abattoir waste is not published or readily available.

In order to optimize the Anaerobic Digestion process, it is important to know the characteristic composition and potential production of biogas of a given substrate material (Raphael and Reckson, 2017). Important substrate characteristics include Total solid, Volatile solids content, Carbon to Nitrogen ratio (C/N), Biogas Potential, and presence of inhibitory substances (Kwietniewska and Tys, 2014; Babaee and Shayegan, 2011). One suggested optimization solution to the anaerobic digestion process is the application of co-digestion of substrate (Wang *et al.,* 2014). In this system, abattoir wastes can be treated together with the other residues generated during other agricultural activities, but still the system needs substrates characterization for maximum efficiency (Jhosané *et al.,* 2015). The main advantages of co-digestion are related to a balanced nutrient supply, better carbon to nitrogen (C/N) ratio, the dilution of inhibitory compounds, as well as to a more efficient utilization of the digester plant to treating several wastes at the same time (Lima *et al.,* 2017).

Energy supply shortages at Kumasi hinder the abattoir's smooth running and operations which reduce its profit both financially and environmentally. During energy shortages the slaughtered animals are alternatively smoldered using spent vehicle tyres while administrative activities becomes slow and inefficient. Unbearable smoke from burning

the tyres pollutes the atmosphere and also adds to greenhouse emissions. The smoldered animals also become harmful for human consumption due to contaminations. Therefore, the aim of this case study, is to investigate the suitability of cattle ruminal content and bloods waste characterization for anaerobic digestion process, as a way of mitigating environmental contamination by producing biogas energy for Kumasi Abattoir.

1.4 OBJECTIVES

1.4.1 Main Objective

To analysis the cattle rumen content and blood abattoir waste for biogas production in Kumasi, Ghana.

1.4.2 Specific Objective

1) To determine the volatile solids and other properties of Abattoir waste for biogas production in Kumasi.

2) To study the variation in the characteristic properties of the Kumasi Abattoir waste.

3) To compare the characteristics of the Kumasi Abattoir waste before and after the anaerobic digestion.

4) To determine the biogas quality of the gas produced from the Kumasi abattoir waste.

1.4.3 Research Questions

1) What are the characteristic values of the Kumasi abattoir waste?

2) Does the waste of cattle slaughtered from the Kumasi abattoir have variation in composition?

3) What is the difference of abattoir waste characteristics before and after anaerobic digestion process?

4) What is the quality of the biogas that can be produced from the Kumasi abattoir waste?

2 LITERATURE REVIEW

2.1 INTRODUCTION

2.1.1 Overview of Biogas

Biogas is a renewable biological gas which is produced when organic materials are broken down by microorganisms in an anaerobic environment (Kayode and Jude, 2015). The gas is mainly composed of varying amount of Methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), hydrogen (H_2), hydrogen sulphide (H_2S) and oxygen (O_2) (Sarikaya and Demirer, 2013; Al Seadi *et al.,* 2008). The energy component of the biogas is carried by Methane composition and the higher the methane content the higher the calorific value of the biogas produced (Raphael and Reckson, 2017). When biogas reacts with oxygen it releases the heat energy which is considered clean. It is named sustainable energy because it is a valuable source of energy in rural areas and can act as an alternative replacement for natural gas (Balat, M. and Balat, H., 2009). The amount of biogas is usually stated in units of normal cubic meters (Nm^3), which is defined as the volume of gas at 0 ^oC and atmospheric pressure (Held *et al.,* 2008). Table 2.1 summarizes the biogas properties.

Table 2.1: Biogas properties.

Property	Quantity	Units
Energy content	6.0 – 6.5	kWh/m^3
Fuel equivalent	0.5 – 0.65L	oil/m^3 biogas
Explosion limits	6 – 12	% biogas in air
Ignition temperature	650 – 750	^oC
Critical pressure	75 – 89	bar
Critical temperature	-82.5	^oC
Normal density	1.2	kg/m^3
Molar mass	16.043	kg/kmol

Source: Modified from Deublein and Steinhauser (2008).

2.1.2 Uses of Biogas

The energy in biogas can be utilised in many ways as it can be used as a fuel for 1) heat generation for cooking or Home heating, 2) Electricity generation through combined heat and power production Technologies and 3) Transportation. The heat generated

could also be used to power industrial boilers which may also generate steam for heat exchangers, while at the abattoir the heat generated may be used for smouldering slaughtered animals (Held *et al.,* 2008). Traditionally, biogas has been burned in internal combustion engines for the electricity production and heat, but its potential use in fuel cells could increase its electric efficiency, especially in applications at low scale, diminishing the NO_x emissions to the atmosphere (Benito *et al.,* 2007). The use of Biogas as a transport fuel requires that impurities such as carbon dioxide and water content to be removed or scrubbed off the biogas. Scrubbing usually increases the calorific value of the gas and usually the Methane enriched biogas results in more than 97% methane content. The enriched methane has an energy value of 36.6 MJ/m^3 and it can replace 1 litre of petrol (Murphy, 2005) hence biogas can have many potential uses.

2.2 THE BIOGAS GENERATION PROCESS

Anaerobic digestion is a biological process in which large organic molecules (carbohydrates, proteins and lipids) are digested or broken down through actions of different groups of microorganisms (Bacteria and Archaea) to produce biogas in the absence of oxygen. The microorganisms involved in the anaerobic digestion process are acidogenesis, acetogenesis and methanogenesis bacteria (Comparetti *et al.,* 2013). The process takes place through a series of parallel and serial-parallel biochemical reactions, in which active microbial community has to work together.

The anaerobic process can occur where there is high concentration of wet organic matter accumulating in the absence of dissolved oxygen. It can occur on natural environments such as bottom sediments of lakes and ponds, in swamps, peat bogs, and intestines of animals and in the anaerobic interiors of landfill sites (Vigneron *et al.,* 2017 and Katsunori *et al.,* 2011). It can also take place in engineering designed environment if required process conditions are provided. The AD process can be divided into four main degradation steps of Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis, as it is schematically shown in the Figure 2.1.

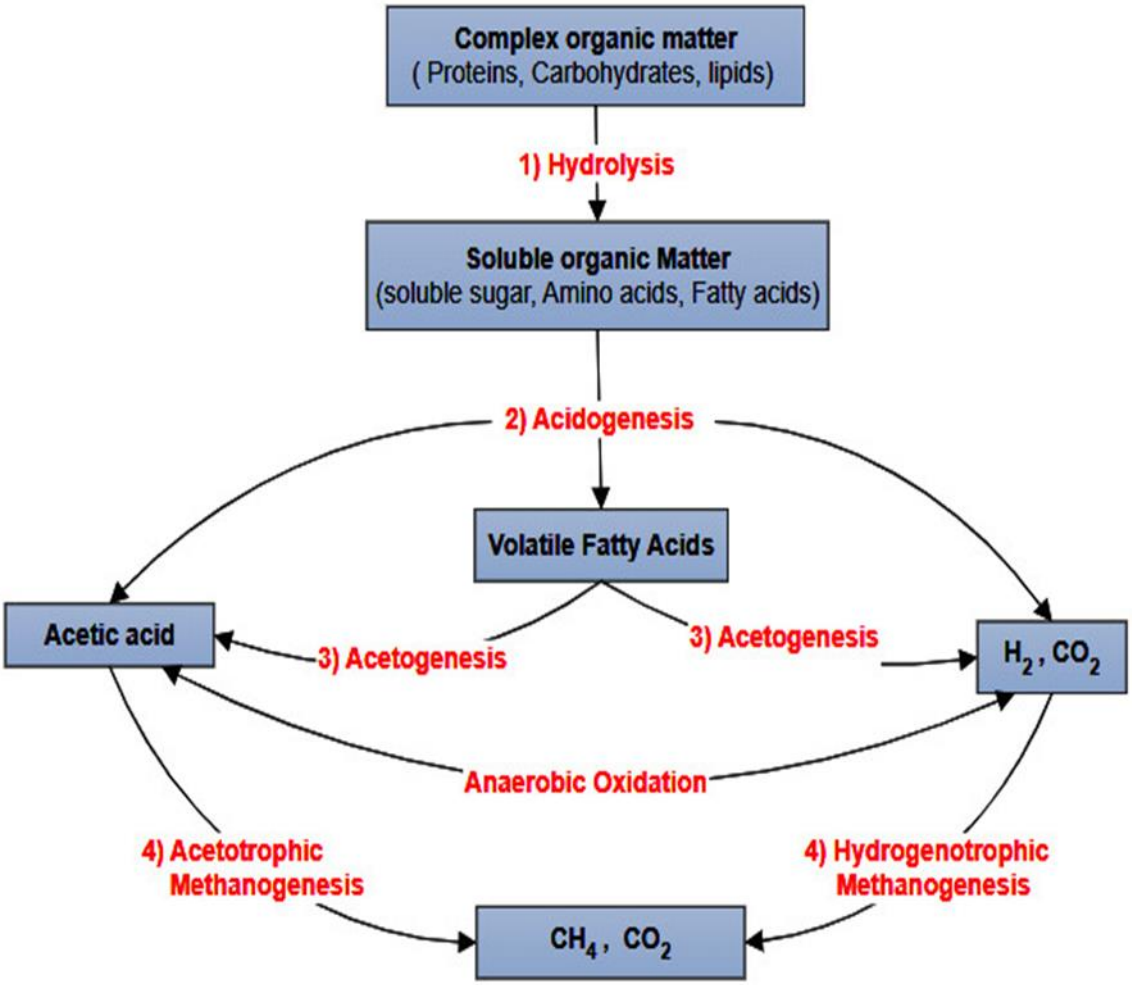

Figure 2.1: Stepwise decomposition of organic matter into biogas.

Source: Modified form Ernesto *et al.*, (2013).

2.2.1 Hydrolysis

In the first step called Hydrolysis, large organic matter containing carbohydrates, proteins and lipids are hydrolysed to produce small organic compounds such as soluble sugar, amino acids, fatty acids and alcohol (Gonzalez *et al.*, 2015). The Hydrolysis is carried out through the action of different extracellular enzymes, such as proteinases and lipases which are excreted by the hydrolytic bacteria (Weiland, 2010). The different extracellular enzymes "cut" the larger molecules (carbohydrates, proteins and lipids) up into smaller pieces that the bacteria microorganism can then take into the cell and use as a source of energy and nutrition (Mahdy *et al.*,2016).

Different Substrate will have different microorganisms which secrete several different enzymes to allow them to hydrolyse different types of organic molecules. The proteins

are first broken down to amino acids by the proteinases, and the carbohydrates are broken by the cellulases, hemicellulases, and amylases to simple sugars (Boontian, 2014). The lipids are broken down to produce glycerol and long-chain fatty acids (LCFAs) by lipases (Ward *et al.*, 2014). Table 2.2 contains a summary of different extracellular enzymes.

Table 2.2: some important groups of hydrolytic enzymes and their functions.

Enzymes	Substrate	Breakdown Products
Proteinase	Proteins	Amino acids
Cellulase	Cellulose	Cellobiose, glucose
Hemicellulase	Hemicellulose	Sugars, such as glucose, xylose, mannose, arabinose
Amylase	Starch	Glucose
Lipase	Fats	Fatty acids and glycerol
Pectinase	Pectin	Sugars such as galactose, polygalactic uronic acid

Source: Modified from Schnürer and Jarvis (2009).

The rate of hydrolysis step dependents on the complexity and nature of the substrate such as substrate concentration, particle size, the pH value and the substrate temperature (Boontian, 2014). In the presence of suitable enzymes produced from microorganisms the Hydrolysis step is relatively rapid. However, if the substrate is hardly accessible for enzymes, the hydrolysis step will be limited for the reason that physical contact between the substrate and enzymes is needed for hydrolysis to happen (Taherzadeh and Karimi, 2008). However, the substrate may be enhanced by mechanical, thermal or chemical pre-treatment to increase the hydrolysis rate and availing the substrate for further digestion process steps (Zupančič and Grilc, 2012).

2.2.2 Acidogenesis

The Acidogenesis is the second step on the biogas process. The soluble organic products of simple sugars, amino acids, and long chain fatty acids formed during the hydrolysis step are further converted by microorganisms into shorter chain organic compounds containing one to five carbon units (Ali Shah *et al.*, 2014). The main products formed

during the acidogenesis step are volatile fatty acids (VFAs), like acetic, propionic, valeric, and butyric acids (Table 2.3) (Lee *et al.*, 2014). During the Acidogenesis many reactions can take place depending on the type of the substrate characteristics, environmental conditions and the types of microorganisms available. Furthermore, products such as alcohol, hydrogen, Carbon dioxide and ammonia are also produced as sugars are turned into volatile fatty acids while amino acids are degraded into acetate, ammonia, carbon dioxide, and hydrogen sulphide (Madigan *et al.,* 2010).

The acids formed are typical in a way that, the charged form (without protons) is in equilibrium with the uncharged form (with protons, Eq 1). The acid constant (pKa) indicates how easily the acid releases its proton. If the pH is below the pKa-value, the majority of the acid is in its uncharged form, while at a pH above the pKa-value it is mainly in the charged form. In a biogas process at pH > 7, acids are mainly in the charged form (anion). At this stage, they tend to form salts with different metals such as sodium and potassium. The acid form and anion have different names (for example acetic acid (acid) and acetate anion, Table 2.3).

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+ \qquad \text{[Eq 1]}$$

Acetic acid is in equilibrium with its anionic form, acetate

Table 2.3: Names of some common acids and their pKa values and chemical structure. The values apply to aqueous solutions at 25°C.

Common name	Systematic name	Anion	pKa	Chemical structure (acid form)
Formic acid	Methanoic Acid	Formate	3.77	HCOOH
Acetic acid	Ethanoic acid	Acetate	4.76	CH 3 COOH
Propionic acid	Propanoic acid	Propionate	4.8	CH 3 CH 2 COOH
Butyric acid	Butanoic acid	Butyrate	4.83	CH 3 CH 2 CH 2 COOH
Valeric acid	Pentanoic acid	Valeriate	4.84	CH 3 CH 2 CH 2 CH 2 COOH
Caprylic acid	Hexanoic acid	Capronate	4.85	CH 3 CH 2 CH 2 CH 2 CH 2 COOH

Source: Modified from Schnürer and Jarvis (2009); Madigan *et al.,* (2003).

2.2.3 Acetogenesis

Acetogenesis is taken to be the third step of the anaerobic digestion process. At this stage, VFA, alcohols and other products from the Acidogenesis step, which could not

be converted to methane directly by methanogenesis bacteria are converted to methanogenic substrates by acetogenic bacteria. VFA, with carbon chains longer than two units and alcohols, with carbon chains longer than one unit, are oxidized into acetate and hydrogen (Al Seadi *et al.,* 2008). The production of hydrogen increases the hydrogen partial pressure. This inhibits the metabolism of the acetogenic bacteria. However, methanogenesis bacteria convert hydrogen into methane thereby reducing the hydrogen partial pressure. Acetogenesis and methanogenesis therefore run as symbiosis of two groups of organisms (Al Seadi *et al.,* 2008).

2.2.4 Methanogenesis

Methanogenesis is the final step of the anaerobic digestion steps. After the Acidogenesis step the Methanogenic bacteria uses acidogenesis products to produce methane. The methanogenesis step can be achieved using two pathways. In one path carbon dioxide and hydrogen are converted to methane. In the other path acetate is converted into methane, hydrogen and carbon dioxide. According to Al Seadi *et al.,* (2008) about 70% of the methane is produced from acetate while the remaining 30% is produced from the conversion of hydrogen and carbon dioxide. However, there are three types of methanogenic bacteria in the methanogenesis step which include hydrogenotrophic methanogens, acetotrophic methanogens and methylotrophic methanogens (Al Seadi *et al.,* 2008). The hydrogenotrophic methanogens use hydrogen to reduce carbon dioxide into methane. As a result they help to reduce hydrogen pressure. The chemical equation shown in Eq 2 summarizes this conversion (Funda, 2011).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{[Eq 2]}$$

The acetotrophic methanogens convert acetate into methane and carbon dioxide. The carbon dioxide produced is used by hydrogenotrophic methanogens. This group of methanogens is affected more by hydrogen pressure. The chemical equation shown in Eq 3 summarizes this conversion (Funda, 2011).

$$4CH_3COOH \rightarrow 4CO_2 + 2H_2 \qquad \text{[Eq 3]}$$

The methylotrophic methanogens convert methyl groups such as methanol and methylamines into methane. Eq 4 summarizes this conversion (Funda, 2011).

$$3CH_3COH + 6H_2 \rightarrow 3CH_4 + 2H_2O \quad \text{[Eq 4]}$$

All types of methanogens can produce methane but they differ in structure, enzymes, substrate utilization and temperature range for growth. Digester overloading, temperature changes or large entry of oxygen can result in termination of methane production.

2.3 FACTORS THAT INFLUENCE ANAEROBIC DIGESTION

Some of the factors which affects the anaerobic digestion inputs, processing and output products, are discussed below.

2.3.1 Microorganisms in anaerobic digestion

A complex microbiological process lies behind the efficient production of biogas. Many different species of microorganisms need to be active in order for biogas to form. In addition, these organisms have to work closely together. A disturbance of this teamwork results in reduced biogas production and, in the worst case scenario, a breakdown of the process. Therefore, the analysis to have an efficient biogas process needs knowledge of the microbiology behind the biogas process and how microorganisms grow and function during the process (Viola, 2015).

Microorganism needs access to an appropriate substrate culture medium in order to thrive and function well (Morris *et al.*, 2013; Viola, 2015). The substrate which acts as the food for microorganism must contain different elements of energy sources, electron acceptors, building blocks for building new cells and different types of vitamins and trace elements (Schnürer and Jarvis, 2009). When the microorganisms get access to a substrate, they metabolize that is building up new cells (anabolism) and produce energy (catabolism) for their growth (Figure 2.2) (Morris *et al.*, 2013). The organic waste treated in the biogas process represents the substrate for various microorganisms. The more varied the composition of the organic material, the more components are available for growth and thus the greater diversity of organisms that can grow (Schnürer and Jarvis, 2009).

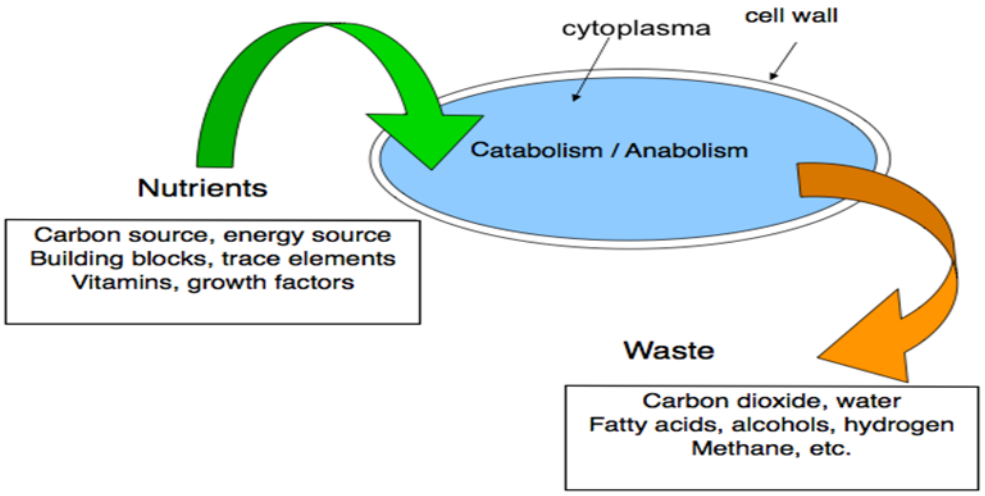

Figure 2.2: Cell metabolism.
Source: Modified form Viola (2015); Schnürer and Jarvis (2009).

However, it is not good if the composition varies too much with time because many of the microorganisms that develop during the process are specialists. That is they grow best on a specific substrate (Schnürer and Jarvis, 2009). When microorganisms utilize substrate, they form new cells and other waste products (Schnürer and Jarvis, 2009). The waste products excreted by a specific organism cannot be used by it any longer but it can serve as a substrate for another microorganism (Morris *et al.*, 2013). This is typical of the biogas process where a series of different microorganisms utilize each other's decomposition products as substrate. Examples of microbial waste products in a biogas process are fatty acids, carbon dioxide, and hydrogen. Methane, which is the end product of a biogas process, is microbial waste product.

In addition to the substrate, the microorganisms require a suitable environment, temperature, pH, oxygen content and salt concentration as factors which help them to function and thrive well (Mutasem *et al.*, 2013). Varies microorganisms have different requirements for those factors in order to be able to grow optimally. It’s typically that microorganisms adapt to their environment (Schnürer and Jarvis, 2009). For example, microorganisms that live and adapted to high temperature environments, often grow best at those temperatures. In a biogas process where many different microorganisms may be active, the reactor environment has to be compatible with the requirements of many

microorganisms as possible. This means that the environment may not be perfect for each microorganism, but still good enough to allow the organisms to grow (Schnürer and Jarvis, 2009).

2.3.2 Substrates for biogas production

The energy source is the material that the organism uses to get energy for both its growth and function, such as movement or the intake of substrate. The energy source for a microorganism can either be a chemical compound or solar energy (Madigan *et al.*, 2015). The organisms in a biogas process use various chemical compounds as energy sources. These can be either inorganic compounds like hydrogen (Muzaffar *et al.*, 2016), or organic compounds such as various types of sugars, fats, and proteins (Schnürer and Jarvis, 2009).

When organisms use a chemical compound as a source of energy, the compound oxidizes and electrons or protons are transferred through a number of intermediate carriers to a final electron acceptor. Energy is formed during this transfer of electrons. The type of energy used by microorganisms is often the chemical compound ATP (Adenosine Triphosphate) (Madigan *et al.*, 2015; Fuchs, 2011).

The most important building blocks are carbon, which provides about 50% of the microorganism biomass, and oxygen, nitrogen, and hydrogen (Table 2.4). Other important building blocks are sulphur, phosphorus, sodium, potassium, magnesium, calcium, and chlorine. When the energy source is organic, it is also common to use it as a source for the building blocks. When the energy source is inorganic, carbon dioxide (CO_2), is the most common source of carbon (Ali Shah *et al.*, 2014), and ammonia (NH_3) is the most common source of nitrogen (Sepideh, 2015). Energy formed by oxidation of the energy source is used to form new cells. The design of synthetic nutrient solutions for growing microorganisms is often based on the structure of the cells. The structure of the cells can also be used as a guideline for the approximate composition of an optimal substrate.

Table 2.4: Approximate composition of a bacterial cell.

Component	*C*	*O*	*N*	*H*	*P*	*S*	*K*	*Na*	*Ca*	*Mg*	*Fe*	*Other*
% of dry Weight	50	20	14	18	3	1	1	1	0.5	0.5	0.5	0.5

Source: Modified from Madigan and Martinko (2006).

2.3.3 Electron Acceptor

Oxygen is the final electron acceptor or electron receiver in aerobic respiration (breathing oxygen). In the absence of oxygen, either fermentation or a so-called anaerobic respiration takes place. Fermentation mainly uses various organic substances as electron acceptors. The end products formed are primarily various acids and alcohols, as well as hydrogen and carbon dioxide. Anaerobic respiration primarily uses inorganic compounds as electron acceptors (Muzaffar *et al.*, 2016).

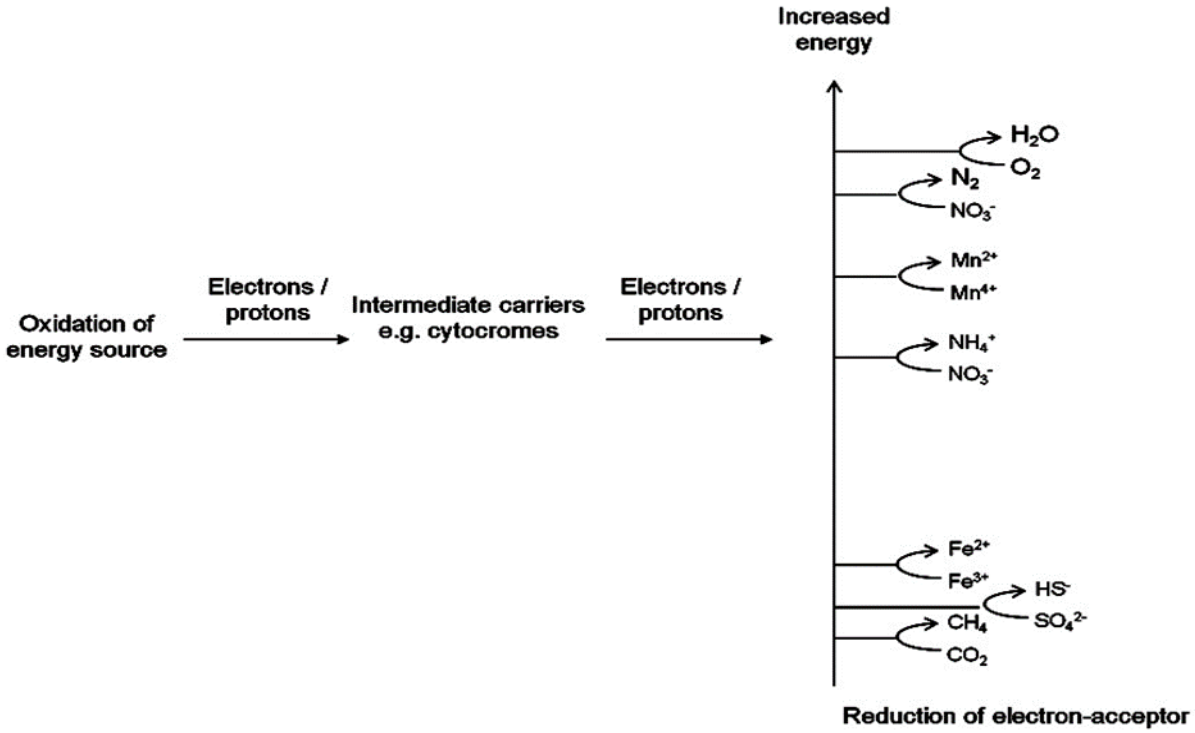

Figure 2.3: Flow of electrons to various electron acceptors during anaerobic respiration.

Source: Modified from Mengmeng *et al.*, (2015); Schnürer and Jarvis (2009).

Substances that can be used for anaerobic respiration include, for example, sulphate (SO_4^2), iron (Fe^{3+}), manganese (Mn^{4+}), nitrate (NO_3-), and carbon dioxide (CO_2) (Beal *et al.*, 2009). Some microorganisms can only use a single type of acceptor, while others can use several different types. Some electron acceptors are more advantageous than others because they enable the formation of more energy, in the order of: O_2> Mn^{4+}> NO_3-> Fe^{3+}> SO_4^{2-}>CO_2, where oxygen (O_2) provides the most energy and carbon dioxide (CO_2) the least (Mengmeng *et al.*, 2015; Schnürer and Jarvis, 2009).

If several electron acceptors are available in the same process, the organisms that utilize the most available energy-generating compounds will dominate. This is exemplified by the biogas process, where there is normally a large amount of carbon dioxide (and carbonates) (Sepideh, 2015). The methane-producing microorganisms dominate here, and they use carbon dioxide as the final electron acceptor.

The process also includes a small number of sulphate-reducing bacteria (Cui *et al.*, 2015 and Hugler and Sievert, 2011). They reduce sulphate into hydrogen sulphide using H_2 as the final electron acceptor (Mengmeng *et al.*, 2015). If large amounts of sulphate were added to a biogas process, sulphate reducers would grow at the expense of methane producers that would decrease in number (Milucka *et al.*, 2012). This is because the sulphate-reducers generally obtain more energy in their metabolism and thus can grow better. Figure 2.3 above summaries the process.

2.3.4 Trace Elements and Vitamins

Microorganisms just like other organisms need different trace elements and vitamins to function (Mutasem *et al.*, 2013). Different organisms have different requirements for these substances (Demirel and Scherer, 2011). Some organisms can form vitamins themselves, while other organisms need to absorb a number of vitamins from their environment. Trace elements are always taken up from the surrounding environment. In a biogas process, the substrate should supply these substances to the microorganisms. However, the occurrence of these substances varies greatly between various types of substrates (Schnürer and Jarvis, 2009).

Despite the importance of trace elements for process stability and the production of biogas, it is very clear that there is still no formula for optimum composition (Mutasem *et al.,* 2013). Trace elements that have been found to be important to methane-producing organisms are iron, zinc, nickel, copper, cobalt, molybdenum, and in some cases selenium and tungsten. Studies show that the addition of trace elements can stimulate the biogas process and enable higher organic loadings (Climenhaga and Banks, 2008). Hence, substrate characteristics can determine whether trace element additions are needed or not. For example, plant-derived materials may limit the biogas process due to the low content of certain trace elements. Several biogas plants that digest plant-based materials without using manure add trace elements to achieve stable operation (Schnürer and Jarvis, 2009).

2.3.5 Temperature

Temperature describes how cold or hot the microorganism's environment is and has a critical influence on their metabolic activity. The optimum temperature or the temperature at which the organism grows rapidly and function most efficiently, varies among species (Gurinderpal *et al.,* 2017). Microorganisms can be divided into different groups depending on the temperature at which they best thrive and grow. Although, anaerobic digestion process can take place at different temperatures. The temperatures are divided into three ranges of psychrophilic (<20°C), mesophilic (30 to 42°C) and thermophilic (43 to 55°C) temperature ranges (Li Hou *et al.,* 2018).

Table 2.5: Thermal stage and typical retention times.

Thermal Stage	*Process Temperature*	*Minimum Retention Time*
Psychrophilic	< 20 °C	70 to 80 days
Mesophilic	30 to 42 °C	30 to 40 days
Thermophilic	43 to 55 °C	15 to 20 days

Source: Modified from Viola (2015); Al Seadi *et al.,* (2008).

At psychrophilic temperatures biogas production is possible but may also result in a lower methane production rate depending on the type of process (Bohn *et al.,* 2007). In the case of high temperatures, there are examples of methane-producing organisms that can handle 110°C (Chaban *et al.,*2006), but stable biogas processes do not seem to

operate above 60°C-70°C (Meena *et al.*,2013). At temperatures above 60°C, the activity of methane producers is reduced to a greater degree than that of acid-forming organisms, which often results in the accumulation of fatty acids in the biogas process (Gurinderpal *et al.,* 2017).

2.3.6 Oxygen Concentration

The importance of oxygen concentration varies greatly for the different microbial communities that comprise the biogas process. Some of the organisms, such as those that produce methane, are very sensitive to oxygen and die if they come in contact with air (Deshai *et al.,* 2009). Others can survive quite low concentrations of oxygen (Zhou *et al.*, 2007), while others grow better if oxygen is present (Lim and Wang, 2013). The free radicals of oxygen are strong oxidising agents that can destroy cells by oxidizing various cell components (Botheju and Bakke, 2011). Microorganisms that can live in the presence of oxygen have different defence systems, that is, various enzymes that can protect the cell against oxidation by oxygen. The organisms that are sensitive to oxygen do not have this enzymatic defence system and are destroyed in the presence of air.

Microorganisms are usually divided into different groups depending on their relationship with oxygen. Both strict anaerobes and so-called facultative aerobes are found in the biogas process. Strict anaerobes only grow in the absence of oxygen. This group includes the methane-producing organisms. On the other hand, facultative aerobes grow in both the presence and absence of oxygen. This group includes numerous fermentative microorganisms. In the presence of oxygen, they can grow by aerobic respiration, but then they switch to fermentation when oxygen is depleted. This means that a temporary air leakage to a biogas process need not be a problem because there are microorganisms that can rapidly consume the incoming oxygen. There are even studies that show that a brief aeration during the biogas process can be a way of reducing the concentration of Hydrogen Sulphide (Montalvo *et al.,* 2016).

2.3.7 Salts Concentration

Salts contains essential building blocks such as Sodium, potassium and chlorine for microorganism's activity and development (Hierholtzer and Akunna, 2014). Normally microorganisms require salts to function well. Waste Substrate like manure (Usack and

Angenent, 2015), Abattoir waste (Franke and Insam, 2013; Pitk *et al.,* 2013) and aquaculture sludge (Zhang *et al.,* 2016) usually have high salt and ammonia nitrogen concentration. However, some waste substrate with high salt concentration may results in the process releasing more salt, which inhibit the biogas production microorganisms Salts generally have a preservative effect hence they inhibit bacterial growth. Too much salt causes water to move outside of the cell, causing the cell to lose both form and functioning (Jo *et al.,* 2017). Especially the methane producing microorganisms are usually the most affected by increasing salt concentrations in the biogas process

Although, some microorganisms can adapt to high salt concentrations if they are allowed to adjust slowly. They often form so-called osmolytes: compounds that help them maintain their function, even in the presence of salt. Organisms that can handle relatively high salt concentrations are called halotolerant, and those that grow even better at high salt concentrations are called halophiles. The most extreme forms of halophile grow best at salt concentrations above 20%-30% sodium chloride (> 3.4mol/L-5.1mol/L) and this group also includes some methane producers (Chaban *et al.,* 2006). In addition, the dilution of substrate with substance like glycerol can be used to avoid problems of microorganisms' inhibition due to the presence of salts such as chloride and sulphates (Vianaa *et al.,* 2012).

2.3.8 pH level

A neutral pH which is estimated to be around pH 7.0-7.5 is the most preferred by microorganisms. However, some organisms are active at both lower and higher pH values (Schnürer and Jarvis, 2009). There are several different organisms in the biogas process, and their pH requirements for optimal growth vary greatly. While fermenting, acid-producing microorganisms manage to live in relatively acidic conditions, down to pH of 5. Most methane producers generally require neutral pH values to be active. Although most methane producers thrive best at neutral pH values (Whitman *et al.,* 2006), methane producing microorganisms are sensitive to low pH. A pH of 6.8-7.2 works satisfactorily for a biogas process but the process efficiency is best when the pH is 7.0-7.2 (Li Hou *et al.,* 2018).

There are known examples of acidophilic methane producers that grow down to pH 4.7 and alkaliphilic methane producers that grow at pH values of up to 10 (Sivakumar *et al.,* 2012). The fact that acid-forming organisms can handle a lower pH is illustrated by the fact that decomposition of the substrate often begins already in the substrate tank, with acid formation and low pH as a result. However, methane production does not usually occur here because the pH is too low (Stavropoulos *et al.,* 2016). Instead, it starts in the digestion tank where the pH is significantly higher. The growth of microorganisms at various pH ranges often follows the same pattern as the growth at various temperatures (Gurinderpal *et al.,* 2017). That is, at all growth intervals, the pH value that generally results in the greatest rate is closest to the pH value that results in cell death.

2.4 BENEFITS OF BIOGAS PRODUCTION

2.4.1 Waste Reduction

The reduction of the quantity and pollution of any waste produced, to eliminating the need for waste handling has been the main concern principle of Waste reduction management (Sofoluwe and Olushola, 2015). Anaerobic digestion reduces organic waste while biogas is produced from the waste (Khald *et al.,* 2011). The use of organic waste substrate for biogas production is an effective way of managing organic waste and reducing its harmful impact on the environment (Dennis and Jing, 2016; Lima *et al.,* 2016). Apart from the health and safety of the people around the waste disposal vicinity areas, the degradation of organic waste releases uncontrolled amount of greenhouse gases which is contributing negatively to global climate change (Eric and Shafiqur, 2012). Therefore biogas technology compared to other technologies, it reduces both solid waste and gases like methane which are supposed to be emitted into the atmosphere by converting them into energy source (Karaglannidis and Perkoulidis, 2009).

2.4.2 Sustainable Renewable Fuel

The use of fossil fuel as a primary energy source leads to climate change, environmental degradation, and human health problems (IEA, 2006). Fossil fuels are dead plants and animals which have been exposed to heat and pressure in the Earth's crust over hundreds of millions of years. For this reason, fossil fuels are non-renewable resources because their reserves are being depleted at faster rate than new ones are being formed (Al Seadi

et al., 2008). Unlike fossil fuels, biogas from Anaerobic Digestion is permanently renewable as it is produced from organic matter which is actually, a living storage of solar energy through photosynthesis. Biogas from anaerobic digestion is not only improve the energy balance of a country but also make an important contribution to the preservation of the natural resources and to environmental protection (Antonio, 2015).

When biogas is combusted, it produces low emissions of carbon dioxide and particulate matter (Chaouki and Isam, 2015). This makes biogas an environmentally friendly fuel. It is capable of replacing petrol and diesel (Matjaž and Bogomir, 2010). Methane is a safer fuel than diesel and petrol due to its lighter weight than air and not poisonous. During leakages, methane quickly dilute in the surrounding air. Calculations indicate that replacing fossil fuel vehicles by biogas reduces the carbon-dioxide emission per unit of energy by 90% (Held *et al.,* 2008). The benefits can be doubled if biogas is produced from manure, since this decreases emissions of both methane and carbon-dioxide from open landfills. The reduction, measured in carbon dioxide equivalents, can then be as large as 180% per unit of energy (Held *et al.,* 2008).

2.4.3 Bio fertilizer

An anaerobic digestion plant does not only produce biogas energy, the digester also produced a digested substrate or residue which can be used as a biological fertiliser (Wagaw, 2016). The digested substrate forms a fertilizer which is rich in nitrogen, phosphorous, potassium and nutrients which are good for soil fertility (Alfa *et al.,* 2014). Compared to other organic fertiliser like cow dung, the digested fertiliser has high homogeneity and nutrient availability, better carbon to Nitrogen ratio and significantly reduced odours (Al Seadi *et al.,* 2008).

Anaerobic digestion provides a closed nutrient and carbon cycle, as it allows organic substrate produced from the soil, to be digested to produce Methane and use the residue back as a fertiliser to the soil. When the Methane is burned, Carbon dioxide is released to the atmosphere and recaptured by plants using photosynthesis. The plants will then prepare their own food, which is organic rich in carbon and when digested to carbon can improve the soil fertility. Therefore biogas production can be integrated into farming

and the digested residue could be used a replacement of chemical fertiliser produced with consumption of large quantities of fossil fuel energy.

2.4.4 Greenhouse Emission Reduction

Naturally greenhouse gases like methane, Carbon dioxide and nitrous oxide are emitted into the atmosphere (Scheehle *et al.,* 2006), their concentration changes due to human activities like burning of fossil fuels. Although agricultural sources are global sources of non-carbon dioxide emissions, livestock is accounted to have 70% of methane emissions is from manure fermentation emitting Nitrous Oxide (Prevention, 2006; Scheehle et al., 2006). These methane emissions can be avoided by digesting the animal waste in digesters where all the methane is collected as biogas for later combustion (Alfa *et al.,* 2014; Saeed *et al.,* 2011 and Amanda and Michael, 2008). It is important in this context that the digested residues are covered, since some methane can still be formed before the bio-manure is incorporated in the soil. Nowadays, the methane produced by many landfills is collected; this further reduces losses to the atmosphere (Held *et al.,* 2008).

2.4.5 Employment Creation

Biogas production from anaerobic digestion process requires labour force for production, collection and transport of substrate, in cases where the biogas plant is not near the source of its substrate feeding (Gauri *et al.,* 2013). Manufacture of technical equipment, construction, operation and maintenance of biogas plants are aspects of the biogas production needs which create employment (Marek, 2014). Therefore developing national biogas sector will contribute to the establishment of new enterprises which may increase the income (Al Seadi *et al.,* 2008), especially for African cities like Kumasi.

2.5 BIOGAS DIGESTERS

An Anaerobic digester is a reactor in which an anaerobic digestion process done by microorganisms take place. Digesters are normally engineered to function using a number of configurations and can be classified as 1) Single stage or Multistage processes, 2) Batch or Continuous process mode, 2) Mesophilic or Thermophilic

temperature conditions, 4) High or Low portion of solids, and 5) Low or High Technology Digesters (Al Seadi *et al.,* 2008).

2.5.1 Batch and Continuous digesters

Anaerobic digestion can be carried out as a batch process or a continuous process. In a batch process the substrate is added to the digester at the beginning of the process and the digester is then sealed for the whole period of the digestion. When the retention time is complete, the digestate is wholly removed and replaced with another substrate (Al Seadi *et al.,* 2008). Batch-fed digesters have the advantage of digesting high solids 20% to 40% Total solids substrate content. In continuous digestion processes the substrate is constantly added in stages to the digester. The substrate end products are periodically removed, hence the biogas becomes constantly produced. A single, or multiple digesters in sequence may be used. Examples of this form of anaerobic digestion include continuous stirred-tank reactors, up flow anaerobic sludge blankets, expanded granular sludge beds, and internal circulation reactors.

2.5.2 Mesophilic and Thermophilic digesters

Mesophilic digesters are digesters in which the anaerobic digestion process operates optimally between 30 ^{o}C to 38 ^{o}C, or at ambient temperatures between 20 ^{o}C and 45 °C. The mesophilic digestion process is done by a large variety of mesophilic bacteria which are more tolerant to the mesophilic process temperature fluctuations thus making the process more stable and robust (Monnet, 2003). Mesophilic digesters may need no heating systems to be installed in tropical zones.

Thermophilic digesters operate optimally in the temperature ranges of 49 °C to 57 °C or at elevated temperatures up to 70 °C where thermophiles are the primary microorganisms functioning (Dara *et al.,* 2016). Heating systems are installed in these plants to provide the thermophilic temperature limits needed in the digester. Thermophilic digestion systems are considered to be less stable and require higher energy input than mesophilic plants (Getachew and Pavel, 2016; Jung *et al.,* 2006). Nevertheless, more energy is produced from the organic matter (Federico *et al.,* 2015). This is because the increased temperatures facilitate faster reaction rates and, hence,

faster gas yields. Operating at higher temperatures also accelerates sterilization of the end substrate product.

2.5.3 High and Low solids digesters

High Solids digesters are engineered to digest substrate with total solids content ranging from 20% to 40% (Abdul and Jerry, 2010) The High solids or dry digester are also designed to digest substrate without adding water, mixing of substrate is not required and impurities like wood, stones or glass material can be handled without removing them as it is the case in other digesters. According to Shefali (2002) high solids digesters has higher organic loading rates (15 kg VS/m^3 per day) with high biogas yield, as compared to wet digesters which have about 6 kg VS/m^3 per day) with high per day.

Low solids digesters are design to digest substrates with less or low total solids content inside the digester (Abdul *et al.,* 2011). Due to the increase in liquid-to-feedstock ratio of the inside low digesters, the digesters normally require a larger amount of volume size and land than high solids digester. Since the low solids operate in a liquid environment it enables the substrate on the digester to be easily circulated. The circulation enables the microorganisms to be more readily in contact and access the substrate on which they feed on, thereby increasing the rate of the digestion (Mang and Li, 2010).

2.5.4 Single and Multistage digesters

In a Single stage digester, all the steps of anaerobic digestion process take place inside one sealed digester (Laurel, 2011). Although the single stage digester has minimum construction costs, the control over the reactions inside the digester is less, because various bacteria microorganisms are involved in subsequent steps and the over activeness of one group of bacteria has an effect on the activities of the other bacteria groups. For example, the acidogenic bacteria produces extra acids which reduces pH, thus this may affect the methanogenic activity of producing biogas on the digester (Praptiningsih *et al.,* 2013).

The Multi-stage or two stage digester allows the anaerobic digestion process to be taking place in separate two or more reactor/ digesters (Yao *et al.,* 2015). The process is

arranged such that the Hydrolysis, acidogenesis and acetogenesis steps occurs within the same first reactor and the last step of methanogenesis occurring on the second or final reactor (Laurel, 2011). Despite the high construction cost of multi-stage digestion system, it has some advantages over single-stage digestion system as it has been reported to improve the process over efficiency (Fernández *et al.,* 2016; Yao *et al.,* 2015; Xie *et al.,* 2008). The rate of hydrolysis and methanogenesis in the multi-stages digesters can be controlled and enhanced making it possible to control the anaerobic digestion process (Monnet, 2003). In spite of all these advantages, it is not possible to completely isolate the different reaction phases; some biogas is often produced in the first digester.

2.5.5 Low and High Technology Digesters

Low Technology small scale digesters are usually used for digesting wet substrates and normally operating at mesophilic temperatures. Building low digester requires less sophisticated techniques while, computerized monitoring systems, heating systems, mechanical feeding and mechanical stirring facilities are not required (Urmila *et al.,* 2008). The main parameters needed to design Low-Technology digesters include the inlet, airtight reactor/ digester, Biogas storage space and an expansion chamber. The Balloon, Floating Drum and Fixed-dome are the three common categories of Low-Technology digesters (Shikun *et al.*, 2014)

High technology digesters are relatively sophisticated technology in feeding, operating and monitoring of the anaerobic digestion process taking place. The system is complex and consists of a variety of elements (Al Seadi *et al.,* 2008). The High Technology digester design largely depends on the type and amounts of substrate or substances fed into it (Bernhard, 2013). Normally High technology digesters are attached to substrate pre-treatment sections, mechanical feeding system, and mechanical stirring devices, heating systems, end products storage systems and computerized process monitoring systems (Christian *et al.,* 2009).

2.6 OPERATIONAL PARAMETERS OF BIOGAS DIGESTORS

The growth and function of microorganisms are affected by operational parameters inside the digesters, which could be described mainly as Organic Loading Rate (OLR), Hydraulic Retention Time (HRT) and Stirring of Slurry or Agitation. The methane

bacteria are fastidious anaerobes, so that the presence of oxygen in the digestion process must be strictly avoided.

2.6.1 Hydraulic Retention Time (HRT)

The Hydraulic Retention Time (HRT) is the period in which the substrate remains in the digester. The estimated optimal retention time for complete biological conversion for thermophilic digester is 12 to 24 days, while for mesophilic digester is 15 to 30 days (Ostrem and Themelis, 2004). For efficient digestion the HRT should be long enough for the microorganisms to completely digest the substrate in order to produce environmentally friendly effluent (Al Seadi *et al.*, 2008; Yadvika *et al.*, 2004). This is very vital when operating in lower temperature (mesophilic) ranges than in higher temperature (thermophilic) ranges. The HRT is very important to be adapted to specific decomposition of the substrate to ensure complete digestion and higher biogas yield (Dareioti and Kornaros, 2014; Ho *et al.*, 2014). Knowing the targeted HRT, the daily feedstock input and the decomposition rate of the substrate, it is possible to calculate the necessary digester volume (Meena *et al.*, 2013). Eq 5 shows the formula for determining of the hydraulic retention time.

$$HRT\ (day) = Vd/V \qquad [Eq\ 5]$$

Where:

HRT = hydraulic retention time (days)

Vd = Volume of digester (m^3)

V = Volume of substrate fed per unit time (m^3/ d).

2.6.2 Organic Loading Rate (OLR)

The Organic Loading Rate, it is the amount of a substrate that is fed on the digester per day, per unit volume of the digester. The rate measures the biological conversion capacity of a digester or the anaerobic digestion system. It is expressed as kg chemical oxygen demand (COD) or volatile solids/organic VS/ODM) per cubic meter of the digester. The ORL can be suggested to be used as Digester design criteria for biogas production (Maizirwan *et al.*, 2015; Azadeh and Jalal, 2011). However, over loading the digester may cause accumulation of fatty acids, acting as inhibitor which results in low

biogas yield. It would cause proliferation of acidogenesis, decrease pH, and mass death of methogenic bacteria (Igoni *et al.,* 2007; Ray *et al.,* 2013). The digester volume is often defined by the relationship between ORL and the HRT. Eq 6 shows the relationship between OLR and the digester volume.

$$OLR = (m \times c)/Vd \qquad [Eq\ 6]$$

Where;

OLR=Organic Loading Rate (kg ODM m^3day)

m = mass of substrate fed per unit time (kg/d)

c = concentration of organic matter

Vd = Digester volume (m^3)

2.6.3 Agitation or Slurry Mixing

Mixing is an important operating parameter for achieving high efficiency biogas production on the digester. The action of mixing allows substrates to provide large surface area for microorganisms to act on it (Al Seadi *et al.,* 2008). Agitation is the turbulence mixing of the substrates which may results in the disturbance of the substrate. Passive agitation usually takes place when a new substrate is added into the digester, while active agitation is usually mechanically stirring or gas recirculation depending upon the substrate total solid concentration inside the digester. However, excessive agitation can disrupt microbes, so slow agitation or mixing is preferred (Khalid *et al.,* 2011). It is known by House (2010), that agitation increases the rate of biogas production by 10% to 15% or 50% in some instances when it is properly done. Active agitation helps to prevent the creation of scum and of sediments, bringing in the microorganisms closer to the substrate particles, facilitate the biogas bubbles up flow, Homogenizing the digester heat distribution and distribution of nutrients within the whole digester. Therefore mixing the substrate is important for biogas production.

2.6.4 Carbon Nitrogen (C/N) Ratio

The ratio of the number of carbon atoms to the number of nitrogen in the substrate is very important in the decomposition of substrates in a biogas process. The ratio is used to determine the stability of the digestion process. Micro-organisms in the biogas

digester feed on carbon and nitrogen to produce biogas. They use carbon as an energy source and nitrogen for building cell structures. If the ratio is too high the bacteria in the process will experience lack nitrogen and the pH of the process will drop quickly from the formation of fatty acids. This will inhibit the methane producing bacteria. If the ratio is too low the process will suffer from ammonia inhibition (Khalid *et al.*, 2011; Hong and David, 2007). It is difficult to state the exact optimal C/N ratio because it varies with different substrates and also with the process conditions. However, for proper biogas production, the C/N ratio of the substrate mix should be between 20 and 30 (Weiland, 2006). The microorganisms use up carbon about 30 times faster than they use nitrogen so they require a carbon-nitrogen ratio closer to 30 (Weiland, 2006). The C/N ratio of co-digesting to or more substrates can theoretically be determined using the Eq 7 for two co-substrates.

$$R = [W1R1 + W2\ R2]/ (W1+W2) \quad \text{[Eq 7]}$$

For digestion of more than two co-substrates, the C/N ratio can be determined as given in Eq 8.

$$R = [W1R1 + W2R2 + W3R3 + W4R4+\ldots] / (W1+W2+ W3+ W4+\ldots) \quad \text{[Eq 8]}$$

Where:

R – C/N ratio

W- Weight of co-substrate

2.7 TYPES OF SUBSTRATE USED FOR BIOGAS PRODUCTION

There are many different organic substrates which can be used for biogas production, but many of the organic substrates can be group into Agricultural, Kitchen, Municipal, Bioenergy Crops and Abattoir or Slaughterhouse Wastes as discussed in the following sub-sections.

2.7.1 Agricultural Waste

Agricultural waste includes waste such as harvest and post-harvest residue, animal waste from poultry and livestock including waste from processing of agricultural products. This waste can be used for biogas production due to their high organic content (Pöschl

et al., 2010). Depending on the origin, agricultural waste can be rich in carbohydrate, protein or fat agricultural form good substrate for biogas production (Sarkar *et al.,* 2012). Pre-treatment may be needed to optimize biogas production from these materials. The retention time of agricultural waste varies based on the source of the waste and pre-treatment (Karellas *et al.,* 2010). Usually, crop wastes are often high in carbohydrates, cellulose, hemicellulose and lignin. This makes digestion of crop wastes to take longer time. Inoculant is often added to crop waste to supply missing nutrients, microorganism and reduce retention time. To optimize operations crop wastes are taking through mechanical size reduction processes before decomposition (Maile and Muzenda, 2014).

2.7.2 Kitchen Waste

Food waste is commonly used for biogas production. Several biogas digesters both at mesophilic and thermophilic temperatures uses food waste from households, food industries and restaurants to produce biogas (Kunwar *et al.,* 2017). Food waste has variable composition it contains proteins, fats, carbohydrates and various trace elements (Ye *et al.,* 2013; Li *et al.,* 2011) Chemical characterization of a food waste from households showed that the balanced composition of protein, carbohydrate and fat with was beneficial for biogas production (Belitz *et al.,* 2009). It therefore has the potential to function very well in a biogas process). However, if the mixture of the waste is not homogeneous operational problems can arise. For example, mixture that contains too much protein will be affected by ammonia inhibition (Fricke *et al.,* 2007). Similarly, too much fat or sugar can cause production of fatty acids, scum and pH reduction (Schnürer and Jarvis, 2009). Food waste that contains a lot of fried food residues can only be digested under stable conditions after the addition of various trace elements (Climenhaga and Banks, 2008).

2.7.3 Municipal Waste

Organic Municipal Wastes are considered to be portions of municipal wastes materials which are biodegradable (Zupančič and Grilc 2012). The biodegradable municipal waste includes green waste, brown waste, food waste, paper waste, and biodegradable plastics. Other municipal waste are human waste, manure, sewage and sometimes slaughterhouse waste. Most organic wastes produced today originate in municipal, industrial and

agricultural sectors (Zupančič and Grilc 2012). The sources of municipal waste are households, institutions, hotels and restaurants. Municipal waste may have varying composition and mixtures. The waste can either be in solid, liquid or slurry state, hence it can be used for anaerobic digestion for biogas production (Cukjati *et al.,* 2012). On the average about 60% of municipal wastes is biodegradable material (ETC/SCP 2009).

2.7.4 Bioenergy Crops

Energy crops are crops that are grown purposely for producing energy. This include crops jatropha and sugarcane. The methane yield of energy crops is affected by the storage process, site properties and time of harvest, since these factors affect the chemical composition of the crop and hence creating abilities of the microorganisms to use those plants as substrates for their growth (Anon *et al.,* 2007). Bioenergy crops often have a relatively high content of dry solids (10-50%) and a high C/N ratio. Mixing with more nitrogen-rich material can achieve optimum process conditions. Co-digestion of energy crops with manure has been shown to generate a 16-65% increase in methane recovery (Lethomäki *et al.,* 2008). Bioenergy crops with high contents of cellulose; hemicellulose and lignin have long retention time due to their complex structure. In order to maximize the digestion rate of cellulose-rich materials, it is beneficial to increase the surface area by shredding them into smaller sizes or apply pre-treatment to break up the complex structure of cellulose and make it more accessible for digestion (Parawira *et al.,* 2008).

2.7.5 Abattoir or Slaughterhouse Waste

Abattoir wastes are the organic waste generated from the abattoirs and can be used for biogas production (Cuetos *et al.,* 2010). The waste from the abattoir include things such as blood, rumen content, waste water, non-edible parts such as spleen and gut and bones forms part of the abattoir waste (European Parliament and Council, 2002). These wastes are produced in considerable amounts each year by abattoirs, especially poultry slaughterhouse waste, due to high consumer demand for poultry meat which is a low calorie nutritious meat. Abattoir wastes have been found to containing high fats and proteins which are very energy rich and have the potential to generate a lot of biogas (Castellucc *et al.,* 2013). However, excessive fat and protein contents lead to increased

concentrations of ammonia, volatile fatty acids and LCFA's, which negatively affects the anaerobic digestion process (Cuetos *et al.,* 2008).

2.8 CHARACTERISTICS OF SUBSTRATES FOR BIOGAS PRODUCTION

Substrates characteristics in this content are properties of substrates that are used the estimate the potential and performance abilities of the substrates to produce biogas. The substrate properties also have other many uses, especially in designing and sizing of biogas plants digesters, monitoring and operation of the anaerobic process also require knowledge of the substrate characteristics. Some of the substrate characteristics are discussed below.

2.8.1 Moisture content of Substrate

Anaerobic digestion is usually facilitated by adding water on the substrate, to obtain a high moisture content on the substrate, although to maintain the same availability of water throughout the digestion cycle might be difficult (Hernandez *et al.,* 2008). As the anaerobic process proceeds initial water added on the digester drops to a certain level hence High water content is likely to influence the process performance by dissolve readily available organic matter (Alnakeeb *et al.,* 2017). Investigation done on 70% and 80% moisture content effects on anaerobic process shows that at 70% Moisture content more Methane was produced than on 80% Moisture content digestion (Hernandez-Berriel *et al.,* 2008). However, water content does not only dissolve substrate to better methane, it may have effects on the digester design and operation by increasing the digester volume due to hydraulic retention time (HRT) limitations. Hence moisture content is an important parameter of a substrate which needs to be determined to better the process of anaerobic digestion.

2.8.2 Total Solids of Substrate

The percentage of the substrate solid component which remaining behind when a substrate moisture content is evaporated is called Total Solids (TS). The Total solids is used to provide a way of measuring the concentration of the substrate fed into a digester as well as a standard unit for measuring the biogas production potential of a substrate (Clemens, 2012). Normally substrate with 12% Total solids are classified as low or wet

solids substrates, while substrate with 15% to 20% Total solids are classified as Medium solids substrates and those with 20% to 40% Total solids classified as High solids substrates (Nizami and Murphy, 2010). Since, Total Solids (TS) forms part of the biodegradable substrate, which affects the pH, temperature and effectiveness of the bacteria microorganisms in the digestion process (Boontian, 2014). Therefore Total Solids are important substrate parameter to be known before feeding substrate to a digester. Total solids concentration also influences the digester design, as for example a Total solids concentration of 7% to 9% is considered suitable for floating dome digesters (Boontian, 2014; Meena *et al.,* 2013).

2.8.3 Volatile Solids of Substrate

The determination of volatile solids when considering biogas production from a substrate is vital. The Volatile Solids concentration of a substrate forms an important part of Total Solids Content, since it represents the portion of solid substrate that could produce biogas (House, 2010). Most municipal wastes and manure have volatile solids of 70% to 90% indicating different biogas potentials from the substrate (Fulford, 2012). Although the Volatile solids is an indicator of potential biogas production it shall be noticed that the Volatile solids acts as an estimation parameter since the concentration percentage also compose other readily organic compounds or evaporative compounds. The complex nature of the composition of any organic waste means that the methane yield might be determined from anaerobic treatability assays on a suitable sample.

2.8.4 Ash Content of Substrate

Ash Content (Ash) or Fixed Solids (FS) is considered the concentration of the amount of solids that remains after burning the substrate in the furnace at temperatures of around $500^{O}C$ and $600^{O}C$. The Ash content is composed of inorganic elements from the substrates and it does not have energy content. The major cations present in ashes from lignocellulosic substrate are Calcium, Potassium and Magnesium. Other elements such as Manganese, Sulphur and Phosphorus are present in minor amounts (Neves *et al.,* 2008). Trace constituents such as Al, Fe, Zn, Cu, Ti, Pb, Ni, V, Co, Ag and Mo) are also found in many substrates. The anions that are usually present are Chloride, Carbonate, Sulphate and Silicate (Belitz *et al.,* 2009). With waste feedstocks (municipal solid

wastes in particular), ashes are often more abundant and more diverse. Therefore, Ash content can be used to indicate the potential of inorganics elements or substrate fertilizer quantity available after the digestion process.

2.8.5 Chemical Composition of Substrate

The chemical composition of the substrate is an important design consideration to determine an efficient design and biogas production potential. The biogas production of substrate depends on composition of proteins, lipids, carbohydrates and cellulose on the substrate. According to Neves *et al.* (2008), methane yield is high in substrate with excess of protein, cellulose followed by carbohydrates, while anaerobic digester having excess of lipids require long retention time. Hence organic substrates such food and animal wastes are considered to have high content of proteins, lipids, carbohydrates and elements such as carbon(c), Hydrogen (H), Nitrogen (N) and Oxygen (o), which are important for anaerobic microorganisms growth and function (Belitz *et al.*, 2009). In addition, Lesteur *et al.* (2008) has shown that the C, H, N and O components composition analysis or overall assessment of can be used for methane potential prediction from the substrate.

3 MATERIALS AND METHODS

3.1 INTRODUCTION

3.1.1 ABATTIOR AND SAMPLING DESCRIPTION

In this study organic samples were taken from the Kumasi Abattoir Company Ltd in Kumasi, Ashanti region. The abattoir is located at Ahinsan in the Kaase Industrial Area. The geographical location of the abattoir is shown in Figure 3.1. The organic wastes collected from the abattoir were: cattle blood and cattle rumen content.

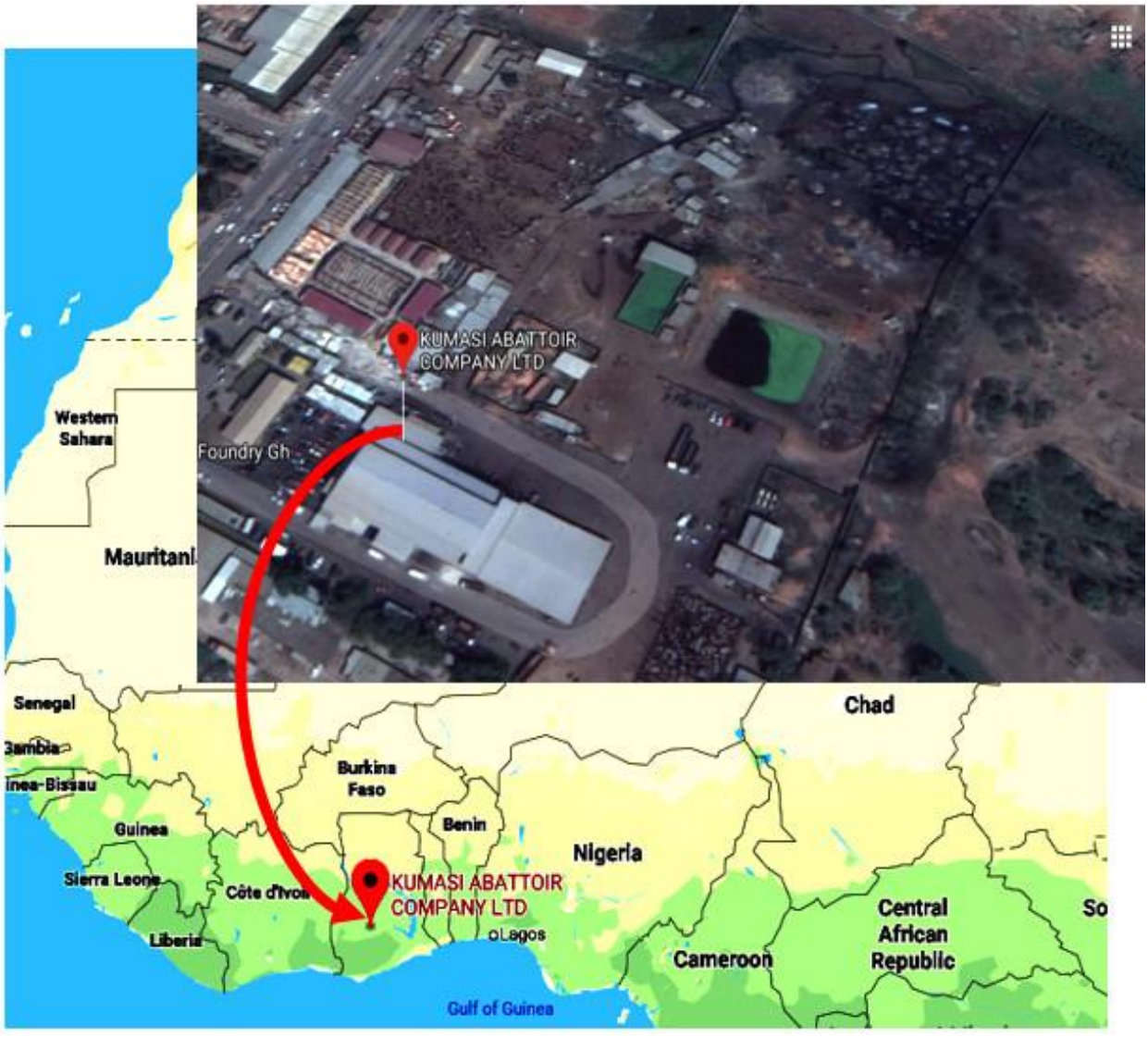

Figure 3.1: Kumasi Abattoir Location and Aerial View.

Samples of the cattle blood and cattle rumen content were randomly collected from the abattoir's premises at exactly 9:00am on each sampling day. The sampling days were over a period of two mouths. The blood and the rumen content were taken directly from

the evisceration unit of the abattoir. The samples were collected into plastic containers and put into a temperature controlled ice-chest to keep the source temperature and then transported to the laboratory for analysis. The Biotechnology Laboratory located at the Chemical Engineering Department of Kwame Nkrumah University of Science and Technology in Kumasi, Ghana was used for the analysis of the study.

3.2 DETERMINATION OF PHYSICAL COMPONENTS

The physical characteristics of the samples determined were moisture content, total solids (TS), ash content, volatile solids (VS) and pH. Three samples of cattle blood and three samples of cattle rumen content were analysed.

3.2.1 pH Measurements

The pH of each of the prepared samples were determined using multi-parameter (HI9829). Each of the samples was stirred at 200rpm using electronic stirrer for 2 minutes. The multi-parameter was held by retort stand and carefully dipped into the prepared samples to measure the pH. Precautions were taken during the measurement to avoid contamination. The multi-parameter was thoroughly cleaned and rinsed with distilled water before using. The multi-parameter was first calibrated with HI9828-0 Quick Calibration Standard Solution before using. The probe's sensors were fully dipped into the samples and allowed for the readings to be stable before recording. All the pH measurements were done at room temperature. The beakers containing the various samples were identified by writing the samples name on the beaker. The correct measured values were carefully recorded.

3.2.2 Total Solids (TS)

To determine the Total Solids (TS), 10g wet samples and their crucibles were put inside a laboratory dryer using tongs. The samples were allowed to completely dry at a temperature of 105 $^{\circ}$C for 12 hours. The dried samples (DS) and their crucibles were then transferred into a desiccator and allowed to cool thoroughly for 24 hours to a humidity of zero percent. The mass of each of the dried samples and their crucibles (MC+DS) was then weighed with analytical mass balance. The weighing was done three times and the average value was taken. The total solids of each sample was calculated

by subtracting the mass of the crucible (Mc) that contains the sample from the mass of the crucible and its dried sample (Mc+DS) and expressed it as a percentage of mass of the wet samples (MWS) as shown in Eq 9.

$$TS\ (\%WS) = ((Mc+DS) - Mc) / MWS) * 100 \quad [Eq\ 9]$$

3.2.3 Moisture Content (MC)

To determine the moisture content of each of the samples. The mass of the Total Solids (TS) of the samples were subtracted from the Mass of the Wet Samples (WS) and expressed as a percentage of the Mass of the WS determined (Cioabla *et al.*, 2012; Samuelsson *et al.*, 2006), as indicated in Eq 10.

$$MC\ (\%WS) = ((MDS - Mc)/MWS) *100 \quad [Eq\ 10]$$

3.2.4 Ash Content (AC)

The ash content was determined by transferring the crucibles with the dried samples from the desiccator into a furnace and burnt to ash for 4 hours a temperature of 550 ^{o}C. The ashes plus the crucibles were then removed from the furnace using tongs and placed inside a desiccator to cool down thoroughly for 40 minutes. The cooled ashes and crucibles were weighed for their masses, using an analytical mass balance, and the results were recorded. The ash content was calculated by subtracting the mass of the crucibles (Mc) from the mass of the ash and the crucibles (Mc+A) and expressed as percentage of mass of total solids (MTS) (Eq 11).

$$AC\ (\%TS) = ((Mc+A) - Mc)/MTS) * 100 \quad [Eq\ 11]$$

3.2.5 Volatile Solids (VS)

To determine VS, the mass of the empty crucibles were subtracted from the mass of the crucibles plus the dried samples and expressed as the percentage of the mass of the total as shown in Eq 12.

$$VS\ (\%TS) = ((Mc+DS)- (Mc+A) / MTS)* 100 \quad [Eq\ 12]$$

3.3 Determination of Chemical Components

To prepare samples for chemical components analysis, the rumen content and blood samples were dried at 75 °C in an oven for two days until samples were dried and then milled to a fine texture.

3.3.1 Total Nitrogen

Total Nitrogen on Rumen Content was done using the Kjeldahl Method, 0.5 g of dried sample was weighted into 250 ml digestion flask on an analytical balance. Thereafter, 0.5 g of Kjeldahi tablet with 0.5 g of $CuSO_4$ were added to the sample. A 12ml of concentrated H_2SO_4 solution was added and the mixture was placed on a digestion block. The digester was put on the mineralization procedure at different temperatures starting from room temperature up to 200°C and kept constant for the 30 minutes. The temperature was increased up from 200°C to 420°C and kept constant for 180 minutes

At the end of the digestion process sample turn into greenish blue showing digestion has come to completion after which samples were left to cool and transferred into a distillation. 100 ml of distilled water and 50 ml of 40 NaOH is added to sample and then distilled into 4% boric acid mixture indicator mixture, distilled for 4 minutes and then titrated with 0.1 HCI to a grey end point. Nitrogen percentage is determined using Eq 13.

$$\% N = \frac{\text{Titre x n x 14.007 X 100}}{\text{Sample Weight in mg}} \qquad \text{[Eq 13]}$$

Where:

N = Nitrogen percentage

n = the normal value of the Acid used

3.3.2 Total Phosphorus

Total phosphorus content was determined by using spectrophotometer Vanado phosphor molybdate method. 1 g of dried and milled sample was weighed into a dried crucible and placed in a Muffle furnace at a temperature of 450 °C for 4 hours. After ashing, an Ash solution was prepared of 10ml 1:2 v/o HNO_3 solution. The sample is filtered using No.1 filter paper into a 100 ml flask and made up to mark with distilled water. 25 ml of the solution is then measured

into another 100 ml volumetric flask and 10 ml of Vanado molybdate reagent added to the sample and made up to the volume. A yellow colour develops which is stable for days and it is read at a wavelength of 420 nm on a spectrophotometer, for the observed absorbance's, Phosphorous content is determined from standard curve (Eq 14).

$$\% P = \frac{Abs}{0.033} 0.04 \qquad \text{[Eq 14]}$$

Where:

0.033 = gradient of the graph

Abs = Absorbance

0.04 = Convention into %, since $\frac{100}{0.25}$ = 400/10000

3.3.3 Sulphur Determination

To determine sulphur content, Samples were weighted first. For example, 0.5 g of rumen content was weighted in a Teflon vessel. For example, 0.5 g of rumen content was weighted in a Teflon vessel. 5ml of concentrated HNO_3 was added to the sample and placed in a microwave digester. At the end of the mineralization, sample transferred to a 100 ml volumetric and make up to the volume with distilled water and filter using filtrate. 5 ml of the filtered sample was taken and 10 ml of Sodium acetate acetic acid buffer added to the sample, to maintain pH, about 4.8 ml of gum acacia and 1 g of BaCl2 crystal are added to the sample and shaken very well, with a volume made up to 25 ml with distilled water. The flask is inverted several times and turbidity measured with spectrophotometer at a wavelength of 440nm using a blue filter.

$$SO4 - S = \frac{Abs}{0.001} \; x \; Dilution \; factor \; (x100) \qquad \text{[Eq 15]}$$

Where:

Abs = Absorbance reading shown by the spectrophotometer.

NB: For Blood sample 1 ml of sample is placed in a Teflon vessel and mineralization is done with the same procedure after which 5 ml is taken for SO_4 analysis. About 50ml for phosphorous analysis. For blood sample dried at 60°C for 3 days was crushed and 0.5 g was weighed into a digestion vessel to undergo the Kjeldal process of digestion

and distillation to determine Nitrogen Content because if normal conventional laboratory were to be used to dry the blood sample the Nitrogen content will evaporate.

3.3.4 Carbon Determination

To determine the organic carbon 10g of each of the samples were measured using analytical mass balance and stirred at 200 rpm for homogeneity. The samples were then dried in a furnace/oven at 40 ^{o}C for 24 hours and ground into powder of particle size of 0.25mm. 0.35g of the dried and ground samples were put into clean carbon free crucibles. The samples were then treated with phosphoric acid in the ratio of 1:1 to completely remove any inorganic carbon. The samples were then reheated in furnace/oven at temperature of 40 ^{o}C for 24 hours. They were then transferred into an oven of temperature 105 ^{o}C (Schumacher, 2002). They were then placed into an LECO CR-412 carbon analyser.

The samples were combusted at a temperature of 1350 ^{o}C in the LECO CR-412 carbon analyser. The organic carbon in the samples was oxidized into CO_2. The gaseous phase was passed through two scrubber tubes. The first scrubber tube is packed with DrieriteÒ ($CaSO_2$) and copper granules to trap water and chlorine gas; the second scrubber tube is packed with Anhydrone ($MgClO_4$) to remove residual moisture. The gaseous phase then flows through a non-dispersive infrared (NDIR) detection cell tuned to selectively respond to CO_2. The integrated area under the signal detected is proportional to the amount of CO passing through the NDIR cell. The weight-corrected result is % C (Schumacher, 2002).

3.4 Determination of Biogas Quality and Potential Yield

To determine biogas potential, three digesters vessels were fed with pure blood waste, another three digesters were feed with pure Rumen Content waste and final another set of three digesters vessel were feed with a mixture of blood and rumen content as a substrate in a 50:50 ratio. All the digester vessels had a maximum volume of 500ml. Each digester was filled with 200g total mixture of water and the substrate. The following equation below (Md. Anisur *et al.*,2017), was modified to determine the amount the amount of blood or Rumen content substrates to be added to form a 200g of

total mixture of water and substrate. A ratio of 1: 5 which means for every 1g of substrate has to form 5g total mixture of water and substrate to be filled inside the digester. Therefore, it also implied that for a mixture substrate 0.5 g of blood was added with 0.5 g Rumen Content to form 1g substrate.

$$\% C_{sub} \times M_{sub} = \%C_{dig} \times M_{dig} \quad \text{[Eq 16]}$$

where:

$\%C_{sub}$ = The substrate total solid concentration in percentage for blood or Rumen Content.

M_{sub} = The mass of the substrate to be weighted or added to form 200g digester mixture.

$\%C_{dig}$ = The total substrate and water mixture concentration inside the digester. For a mixture of Blood and Rumen content samples, a percentage of 2.5% was used for each.

M_{dig} = 200g = The total mass of substrate and water to be filled in the digester.

Measurements of sample into the digesters were done using the analytical balance scale. Water was added into the digester vessels to make a total mass of 200g. In all the digesters, inoculum and artificial nutrients were not added. The digesters were sealed to allow minimum oxygen entry into the digester. All the digester vessels were placed on an incubator to allow the substrates to be digested at a mesophilic temperature of 37 ^{o}C. A biogas Analyser was used in all digesters to measure the percentage of Methane content (CH_4), Carbon Dioxide (CO_2), Oxygen (O_2), Hydrogen Sulphide (H_2O) and Nitrogen (N_2). The measurements were taken every after 3 days for a period of 1 mouth. The values for the 3 pure blood digesters were recorded and summed to give one value, the same was done with the Pure Rumen Content and Mixed Substrate digesters.

3.5 Statistical Analysis

The data collected for each characteristic of cattle blood and rumen content were presented using descriptive statistics. To determine the significant difference between samples collected between days, a one-way analysis of variance (ANOVA) was used on the data collected. All the statistical Analysis were done on Microsoft spreadsheet software.

4 RESULTS AND DISCUSSIONS

4.1 BLOOD WASTE COMPOSITIONAL ANALYSIS

4.1.1 Moisture Content (MC)

The moisture content of the blood waste analysed for 34 days is shown in Figure 4.1. Moisture content varied from 78% to 86%, the moisture contents seems to be higher and consistent with results obtained by Ward and McKague, (2007), who found 80% moisture content for blood waste. Since water important characteristic for anaerobic digestion, the Kumasi abattoir blood waste should be suitable for biogas production as wet substrate makes it easy for microorganisms to act on it.

A one-way analysis of variance was performed on the mean blood moisture content to test the null hypothesis of no statistical significance difference between the Blood waste moisture content means determined from the blood samples collected. As it can be seen from Table 4.1, since the statistical value P = 1.04 × 10-13 is far less compare to the significance level of α = 0.05, therefore, there is a very strong evidence that the Blood waste moisture content mean are not constant or the same. Hence for biogas process monitoring and operation purposes the blood moisture contents will need to be determined each time before feeding the blood waste into the digester.

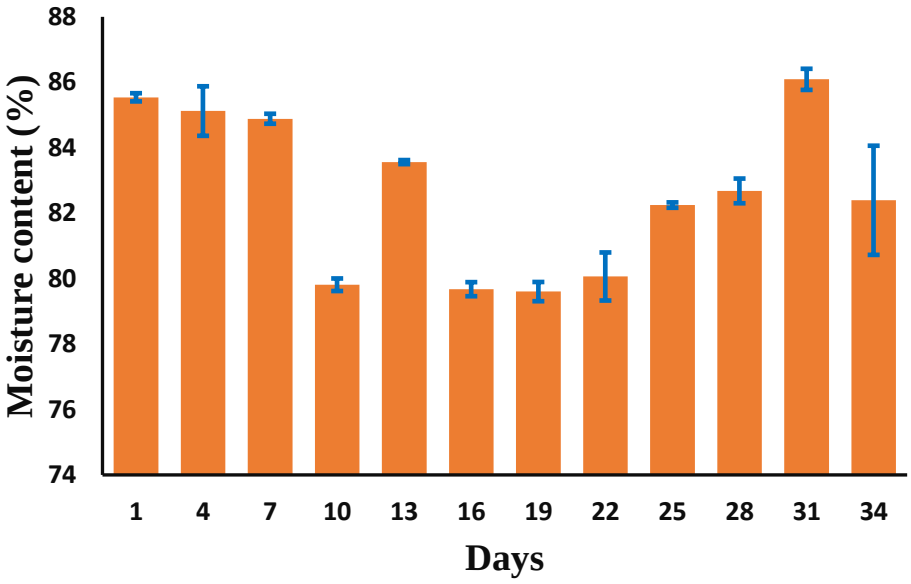

Figure 4.1: Moisture Content of Blood wastes

Table 4.1: Moisture content Blood Analysis

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	195.6582	11	17.78711	48.93667	1.04E-13	2.216309
Within Groups	8.723328	24	0.363472			
Total	204.3815	35				

4.1.2 Total Solids (TS)

Figure 4.2, shows the mean of the total solids of blood waste collected from Kumasi abattoir. The results shows the blood waste has total solids ranging between 14% and 20%. Since total solids content is considered as reflection of the total amount of solids including nutrients, the total solids might be considered low therefore co-digestion of the blood with other might be considered for anaerobic digestion.

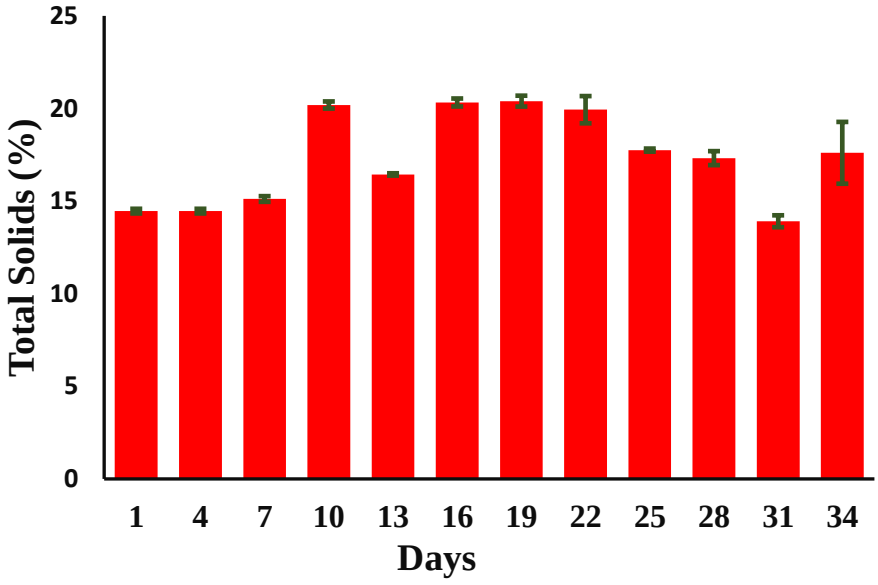

Figure 4.2: Total Solids concentration on blood wastes

The blood total solid means was subjected to one-way analysis of variance, with a null hypothesis that the Blood waste total solids means are constantly the same. The analysis of variance yielded a statistically significant effect of F (2.2163) = 58.033. Since the Fcritical (= 2,216) was less than F (= 58.0) the null hypothesis was rejected as seen from the result in Table 4.2. Consequently, the variation of the blood waste total solid could be due to variance of age, breeds and feeding patterns of cattle slaughtered at the Kumasi abattoir. Therefore, there is a need to always determine the blood waste total solid from

the abattoir before feeding it into the digester for proper monitoring and operation of the anaerobic process.

Table 4.2: Analysis of blood Total Solids

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	202.3953	11	18.39957	58.03316	1.5E-14	2.216309
Within Groups	7.609266	24	0.317053			
Total	210.0046	35				

4.1.3 Ash Content (Ash) or Fixed Solids (FS)

Figure 4.3 shows that the blood samples has ash content generally less than 5% percent, although on day 4, the blood waste substrate proved to have contained more than 5% ash content. In general, the ash content reflects the amount of solids that could remain after the anaerobic digestion, Hence less bio fertilisers could be obtained from the anaerobic digestion of blood waste alone.

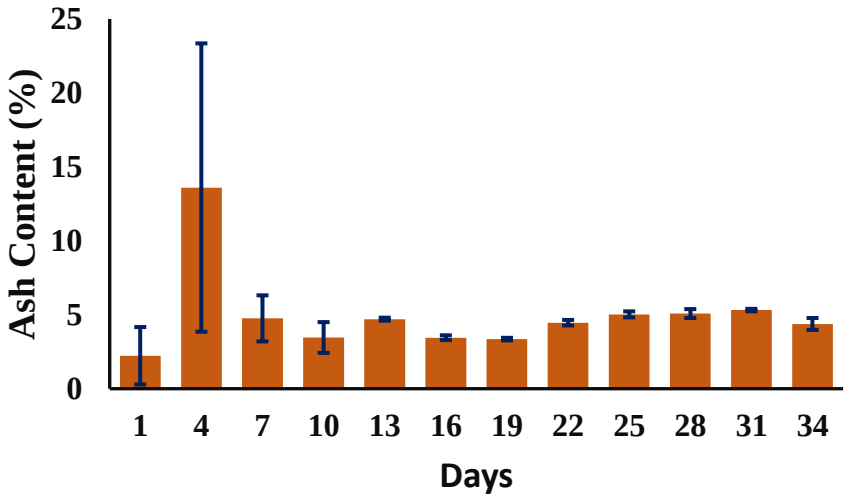

Figure 4.3: Ash Content of Blood wastes

From Table 4.3, Since Fcritical (=2.216) is less than the F (= 2.870) the assumption of the Kumasi abattoir blood waste having the same consistent Ash content average means is rejected. Hence the statistical significant variance provide evidence that the anaerobic digestion will not always output the same amount of solids.

Table 4.3: Analysis of Blood Ash Content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	270.1848	11	24.56225	2.870208	0.014903	2.216309
Within Groups	205.3838	24	8.557657			
Total	475.5686	35				

4.1.4 Volatile Solids (VS)

The data presented in Figure 4.4 shows that the blood waste at the Kumasi abattoir has most of volatile solids ranging from 90% to 100%, while the blood sample collected on day 4 had less than 90% volatile solids. Higher volatile solids content is important as they represent the amount of the total gases that can be produced from the substrate. Hence, this proves that the blood can be used to produce biogas.

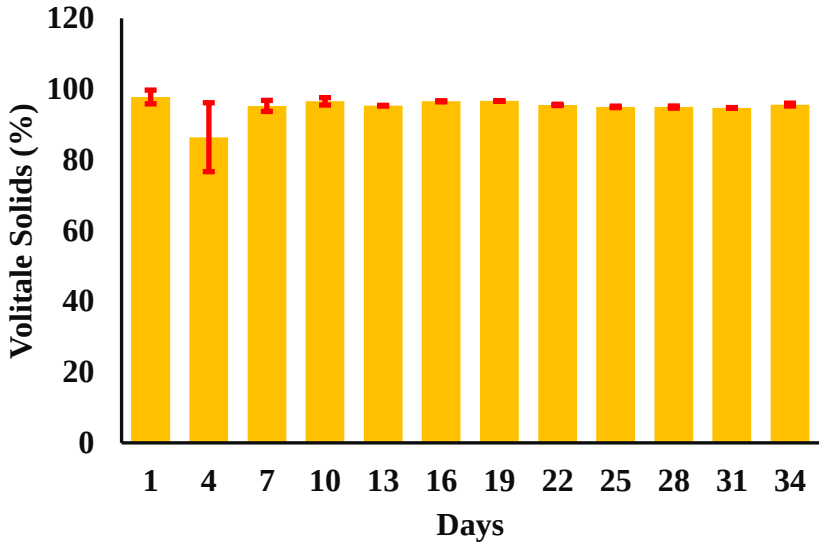

Figure 4.4: Volatile solids of Blood wastes

Since the statistically significant difference determined using one-way ANOVA resulted in F (11, 24) = 2.87, *P* = 0.014. Therefore the assumption that Blood waste volatile solids means are the consistently the same, has been rejected since the F critical (= 2.216) is less than the F (= 2.87) as shown in Table 4.4. This gives evidence that the blood waste has the potential to provide varying amounts of biogas.

Table 4.4: Analysis of Blood volatile Solids

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	270.1848	11	24.56225	2.870208	0.014903	2.216309
Within Groups	205.3838	24	8.557657			
Total	475.5686	35				

4.1.5 pH Measurements

The blood waste has most considerable pH level ranging from 7 to 8 as shown in Figure 4.5. The levels are considered suitable for biogas production (Deepanraj *et al.*, 2015). In general, the pH for the blood waste can be considered to be good for the biogas microorganisms to action and function on the substrate, as it can be observed form Figure 4.5, the blood wastes do not have much variation of pH.

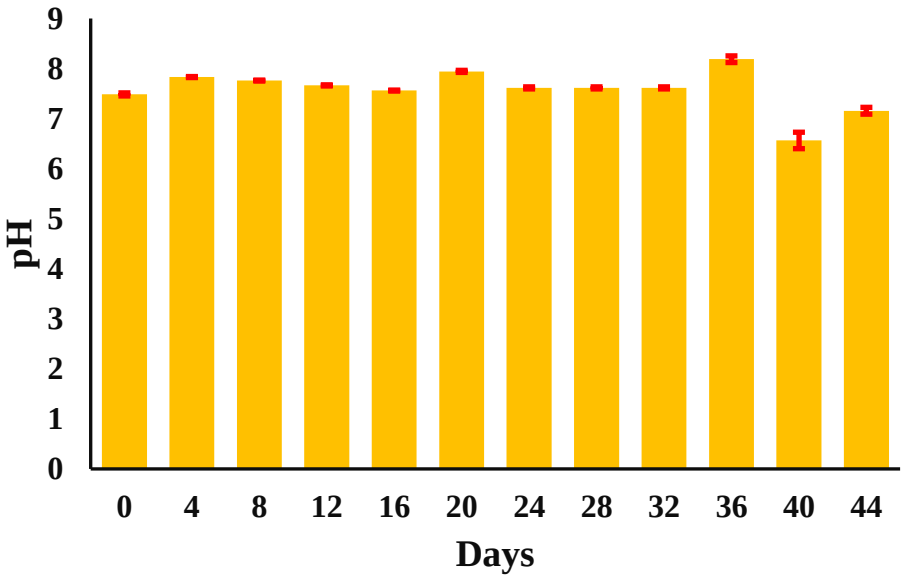

Figure 4.5: The pH of the Blood wastes

Although the blood has suitable pH levels, a one-way ANOVA has shown that F (= 152.594) is greater than F-critical (= 2.216), therefore the null hypothesis that the means pH level of the blood waste from the Kumasi abattoir is constantly the same, was rejected as shown in Table 4.5. This proves that cattle blood waste has a small pH variation which is shown by observation from Table 4.5.

Table 4.5: Analysis of Blood waste pH

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.483222	11	0.498475	152.5943	1.98E-19	2.216309
Within Groups	0.0784	24	0.003267			
Total	5.561622	35				

4.2 BLOOD CHEMICAL COMPOSITIONAL ANALYSIS

4.2.1 Phosphorous and Carbon Composition

Figure 4.6 shows that the blood waste contains phosphorus which is varying from 40 ppm to 160 ppm. In days 8 and 11 the blood samples showed high content of phosphorus while in days 16 and 20 it was less. Hence by observing Figure 4.6 phosphorus composition in cattle blood waste is not consistently the same. Therefore the determination of Phosphorous need to be done on blood waste before feeding on the digester. Beside Lei et al. (2010) investigations shows that addition of phosphorous onto digesters increases methane production. Hence if the blood phosphorous content is known, there might be no need to add phosphorus on the digester.

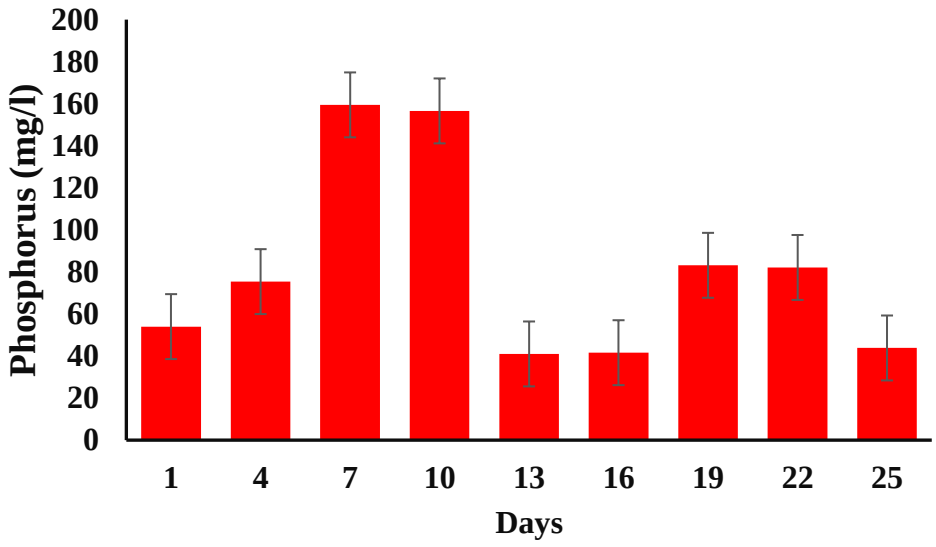

Figure 4.6: blood waste phosphorus concentration

In Figure 4.7, the blood samples contain carbon ranging mostly from 40% to 55%. Overall, the blood from the Kumasi abattoir could be considered to be having enough

energy source for the biogas microorganism, as the phosphorus and carbon are considered to be good nutrients (Schnürer and Jarvis, 2009).

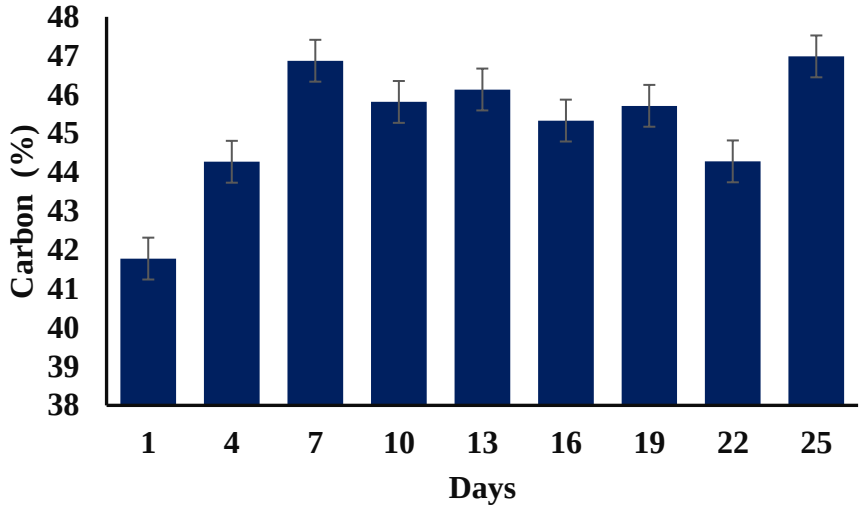

Figure 4.7: Blood Carbon Concentration

4.2.2 Nitrogen and Sulphide Composition

Figure 4.8 shows that the blood contains Nitrogen levels which range mostly between 12% and 14%, even though blood sample on day 0, 4 and 32 had Nitrogen levels less than 3%. This could be considered a good substrate as too much Nitrogen could be turned to Ammonium Nitrate which inhabits biogas microorganism growth (Na Li, 2018).

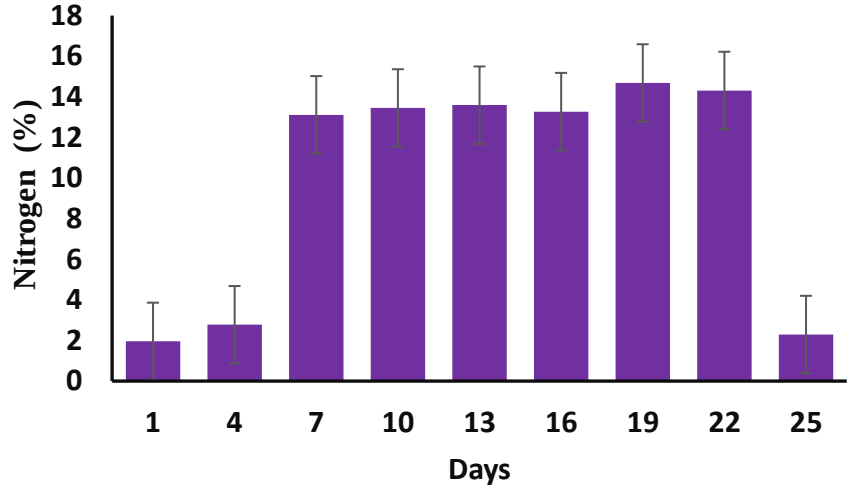

Figure 4.8: Blood waste Nitrogen concentration

The blood Sulphur content is relatively low, ranging from 0.2% to 0.55% as shown in Figure 4.9. Too much sulphur is not recommended as it could react with hydrogen during the digestion process to form Hydrogen Sulphide, which reduces the quality of the Biogas (Ernesto et al., 2013). Since there is no much recommended literature on sulphur levels on anaerobic digestion, further studies need to be done to cover the gap.

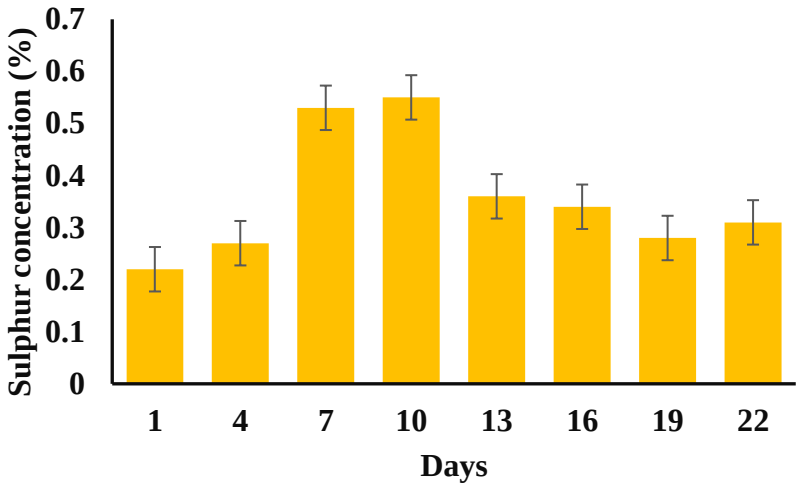

Figure 4.9: Blood waste Sulphur concentration

4.2.3 The Protein Composition

The cattle blood has higher protein content. As shown in Figure 4.10, protein content ranges from 80% to 90%, although on day 0, 4 and 32 the protein level are below 20%. It shows that the blood protein levels are inconsistent and could be subjected to variation.

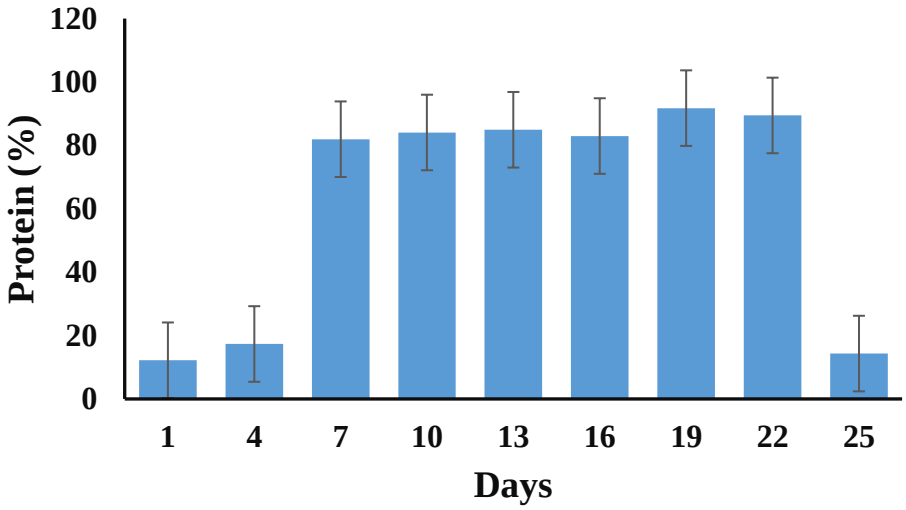

Figure 4.10: Blood waste Protein content

4.3 Blood Waste Analysis Composition Summary

Statistically significant effect on all the blood samples has rejected that each blood characteristic has the same consistent mean. As shown in Table 4.6, The blood collected from the Kumasi abattoir has 82.64 ± 0.82% moisture content, 17.36±0.82% Total Solids, 4.99±1.25% Ash content, 95.02±1.25% Volatile Solids, 7.68±0.14 pH, 81.90±35.60 mg/l phosphorus, 40.80±4.44% Carbon, 9.90±0.09% Nitrogen, 0.36±0.08% Sulphur and 61.89±27.75% Proteins average means. Previous studies found Pigs Blood has 76.68% Water content, 23.32% total solids, 15.1% Nitrogen and 94.4% Proteins (Etelka *et al.,* 2013), which is closer to the Kumasi abattoir cattle blood except that the pig blood had more Proteins. Hence in regard to protein content of blood from the abattoir may be considered a better substrate for co-digestion

Table 4.6: Summary of Blood waste Analysis

	MC %	*TS%*	*Ash %*	*VS %*	*Ph*	*P (mg/l)*	*C %*	*N %*	*S %*	*Protein %*
Mean	82.639	17.361	4.985	95.015	7.582	81.904	40.796	9.903	0.363	61.891
Standard Error	0.403	0.403	0.614	0.614	0.066	15.438	4.255	1.925	0.038	12.033
Median	82.611	17.389	4.641	95.359	7.625	75.399	45.330	13.270	0.340	82.938
Standard Deviation	2.416	2.416	3.686	3.686	0.399	46.314	12.766	5.776	0.114	36.098
Kurtosis	-1.506	-1.506	24.095	24.095	2.297	-0.181	8.654	-1.671	-0.568	-1.671
Skewness	-0.004	0.004	4.534	-4.534	-1.287	1.087	-2.925	-0.838	0.726	-0.838
Range	7.133	7.133	23.616	23.616	1.790	118.451	39.888	13.027	0.330	81.416
Largest	86.382	20.751	24.587	99.029	8.230	159.453	46.870	14.679	0.550	91.746
Smallest	79.249	13.618	0.971	75.413	6.440	41.002	6.982	1.653	0.220	10.330
CL(95.0%)	0.818	0.818	1.247	1.247	0.135	35.600	9.813	4.440	0.088	27.747
CL(95.0%)	0.818	0.818	1.247	1.247	0.135	35.600	9.813	4.440	0.088	27.747

4.4 RUMEN CONTENT WASTE COMPOSITIONAL ANALYSIS

4.4.1 Moisture Content (MC)

Moisture content of cattle rumen content is shown in Figure 4.11. The Moisture content for cattle rumen content waste varies between 78% and 88%. The range is slightly high to the moisture content of Blood waste. In general, the results show that blood and rumen contents haves high values of moisture as cattle drink large quantities of water to help with digestion. Hence the blood and rumen content will be suitable for biogas production as wet substrate makes it easy for microorganism to act on it.

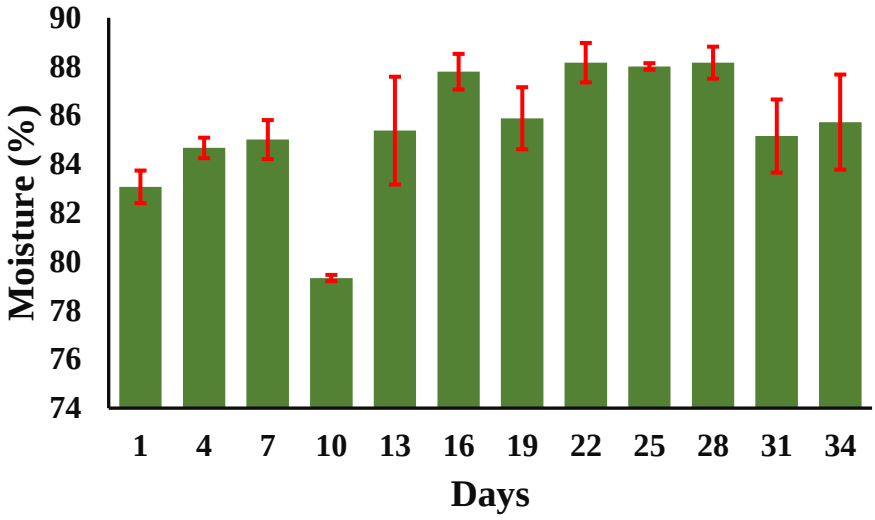

Figure 4.11: Moisture content of Rumen Content wastes

A statistically significant difference between the Rumen waste Moisture content mean was determined by one-way ANOVA as shown in Table 4.7. Since the F critical (=2.216) is less than the F (= 15), there is a very strong evidence that the cattle Rumen waste Moisture content mean collected from the Abattoir have different means. Therefore determination of moisture content needs to be done for biogas production efficiency purposes.

Table 4.7: Moisture Content Rumen Content Analysis

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	212.6138	11	19.32853	15.00017	3.43E-08	2.216309
Within Groups	30.9253	24	1.288554			
Total	243.5391	35				

4.4.2 Total Solids (TS)

The results from Figure 4.12, shows the mean total solids of Rumen Content collected from Kumasi abattoir. The values for rumen content ranged from 10% to 20% while most of the sample total solids were below 15%. Investigations done by Na Li *et al.*, (2018) and Deepanraj *et al.*, (2015) shows that rumen waste content which has 10% total solids is best suitable for anaerobic digestion. Hence, in general the Kumasi abattoir Rumen Content total solids may be considered to be good for Biogas production as it mostly have total solids closer to 10%. Alternatively, the Kumasi rumen waste should be diluted with water to reduce the total solid concentration to 10%.

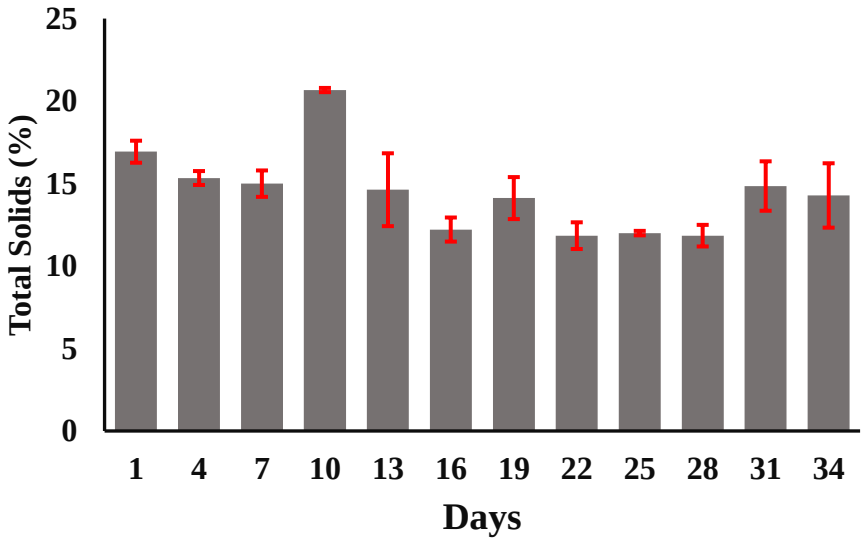

Figure 4.12: Total Solids of Rumen Content wastes

The Rumen waste total solid means were subject to analysis of variance, with a null hypothesis that the average mean Rumen waste total solids at the abattoir have the same average total solids composition (Table 4.8). A one-way analysis of variance test yield a statistically significant effect of F (2,24) = 15.0, $P = 3.43\times10^{-08}$. Since the Fcritical (= 2.216) is less than F (= 15), the assumption that the mean of the Rumen waste Total solids from the abattoir is the same was rejected statistically. This implies that cattle slaughtered at the Kumasi abattoir might be grazing on different grass or feed with different types of solids feeds. Therefore, since there is a significant variation in total solid of Rumen content to be fed into the digester, there is a need to determine total solid on substrates on avoid over loading.

Table 4.8: Analysis of Rumen Content Total Solids

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	212.6138	11	19.32853	15.00017	3.43E-08	2.216309
Within Groups	30.9253	24	1.288554			
Total	243.5391	35				

4.4.3 Ash Content (Ash) or Fixed Solids (FS)

Figure 4.13, Shows that the cattle rumen waste has ash content ranging from 10% to 20%. Since the Ash content does not have direct impact on the quality of the biogas to be produced, the Ash content simply reflects the amount of fixed solids substrate that will be the part of the anaerobic digestion output. Since the substrate will be having less solids, it will be best utilised as a liquid fertiliser.

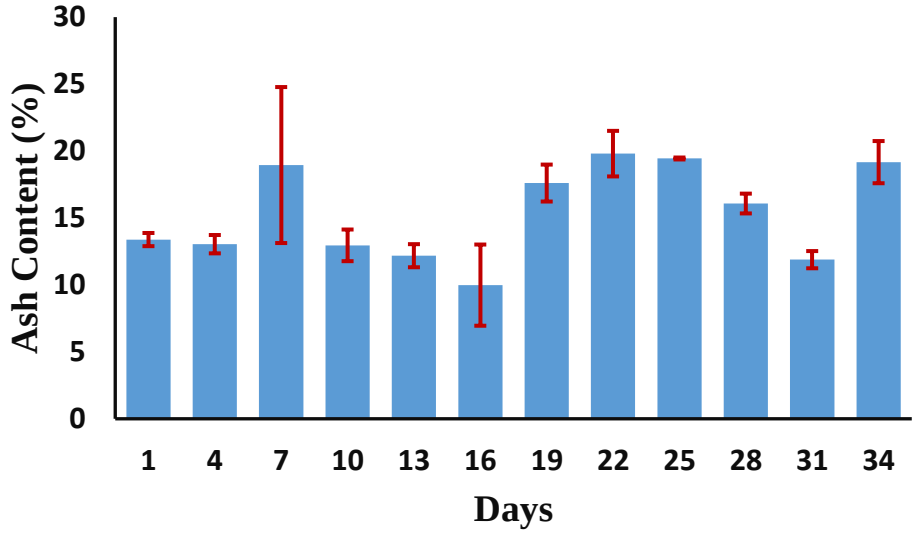

Figure 4.13: Ash Content of Rumen Content Waste

From Table 4.9, since Fcritical (= 2.216) is less than the F (= 8.18), the assumption that the Rumen Ash content average means from the abattoir is constant is rejected, Due to differences of cattle background of feeding pattern, breed type and age, the cattle waste will have different solid waste, since the ash content reflects amount of fixed solid on Rumen waste.

Table 4.9: Analysis of Rumen content Ash content

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	406.6481	11	36.96801	8.178568	9.85E-06	2.216309
Within Groups	108.4826	24	4.520108			
Total	515.1306	35				

4.4.4 Volatile Solids (VS)

Figure 4.14, shows that Rumen content collected at the abattoir had volatile solids between 70% and 90%. Higher volatile solids content are important as they reflect the amount of the total gases that can be produced from the substrate. Hence, this proves that the blood and the rumen can be used to produce biogas.

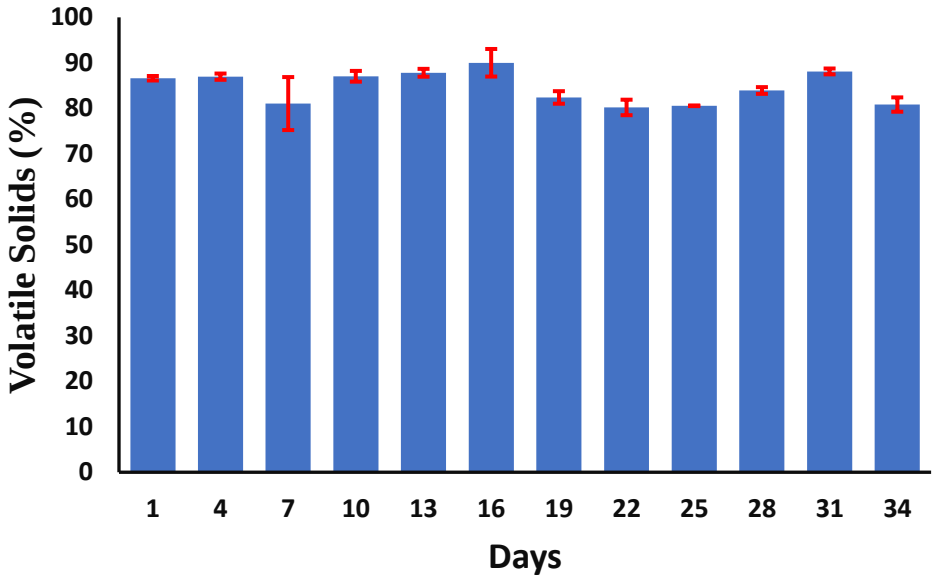

Figure 4.14: Volatile Solids of Rumen Content waste

The rumen content volatile solids means was subject to one-way analysis of variance (Table 4.10). Since the statistically significant difference results yielded Fcritical (=2.216) < F (= 8.1786), the assumption that the Cattle rumen waste has constantly the same average mean Volatile Solids is rejected. Hence the expectation for different amounts of biogas potential should be accepted.

Table 4.10: Analysis of Rumen Content Volatile Solids

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	406.6481	11	36.96801	8.178568	9.85E-06	2.216309
Within Groups	108.4826	24	4.520108			
Total	515.1306	35				

4.4.5 pH Measurements

Figure 4.15 shows that the rumen content has a pH levels ranging mostly from ph6 to ph8. In general the pH for the Rumen waste can be considered to be good for the biogas microorganisms to act and function on the rumen waste.

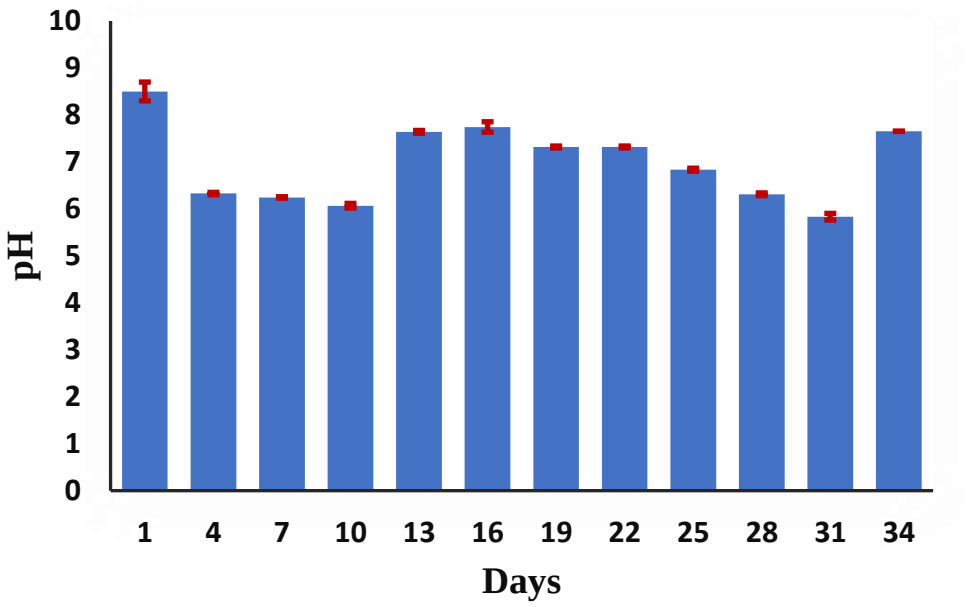

Figure 4.15: The pH of the Rumen Content Wastes

From Table 4.11, since the statistically significant value Fcritical (= 2.216) < F (= 384.88), the assumption that the average mean Rumen content waste pH is constantly the same is rejected. Therefore expectation is that the pH of the Rumen waste can change hence need for determination and monitoring during the anaerobic process.

Table 4.11: Analysis of Rumen Content pH

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22.77957	11	2.07087	384.8803	3.43E-24	2.216309
Within Groups	0.129133	24	0.005381			
Total	22.9087	35				

4.5 RUMEN WASTE CHEMICAL COMPOSITION ANALYSIS

4.5.1 Nitrogen and Sulphur Composition

Figure 4.16, shows that Rumen Content has lower Nitrogen levels of mostly 1.8% to 2.8%, compared to the blood which ranged between 12% and 14%. Therefore the Rumen content might be considered more suitable than the Blood, as it contains low Nitrogen, to form low Ammonium Nitrate. High Ammonium Nitrate inhibits biogas production from growth and function. As for Sulphur the Rumen content has levels ranging from 4% to 7.5% as shown in Figure 4.17. Although sulphur content low in rumen waste, ways of removing sulphur are suggested to avoid it form reacting with hydrogen a reaction which forms Hydrogen sulphide (Ernesto et al., 2013).

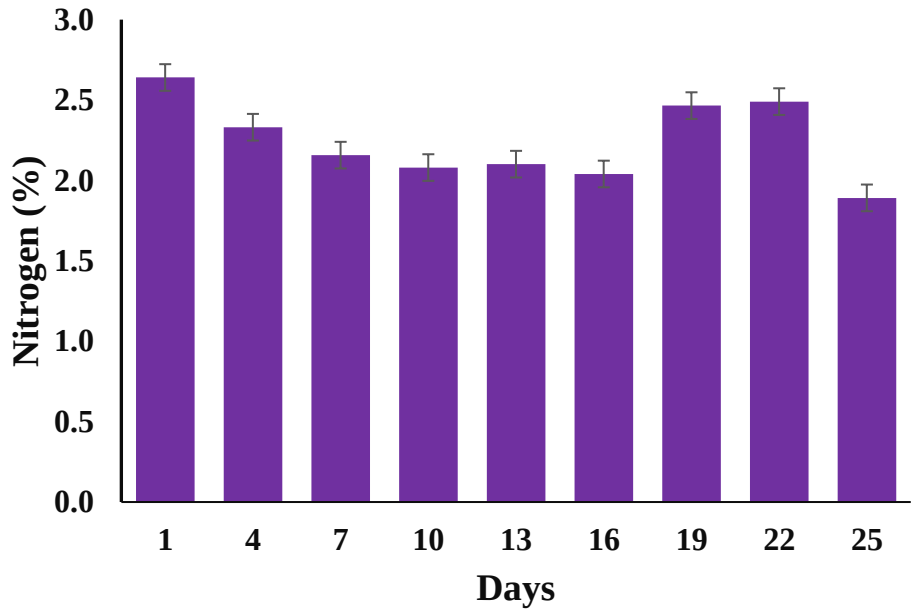

Figure 4.16 Rumen Content waste Nitrogen concentration

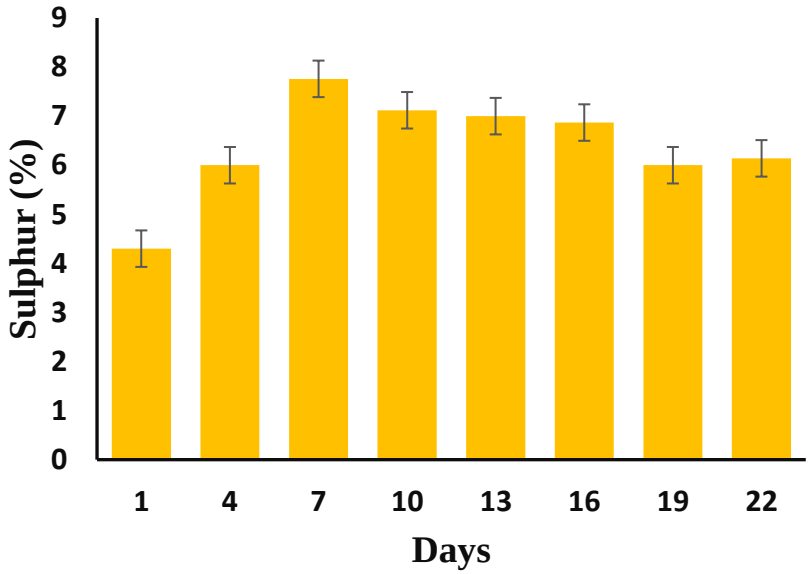

Figure 4.17: Rumen Content waste Sulphur Concentration

4.5.2 Phosphorous and Carbon Composition

The cattle rumen content phosphorus in Kumasi was found to be range from 0.3% to 0.6% as shown in Figure 4.18. The carbon content level vary mostly from 40% to 50%, while on day 32 the carbon level was between 35% and 40%. The rumen content might be considered a suitable substrate.

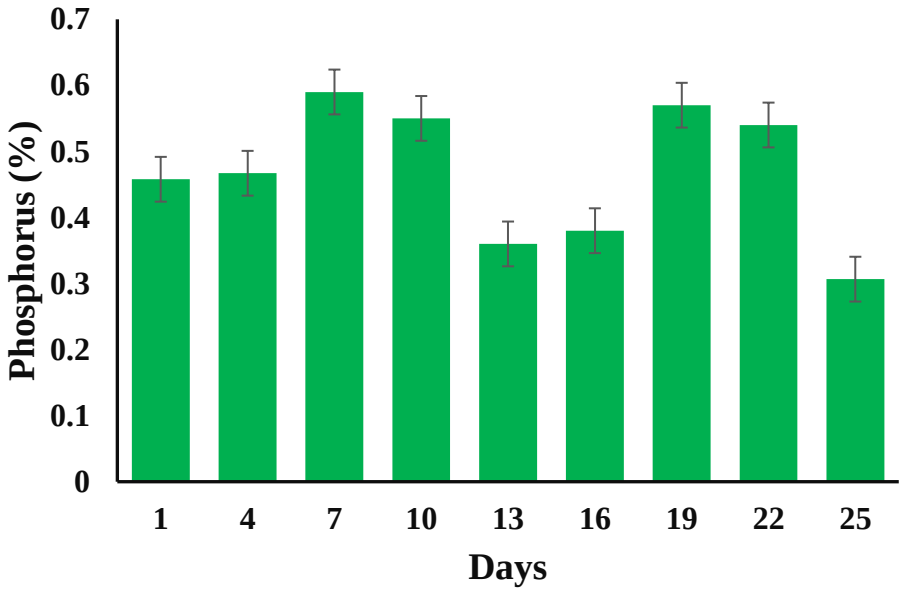

Figure 4.18: Rumen content waste phosphorus concentration

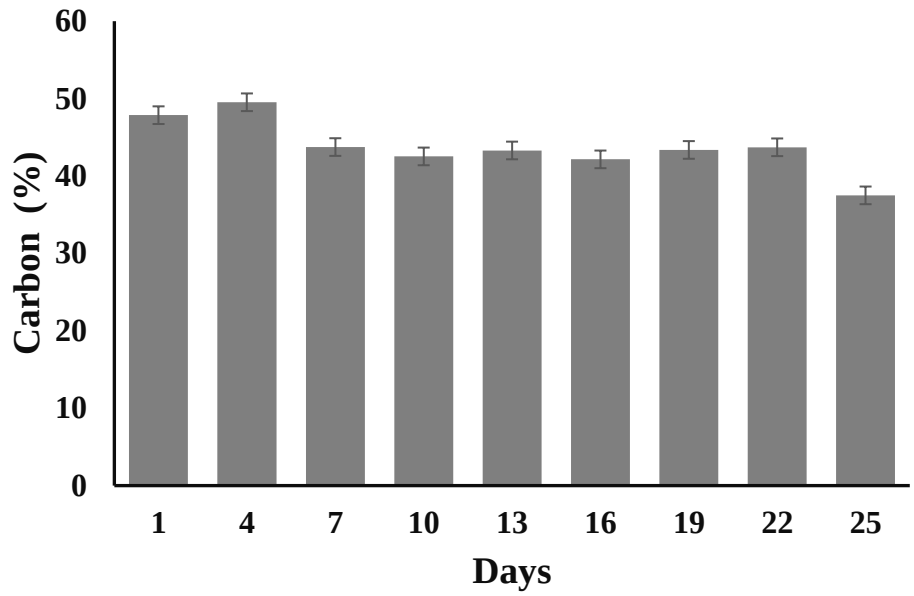

Figure 4.19 Rumen Content Carbon Concentration

4.5.3 Protein Composition

The Rumen content has low protein content ranging mostly from 12% to 17% as shown in Figure 4.20. From day 7 to day16 the rumen waste has moisture content of about 13%. Nitrogen in important as a substrate nutrient, the rumen waste will be a better biogas production substrate by observation from Figure 4.20.

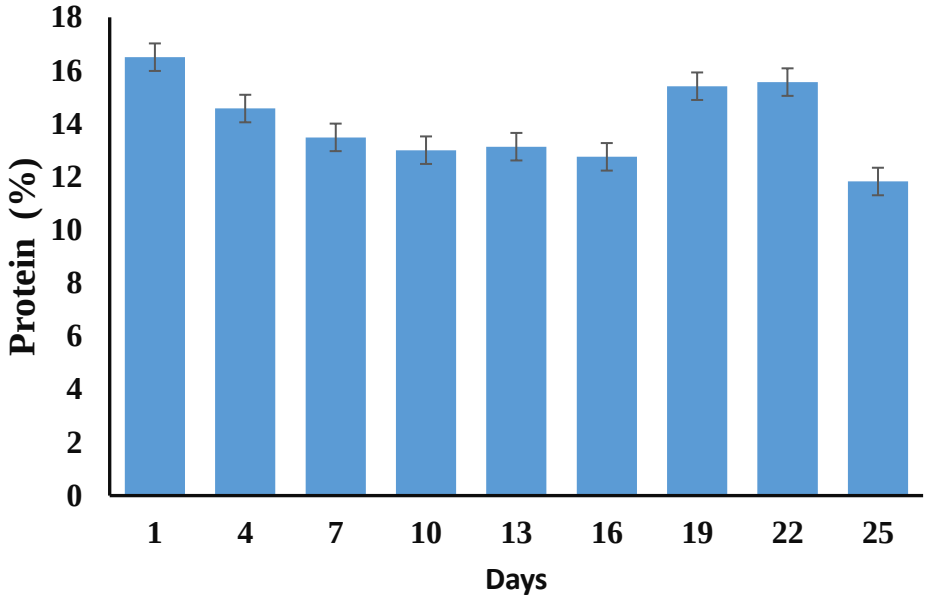

Figure 4.20: Rumen Content waste Protein Concentration

4.6 Rumen Waste Analysis Composition Summary

Since the hypotheses that each rumen content characteristic has same consistent mean has been rejected. The average means of the Kumasi abattoir cattle Rumen content, as shown on Table 4.12 ranges from 85.53±0.89% for moisture content, 14.47±0.89% for Total Solids, 15.37±1.30% Ash content, 84.63±1.30% Volatile Solids, 6.98±0.27 pH, 0.35± 0.19% phosphorus, 38.43±11.32% for Carbon, 4.90±4.17% for Nitrogen, 5.40±0.08% for Sulphur and 30.64±27.75% for Proteins.

Comparing the results, investigation done by Gammaa et al. (2015) shows that Cattle manure had 73.2% moisture content, 36.0% total solids, 26.8% Ash content, 73.2% volatile solids, 54.3% carbon and 1.6% nitrogen content. The Kumasi cattle rumen content characteristic are not much different to cattle manure and can be used as anaerobic digester substrate.

Table 4.12 Summary of Rumen Content Analysis

	MC %	*TS%*	*Ash %*	*VS %*	*Ph*	*P %*	*C %*	*N %*	*S %*	*Protein %*
Mean	85.527	14.473	15.374	84.626	6.982	0.349	38.427	4.904	5.402	30.649
Standard Error	0.440	0.440	0.639	0.639	0.135	0.080	4.908	1.806	0.977	11.290
Median	85.771	14.229	14.701	85.299	7.085	0.380	43.300	2.157	6.870	13.482
Standard Deviation	2.638	2.638	3.836	3.836	0.809	0.241	14.725	5.419	2.932	33.870
Kurtosis	0.414	0.414	0.202	0.202	-0.988	-1.569	7.938	0.732	0.512	0.732
Skewness	-0.918	0.918	0.408	-0.408	0.249	-0.561	-2.751	1.615	-1.484	1.615
Range	9.822	9.822	18.667	18.667	2.930	0.585	49.512	12.732	7.560	79.574
Largest	89.010	20.812	25.675	92.992	8.700	0.590	49.526	14.623	7.760	91.396
Smallest	79.188	10.990	7.008	74.325	5.770	0.005	0.015	1.891	0.200	11.821
CL (95.0%)	0.893	0.893	1.298	1.298	0.274	0.185	11.319	4.166	2.254	26.035

4.7 BIOGAS POTENTIAL AND QUALITY ANALYSIS OF ABATTOIR WASTE

4.7.1 Methane Concentration

The maximum methane content produced by Kumasi Abattoir cattle Rumen content at constant 37°C temperature is 46% as shown in Figure 4.21, while the Mixture of Blood and the Rumen content produce a maximum methane content of 40%. The anaerobic digestion of the blood alone, has proved to produce less methane content which is less than 5% as shown in Figure 4.21. According to Saeed *et al.*, (2011) methane content ranges from 40% to 75%, indicating that the anaerobic digestion of Rumen Content or a Mixture substrate would be preferable for biogas generation compared to pure blood alone. Although other studies conducted by Christy *et al.*, (2013) and Al Seadi *et al.*, (2008) shows that the biogas methane content should be approximately to 50% to 80%, still the methane concentration of 46% and 40% is closer to the these recommended values.

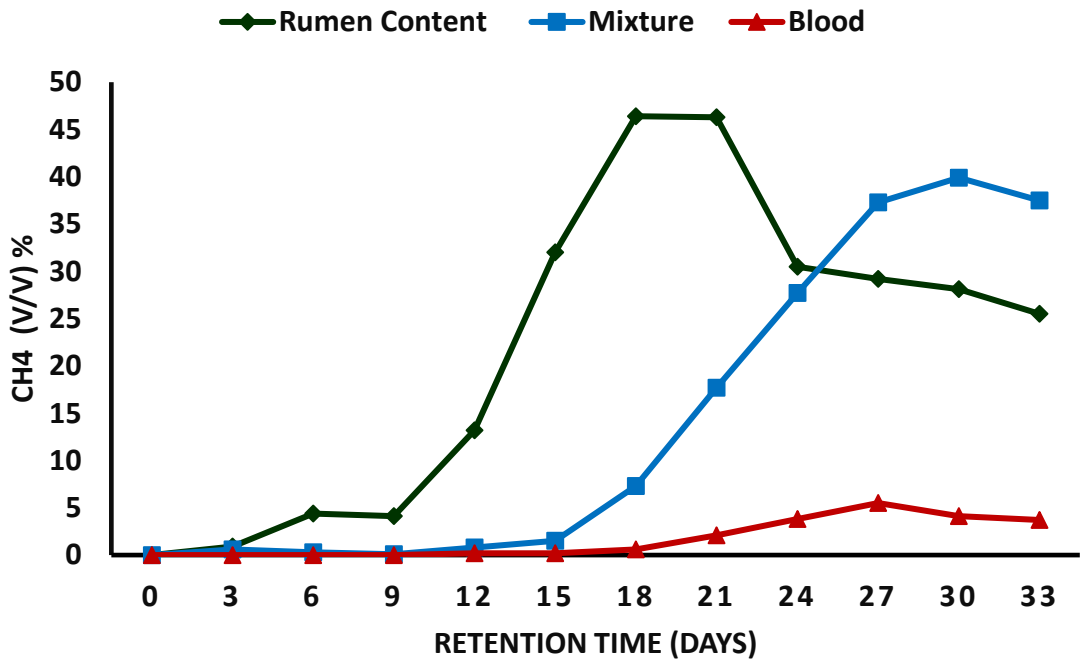

Figure 4.21: Methane Content of Rumen Content, Mixture and Blood Waste Biogas

The low methane values could be caused by 5% low Total solid content substrate feed on the digester. According to Na Li *et al.*, (2018) 10% total solids of rumen waste was

fed on the digester and optimum biogas was obtained. Similar results were obtained by Deepanraj *et al.,* (2015) for food waste and Budiyono *et al.,* (2010) for rumen content.

In comparing the results from Figure 4.21, the digestion of Rumen Content performed well with 46% Methane Content compared to the Co-digestion of Blood and Rumen Content. This is similar to results obtained by Mirzaman et al. (2017) on food waste. The anaerobic digesters fed solely food waste performed better than the co-digesters (food waste and cow manure). This indicate the co-digestion of substrates may not always be best for biogas production form substrates as reported by Eric and Shafiqur (2012).

4.7.2 Carbon Dioxide Content

Carbon Dioxide is one of the gases produced as can be seen from the graphs in Figure 4.22. The Mixture of Blood and Rumen substrate seemed to have produced high Carbon dioxide during the first 9 days of the retention time, but reduced afterwards. Hence co-digestion could be considered advantages in producing biogas with less Carbon dioxide.

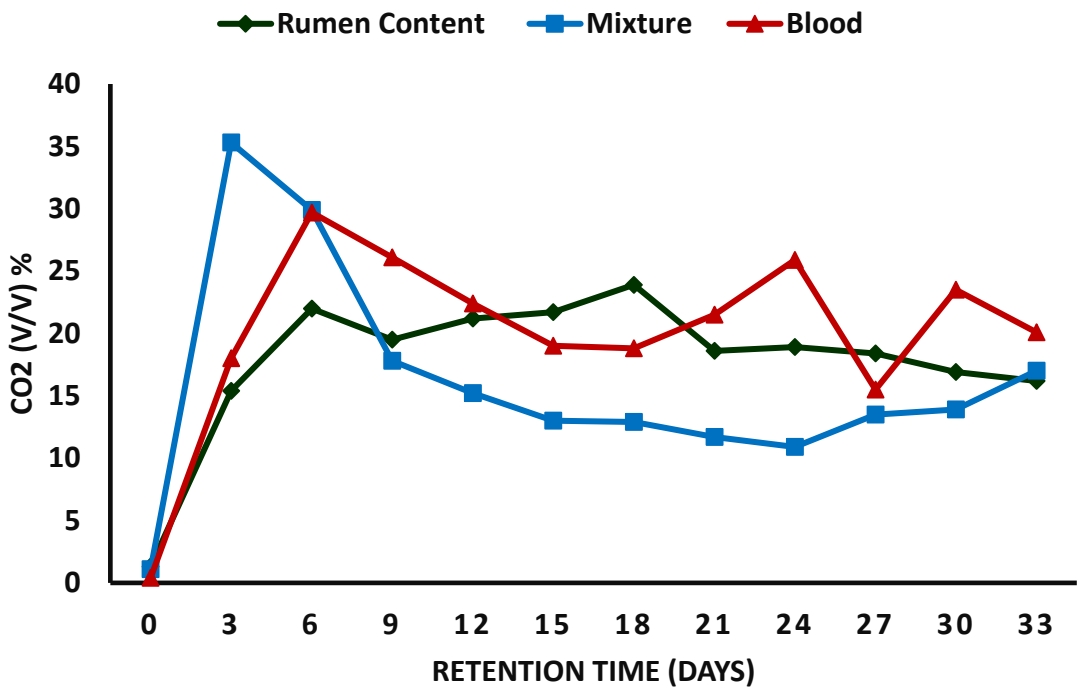

Figure 4.22: Carbon Dioxide Content from Rumen Content, Mixture and Blood Waste

4.7.3 Oxygen Content

The Figure 4.23, shows the Biogas oxygen content pattern inside the digester at 37°C temperature. As it can be seen from the day 0 the oxygen levels are high. The reason is that in the beginning some of the oxygen goes inside the digester thorough new substrate feeding. The oxygen will be utilised by other aerobic microorganisms. Once oxygen levels decreases lower down as indicated on day 3 and 6, the anaerobic digestion microorganisms will start working on the Rumen content or substrate. Oxygen levels are important for monitoring the biogas production process. Between day 9 and 27 high levels of Oxygen levels resulted in the blood waste digester producing lower methane whereas the rumen waste digester produced higher methane with less Oxygen. Oxygen is dissolved into the blood during the cattle breathing period, hence it explains the oxygen high content in the blood waste, assuming no air leaks during the digestion process. Therefore the blood waste performs better are a co-digestion substrate.

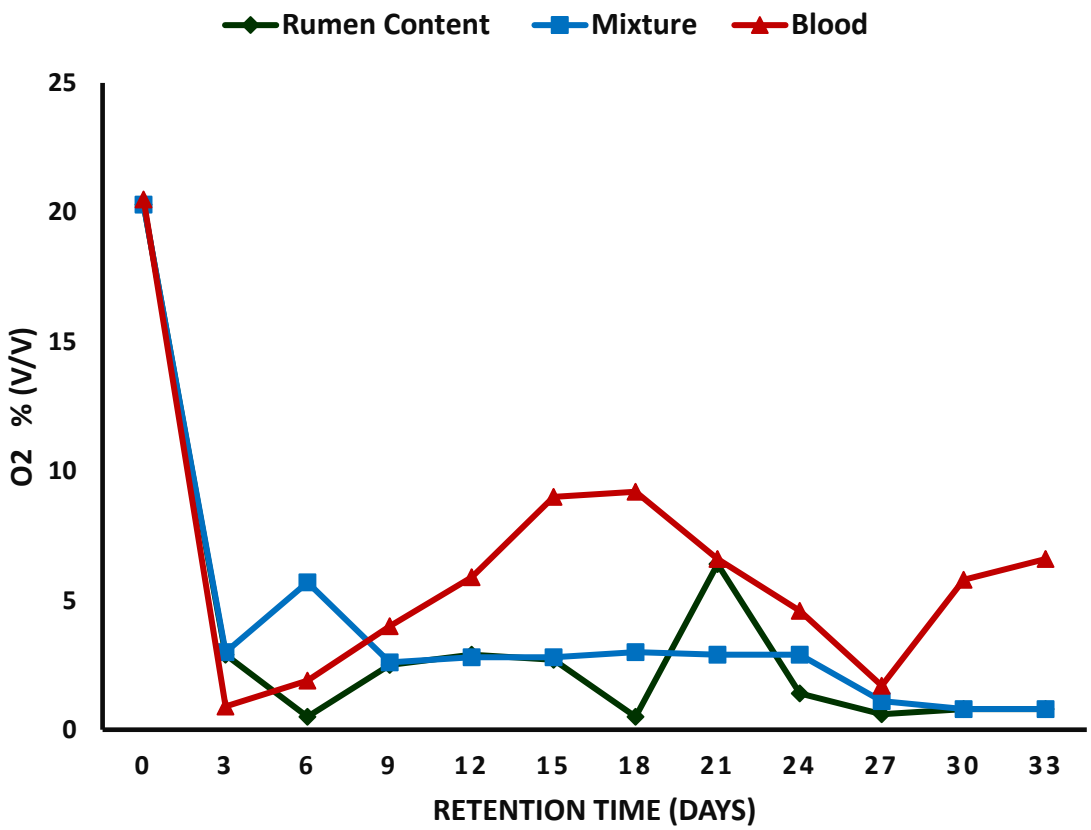

Figure 4.23: Oxygen Content on Rumen Content, Mixture and Blood Waste

4.7.4 Hydrogen Sulphide Content

As shown in Figure 4.24, the period between day 0 and day 9, the blood substrate digester produced large quantities of Hydrogen Sulphide (5000 ppm) while Blood and Rumen Mixture produced 3000 ppm of Hydrogen Sulphide. After day 9, as methane production started, the Hydrogen Sulphide reduced to levels below 1000 ppm, but still the blood substrate continued to have more Hydrogen sulphur compared to other substrate. Since Hydrogen Sulphide is considered as corrosive, toxic and preferable to be removed from any utilization of the biogas (Ernesto et al., 2013), Rumen Content can be used to produce biogas with less hydrogen sulphide compared to blood substrate.

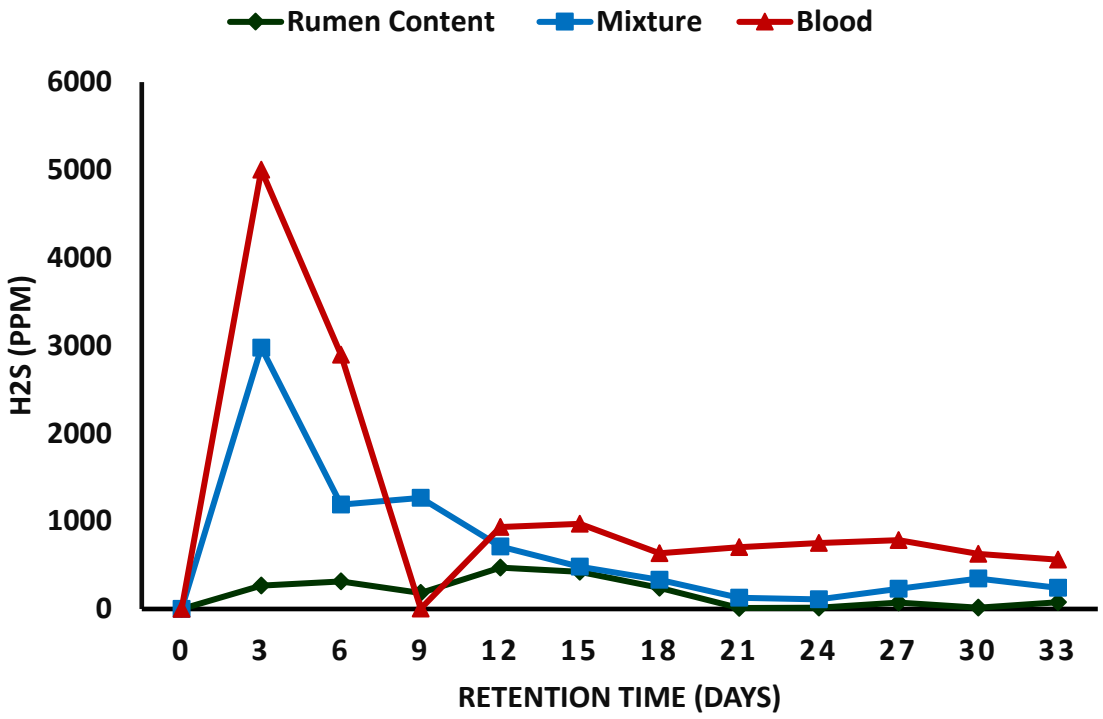

Figure 4.24: Hydrogen Sulphide content on Rumen Content, Mixture and Blood Waste

4.7.5 Residual Nitrogen Content

Figure 4.25 show the Nitrogen content with the time the substrate is digested. From day 9 to day 24, the Nitrogen content is decreasing in the Rumen content and Blood substrate digested while in the Mixture substrate they is slight increase. Nitrogen is important for anaerobic digestion as it can indicate the amount of ammonium being formed inside the digester.

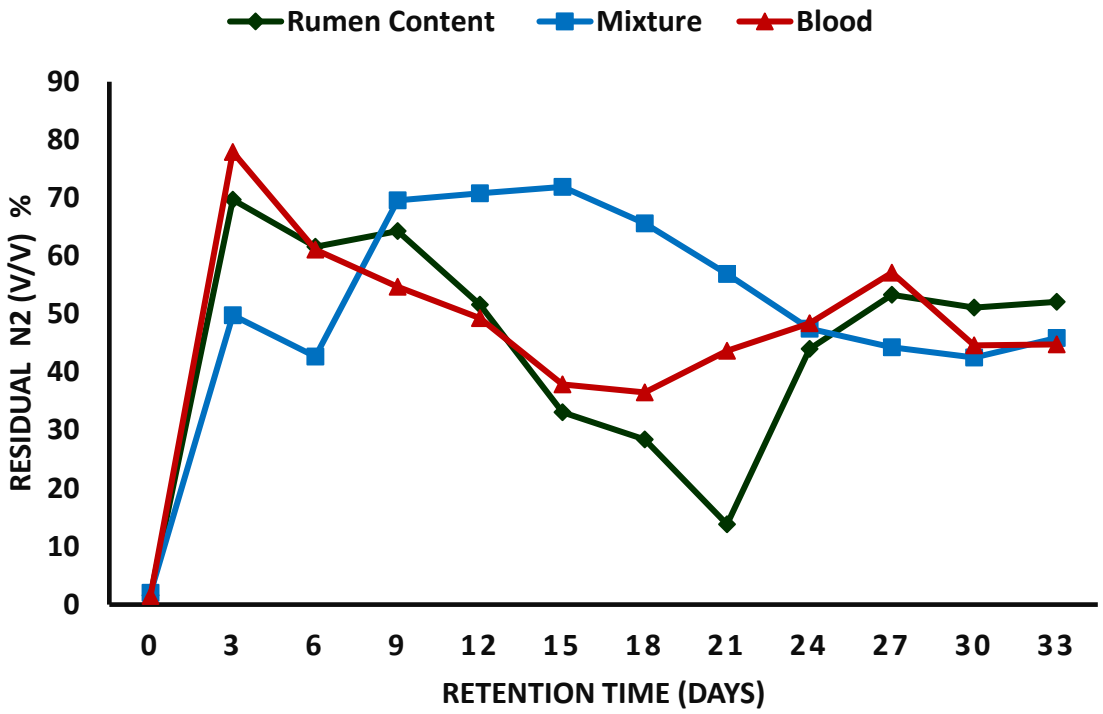

Figure 4.25: Nitrogen Content on Rumen Content, Mixture and Blood Waste

Therefore substrate which produce too high Nitrogen during the methane produce are not good, as it can be seen from Figure 4.25. Mixture substrate produced high Nitrogen. Alternatives from other substrate may be considered to co-digest the blood with other substrates with less nitrogen content.

4.8 COMPARISON OF ABATTOIR WASTE AFTER ANAEROBIC DIGESTION

4.8.1 Anaerobic Digestion of Cattle Blood Summary

The decrease in Blood substrate total solids (TS) from (17.75±0.20)% to (1.42±0.42)% as shown in Table 4.13, could be considered as an indicator of an anaerobic digestion effectiveness in treating or reducing abattoir blood waste. The Volatile Solids (VS) was also decreased from (94.97±0.51)% to (83.11±2.26)%, this could be seen as the potential biogas from those volatile gases or use for other new purposes. The increase in the Moisture content form (82.25±0.21)% to (98.92±1.10)% is due to water added to the substrate for better microorganism digestion could be considered good as it forms a better liquid fertiliser. Table 4.13 also shows an increased in Ash content from (5.03±0.51)% to (16.89±2.26)%, this is also good as it show the potential increase in fixed solids such as potassium which for crops.

Table 4.13 Summary of Blood waste anaerobic digestion Analysis

	Before Digestion					After Digestion				
	MC%	*TS%*	*Ash%*	*VS %*	*pH*	*MC%*	*TS%*	*Ash%*	*VS %*	*pH*
Mean	82.247	17.753	5.027	94.973	7.613	98.921	1.417	16.893	83.107	7.760
Standard Error	0.048	0.048	0.118	0.118	0.012	0.345	0.097	0.709	0.709	0.056
Median	82.210	17.790	5.105	94.895	7.620	98.663	1.431	16.527	83.473	7.750
Standard Deviation	0.082	0.082	0.204	0.204	0.021	0.691	0.167	1.417	1.417	0.112
Sample Variance	0.007	0.007	0.041	0.041	0.000	0.477	0.028	2.009	2.009	0.012
Skewness	1.605	-1.605	-1.483	1.483	-1.293	1.771	-0.375	0.850	-0.850	0.164
Range	0.152	0.152	0.383	0.383	0.040	1.514	0.334	2.968	2.968	0.220
Largest	82.341	17.811	5.179	95.205	7.630	99.937	1.577	18.742	84.226	7.880
Smallest	82.189	17.659	4.795	94.821	7.590	98.423	1.243	15.774	81.258	7.660
CL (95.0%)	0.204	0.204	0.506	0.506	0.052	1.099	0.415	2.255	2.255	0.178

4.8.2 Anaerobic Digestion of Rumen Content Summary

The increase in Rumen Moisture content (MC) waste form (85.01±1.99)% to (96.23±1.63)% as shown in Table 4.14, is due to water added on the digester. Together with the increase of Ash content (AC) from (14.90±3.03) % to (18.04±2.72)% the

Rumen content after anaerobic digestion would serve as a liquid fertiliser. The decrease in Rumen content Total Solids (TS) from (14.99±1.99)% to (3.77±1.63)% as shown in Table 4.14, like other substrate its indicate solid waste reduction of rumen content. Anaerobic digestion has also shown the ability to turn greenhouse gases into renewable and sustainable biogas energy. As can be seen from Table 4.14, Volatile Solid (VS) which compose volatile gases has been reduced from (85.10±3.03)% to (81.96±2.72)%. Hence providing solution to mitigating climate change impacts. The increase of Rumen content substrate pH level from (6.24±0.04) pH to (7.40±0.04) pH as indicated in Table 4.14, it shows the anaerobic digestion process operate best at neutral pH of 7.

Table 4.14: Summary of Rumen Content Waste Anaerobic Digestion Analysis

	Before Digestion					After Digestion				
	MC%	*TS%*	*Ash%*	*VS %*	*pH*	*MC%*	*TS%*	*Ash%*	*VS %*	*pH*
Mean	85.007	14.993	14.901	85.099	6.243	96.234	3.766	18.040	81.960	7.395
Standard Error	0.462	0.462	0.705	0.705	0.009	0.513	0.513	0.853	0.853	0.013
Median	85.331	14.669	15.356	84.644	6.240	96.070	3.930	17.994	82.006	7.400
Standard Deviation	0.800	0.800	1.221	1.221	0.015	1.026	1.026	1.707	1.707	0.026
Sample Variance	0.641	0.641	1.490	1.490	0.000	1.052	1.052	2.912	2.912	0.001
Skewness	-1.525	1.525	-1.444	1.444	0.935	0.710	-0.710	0.095	-0.095	-0.864
Range	1.499	1.499	2.311	2.311	0.030	2.319	2.319	3.716	3.716	0.060
Largest	85.594	15.905	15.829	86.482	6.260	97.558	4.760	19.945	83.771	7.420
Smallest	84.095	14.406	13.518	84.171	6.230	95.240	2.442	16.229	80.055	7.360
CL (95.0%)	1.988	1.988	3.032	3.032	0.038	1.632	1.632	2.716	2.716	0.042

4.8.3 Anaerobic Digestion of Rumen Content and Blood Waste Mixture.

A co-digestion of cattle blood and Rumen content substrates mixture as shown in Table 4.15, has decreased its Total Solids (TS) from (16.23±1.01)% to (1.61±0.51)% as an average indication of solid waste reduction, if anaerobic digestion could be implemented as waste treatment at Kumasi abattoir. The mixture substrate also has a Volatile solids (VS) reduction from (85.61±2.58)% to (80.58±3.22)% as an indication of the potential amount of Volatile gases or biogas that can be produced from the mixture substrate.

Table 4.15 also show an increase in pH level from (6.93±0.01) pH to (8.06±0.07)%, as a results the pH of 8 level could be considered suitable for co-digestion of blood and rumen content substrate. In summary the Ash Content has increased from (14.39±2.58)% to (19.42±3.22)% while the Moisture content increased from (83.77±1.01)% to (98.39±0.51)%. This indicate the remaining substrate has high moisture content and fixed solids containing phosphorus, potassium and nitrogen which is considered good for crop growth.

Table 4.15: Summary of Rumen Content and Blood Waste Mixture Analysis

	Before Digestion					After Digestion				
	MC%	*TS%*	*Ash%*	*VS %*	*pH*	*MC%*	*TS%*	*Ash%*	*VS %*	*pH*
Mean	83.771	16.229	14.390	85.610	6.928	98.393	1.607	19.420	80.580	8.058
Standard Error	0.233	0.233	0.599	0.599	0.003	0.159	0.159	1.013	1.013	0.022
Median	83.691	16.309	14.565	85.435	6.925	98.401	1.599	18.907	81.093	8.055
Standard Deviation	0.404	0.404	1.037	1.037	0.006	0.319	0.319	2.025	2.025	0.043
Sample Variance	0.164	0.164	1.076	1.076	0.000	0.101	0.101	4.101	4.101	0.002
Skewness	0.856	-0.856	-0.736	0.736	1.732	-0.084	0.084	0.971	-0.971	0.046
Range	0.797	0.797	2.053	2.053	0.010	0.691	0.691	4.373	4.373	0.080
Largest	84.209	16.588	15.329	86.724	6.935	98.731	1.960	22.119	82.254	8.100
Smallest	83.412	15.791	13.276	84.671	6.925	98.040	1.269	17.746	77.881	8.020
CL (95.0%)	1.005	1.005	2.577	2.577	0.014	0.507	0.507	3.222	3.222	0.069

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

During anaerobic digestion process, feedstock quality can have significant impacts on anaerobic process efficiency, operational costs, and economic profitability. This study analysed abattoir waste for biogas production in Kumasi, Ghana. The specific objectives were to determine the characteristics of the Kumasi abattoir waste, analyse the statistical significance on the means of the characteristics, and estimate potential quantity and quality of biogas. The conclusions are as follows.

- The cattle rumen content has average total solids of 14.47±0.89%, Volatile Solids of 84.63±1.30%, Carbon Content of 38.43±11.32% and Nitrogen content of 4.90±4.17%.
- The cattle blood waste has average total solids of 17.36±0.82%, Volatile Solids of 95.02±1.25%, 40.80±4.44% Carbon content, and Nitrogen Content of 9.90±0.09%.
- The analysis of variance of both the cattle blood and rumen waste characteristics concludes that there is a statistically significant difference between the mean of the characteristics, hence all null hypothesis were rejected.
- With regards to quality, cattle rumen content waste produced the highest methane of 46%, followed by co-substrate of blood and rumen waste with 39.9% and blood waste producing the least of 5.5% methane.

5.2 Recommendations

The following recommendations are made.

1. It is recommended that each time the rumen content and blood waste from the Kumasi abattoir is to be fed into an anaerobic digester, the characteristics of the specific rumen content and the blood waste should be determined for better anaerobic digestion process monitoring and operation. This is because of the inconsistency in the samples analysed.

2. It is recommended that the Kumasi abattoir explore the use of anaerobic digestion biogas technology as a waste treatment technology as they can generate biogas from the process.
3. Use of addictive or consideration of multi-substrate digestion is recommended to improve the biogas quality and quantity, because the combination of rumen content and blood produced the highest methane content.

5.3 Contributions and Further Research

This study has analysed Kumasi abattoir cattle rumen content and blood waste characteristics for biogas production. The substrate characteristics information is vital for designing anaerobic digestion digester, monitoring process and for the operation of the biogas production. Further studies could be in anaerobic digestion digesters design selection and sizing for utilization of the Kumasi abattoir waste. The studies may include optimization strategies, including co-digestion of other waste substrate that might be available to obtain the maximum biogas output and quality from the abattoir waste. The extent of the Kumasi abattoir waste impact on the environment and climate is still not clear, therefore further research could be used to cover the research gap.

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ANNEX

Table of Expenditure

SECTION	ITEMS	QUANTIY	COST(DA)	COST(GHc)	COST (USD)
Transportation					
	Flight ticket for research (Round way)	1	128662		1205.94245
	Local transport from Tlemcen to Algiers	1	5000		47
	Visa for Ghana	1	8000	50	74
	Ghana Entry Vaccine	1			20
	Local transport from Accra (Airport) to Kuamsi (internship city)	1		145	36.25
	Local transport from Kumasi (internship city) to Acra (Airport)	1		145	36.25
	Local transport for data collection	45		2250	562.5
Laboratory					
	Lab Coat			200	50
	Safety Shoes			500	125
	Safety glasses			80	20
	Gloves			50	
	KNUST Lab fee			1238.16	309.54
	Soil Research Lab fee			880	220
Reserch Preparation					
	Internet for research	4		1040	260
	Printing of master thesis+binding (100 pages)	4		392	98
Total					3000.00