GLYCEROL SUPPLEMENTATION EFFECT ON BIOGAS PRODUCTION FROM CATTLE AND PIG MANURE SUBSTRATES

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Glycerol Supplementation Effect on Biogas Production from Cattle and Pig Manure Substrates

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature…….. Date……

Peter Ngugi Kariuki

This thesis has been submitted for examination with our approval as University supervisors.

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Signature…….. Date……

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DEDICATION

This work is dedicated to my entire family, most importantly to my beloved parents, Elizabeth Wanjiru Kariuki and the late John Kariuki Ngugi, sister, Mary Wambui Kariuki, brothers, Moses Mwaniki Kariuki and Joseph Karanja Kariuki, my wife, Rita Muthoni Njuguna and our lovely daughter, Hailey Wanjiru Ngugi.

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LIST OF ABBREVIATIONS AND ACRONYMS

AD	Anaerobic Digestion
APHA	American Public Health Association
COD	Chemical Oxygen Demand
C/N	Carbon to Nitrogen ratio
GHG(s)	Greenhouse Gases
HRT	Hydraulic Retention Time
LPG	Liquid Petroleum Gas
MC	Moisture Content
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
pH	Potential of Hydrogen
RT(s)	Retention Time(s)
SRT	Solid Retention Time
TC	Total Carbon
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VFAs	Volatile Fatty Acids
VS	Volatile Solids
v/v	Volume by Volume
m/v	Mass to Volume Ratio
m/m	Mass in Mass

ABSTRACT

Organic wastes are potential sources of biogas and high-quality bio-fertilizers. Biogas is an appealing energy product that can be used directly as a renewable alternative source of energy. Some of the most commonly used substrates for biogas production are cattle and pig manure. Technology for biogas production is an active research area. Studies on optimization of biogas production are being undertaken with a view to making the process fully cost-effective. This study was conducted to investigate the effect of glycerol supplementation on biogas yield under the respective appropriate optimum mesophilic temperatures and substrates to water dilution ratios of both cattle and pig manure substrates. Analysis of substrates and inoculum for various physico-chemical characteristics was carried out. The optimum mesophilic temperature for both cattle and pig manure substrates was determined to be 40 ºC. The optimum cattle manure substrate to water dilution ratio was established to be 17.5:7.5 (m/v) and 5:20 (m/v) for pig manure substrate, respectively. On supplementing the optimum cattle manure substrate to water dilution ratio of 17.5:7.5 (m/v) with 0.1, 0.2 and 0.3 g of glycerol, the respective percentage increases in biogas yields over the control were calculated to be 6.4, 12.5 and 21.9%. Further, for the established optimum pig manure substrate to water dilution ratio of 5:20 (m/v) supplemented with 0.03, 0.05 and 0.08 g of glycerol, the respective percentage increases in biogas yields over the control were calculated to be 10.0, 17.65 and 29.6%. The study shows that, one, the upper limit of the mesophilic range (30-40 °C) gives a higher biogas yield; two, over-diluting or under-diluting substrates with water influences biogas yields; and three, glycerol which is also a by-product of biodiesel manufacturing can be advantageously utilized as a supplement to boost biogas production. Use of optimum conditions of temperature and dilution ratios would significantly improve biogas production.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

One of the ongoing discussions in the new global economy, that is difficult to ignore, is the fact that gas and oil reserves continue to dwindle worldwide, and will definitely run out in the not too distant future. The increase in the global demand for energy resources is currently exceeding the rate of local supply sources, and new discoveries are not keeping pace with the ever-increasing demand. Furthermore, due to fluctuation in energy prices, there is a growing importance to keep to a minimum our dependence on the world's diminishing supplies of fossil fuel, thereby a look beyond the fossils is of the utmost importance for long-term economic development and enhanced global energy efficiency and security (Kunatsa *et al.,* 2013; Minde *et al.,* 2013: Islam *et al.,* 2014; Yemata *et al.,* 2014).

Emission of greenhouse gases (GHGs), which play a significant part in global warming and climate change, is also an issue of global concern. The increasing concentration of atmospheric GHGs owing to culpable human activities, such as fossil-fuel exploitation and use as well as waste production and management represents the root of the problem (Lassey, 2008). Additionally, mass deforestation is a problem that mainly concerns developing countries, where most of the households rely on wood and charcoal excessively for fuel supply which implies cutting down of trees. This has often led to increased soil erosion, thereby decreasing fertility and agronomic value of agricultural land (Khoiyangbam, 2010). With the global energy demand expected to double by 2050 (Edomah, 2013), there is need to develop alternative energies.

Accordingly, we will need to view future energy needs as high priority and think of sustainable ways of providing additional sources of eco-friendly energy. Hence, in an effort to address the concerns regarding the future global energy needs, amid increasing

pressure for more energy, wide-ranging research work is being carried out worldwide to improve the situation.

1.1.1 Biogas in Energy Production

Anaerobic digestion (AD), a process that has been used in the treatment of organic wastes for decades, offers part of the clean and renewable energy solution by providing biogas energy. The term AD is broadly understood to mean a multi-step biological process during which the organic carbon is converted to its most oxidised (carbon dioxide, CO_2) and most reduced (methane, CH_4) state by the concerted action of a wide range of microorganisms in the absence of air (Sotirios *et al.,* 2009). Anaerobic digestion process uses biomass materials such as virgin wood, energy crops, agricultural residues, food waste and industrial waste and co-products to produce biogas and reduce GHG emissions as well as supply farmers with high quality bio-fertilizer, digestate, (Shahinzadeh *et al.,* 2012; Ngumah *et al.,* 2013).

Biogas is an appealing energy product that can be used directly as a source of energy (Torres-Castillo *et al.,* 1995). It's distinct from other renewable energies since it does not have any geographical limitations nor does it require advanced technology to produce. On top of that, it's also very simple to use and apply (Joy *et al.,* 2014; Santhosh & Revathi, 2014). It's a well-established fuel for cooking, heating, lighting and utilization as an alternative vehicle fuel in a number of countries (Sagagi *et al.,* 2009; Alexopoulos, 2012; Al Imam *et al.,* 2013; Raboni & Urbini, 2014).

1.1.2 Composition and Characteristics of Biogas

Biogas may be broadly defined as a combination of gases produced during AD of organic materials of plant origin. The overall result of AD is a nearly sheer conversion of the biodegradable organic matter into biogas (Sotirios *et al.,* 2009). Depending on the feedstock, biogas is predominantly a mixture of gases, which are; methane (CH_4), carbon dioxide (CO_2) and minute traces of hydrogen sulphide (H_2S) and hydrogen (H_2), nitrogen (N_2) and in addition ammonia (NH_3), Table 1.1.

Table 1.1: Typical composition of biogas*

Component and Formula	Concentration (% by vol.)
Methane (CH_4)	55-60
Carbon dioxide (CO_2)	35-40
Water (H_2O)	2-7
Hydrogen sulphide (H_2S)	20-20,000 ppm (2%)
Ammonia (NH_3)	0-0.05
Nitrogen (N_2)	0-2
Oxygen (O_2)	0-2
Hydrogen (H_2)	0-1

***Source: Karthick *et al.*, 2014; Santhosh & Revathi, 2014**

Methane is the only constituent of biogas with significant fuel value. The inert diluents of carbon dioxide and nitrogen lower the calorific content of the gas, whilst hydrogen sulphide corrosive nature wears down the anaerobic digester and pipes involved in the gas distribution (Hosseini *et al.,* 2013; Mushtaq *et al.,* 2013; Subbukrishna *et al.,* 2014). Methane is produced by a few kinds of microorganisms, which thrive in anaerobic conditions; that is conditions in which there is hardly any oxygen (Werner *et al.,* 2004). Biogas is about 20% lighter than air and has an ignition temperature in range of 650 °C to 750 °C (Deublein & Steinhauser, 2008; Joy *et al.,* 2014; Shamalan *et al.,* 2014), Table 1.2.

Table 1.2: General features of biogas*

Property	Value/Limit
Energy content	6-6.5 kWh/m^3
Fuel equivalent	0.6-0.65 l oil/m^3 biogas
Explosion limits	6-12% biogas in air
Ignition temperature	650-750 °C
Critical pressure	75-89 bar
Critical temperature	-82.5 °C
Normal density	1.2 kg/m^3
Smell	Bad eggs
Molar Mass	16.043 kg $kmol^{-1}$

***Source: Deublein & Steinhauser, 2008**

Biogas is a light, colourless and highly inflammable gas, second only to hydrogen in the energy released per gram of fuel burnt, hence its suitability for use as an energy source. Its average calorific value is 20 MJ/m^3 (or 4713 Kcal/m^3) at 0.01 atm and it usually burns with 60% efficiency in a conventional biogas stove with a blue flame similar to liquid petroleum gas (LPG). Besides, LPG has a comparatively higher calorific value of 94 MJ/m3, at atmospheric pressure. The energy available from the combustion of biogas is between 60% and 90% of the dry matter heat of combustion of the plant input material. However, the gas is obtainable from slurries of up to 95% water, so in practice biogas energy is often available where none would otherwise have been obtained (Godfrey, 2004).

1.1.3 Key Drivers of Biogas Production

There are three major influential key drivers of biogas production. These are: (a) oil independence; (b) reduced emissions, and; (c) rural development and value-added agriculture. For sustainable energy supply, reliable and diverse energy resources are essential both for short-term and long-term utilization. It is, therefore, crucial that we

develop and make attractive renewable alternative energies, taking into account the current and future energy consumption along with pollution reduction. It is of the utmost importance therefore that these alternative renewable energies are affordable, environment-friendly and are constantly available (Faaij, 2006; Karekezi *et al.,* 2009).

1.1.4 Benefits of Biogas Technology

Well-functioning biogas systems offer a wide range of benefits for their users and the environment that you don't get from other renewable technologies. Firstly, biogas systems eliminate and convert organic wastes into biogas and bio-fertilizer. As a result, the technology provides a relatively cheaper alternative to fossil fuel. Secondly, the digestate is a high-quality organic fertilizer. It has superior nutrient qualities over normal organic fertilizer and is an excellent bio-fertilizer for replacement of inorganic fertilizer. Thirdly, biogas technology is useful in waste management and sanitation in every respect. Biogas systems function as waste disposal systems, especially for human wastes, and can, for that reason, prevent potential sources of environmental contamination and the spread of pathogens (Khoiyangbam, 2010). Fourthly, setting up a biogas plant reduces GHG emissions at landfills and is, therefore, a necessary mitigation measure to limit global warming. Moreover, the technology's contribution to conservation through the protection of soil, water, air and woody vegetation is an environmental advantage. The technology also has the potential to create job opportunities for thousands of people and assist them in terms of economic development. Consequently, the standard of living can be measurably enhanced which directly contributes to social and economic development of a country (Khoiyangbam, 2010).

Other benefits of biogas technology include: (a) flexibility to use different feedstock and efficient end use of biogas; (b) reduced odours; (c) improved crop-livestock-tree system through nutrient cycling; (d) reduced time and workload of collecting fuel wood; and (e) reduced kitchen smoke-pollution thereby promoting human health (Al Seadi, 2008; Khoiyangbam, 2010; Mkiramweni, 2012; Minde *et al.,* 2013).

1.2 Anaerobic Digestion Process

Anaerobic digestion process is an active area of research, and advanced technologies are always being developed (Palfrey, 2013; Pietsch, 2014). Several AD technologies are commercially available and have been demonstrated for use with agricultural wastes and for treating municipal and industrial wastewater. Further, AD process has been used to convert sludge to end products of liquid and gases while generating as little biomass as possible. The AD process can be used to treat any carbon-containing material with varying degrees of degradation. Compared to aerobic process, AD process is far much more economic (Carlos *et al.,* 1998).

Literature defines AD as a naturally occurring process, by which anaerobic microorganisms convert biodegradable organic matter into biogas, in the absence of oxygen. Anaerobic processes could either occur naturally or in a controlled environment such as a biogas plant (Osueke & Ezugwu, 2011). The process is synergistic and involves a consortium of microbes that can be considered as a series of metabolic pathways. Besides, the process is carried out by facultative and obligatory anaerobic bacteria. The overall result of anaerobic degradation is a nearly sheer conversion of the biodegradable organic matter into biogas (Veeken *et al.,* 2000; Kelleher *et al.,* 2002; Gallert & Winter 2005). An advantage of AD process is the production of biogas, a high energy fuel that may be used to produce environmentally-friendly energy. Further, AD results in a product that contains stabilized solids, as well as some available forms of nutrients such as ammonia-nitrogen. It is essentially for these reasons that scientists and power industry companies have been keen on AD for a number of years (Fayyaz *et al.,* 2014).

1.2.1 Microbiological Processes in Anaerobic Digestion

The transformation of organic material into methane gas, often referred to as biomethanation or biomethanization, is a multi-step process divided into four key biological and chemical stages: Hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Digestion is hardly fully complete until the substrate has gone through

all of these stages, each of which has a physiologically distinctive bacteria population responsible and require disparate environmental conditions (Khanna & Mohan, 1995). Table 1.3 illustrates in detail the types of microorganisms and populations involved in the anaerobic digester (Henze, 2008).

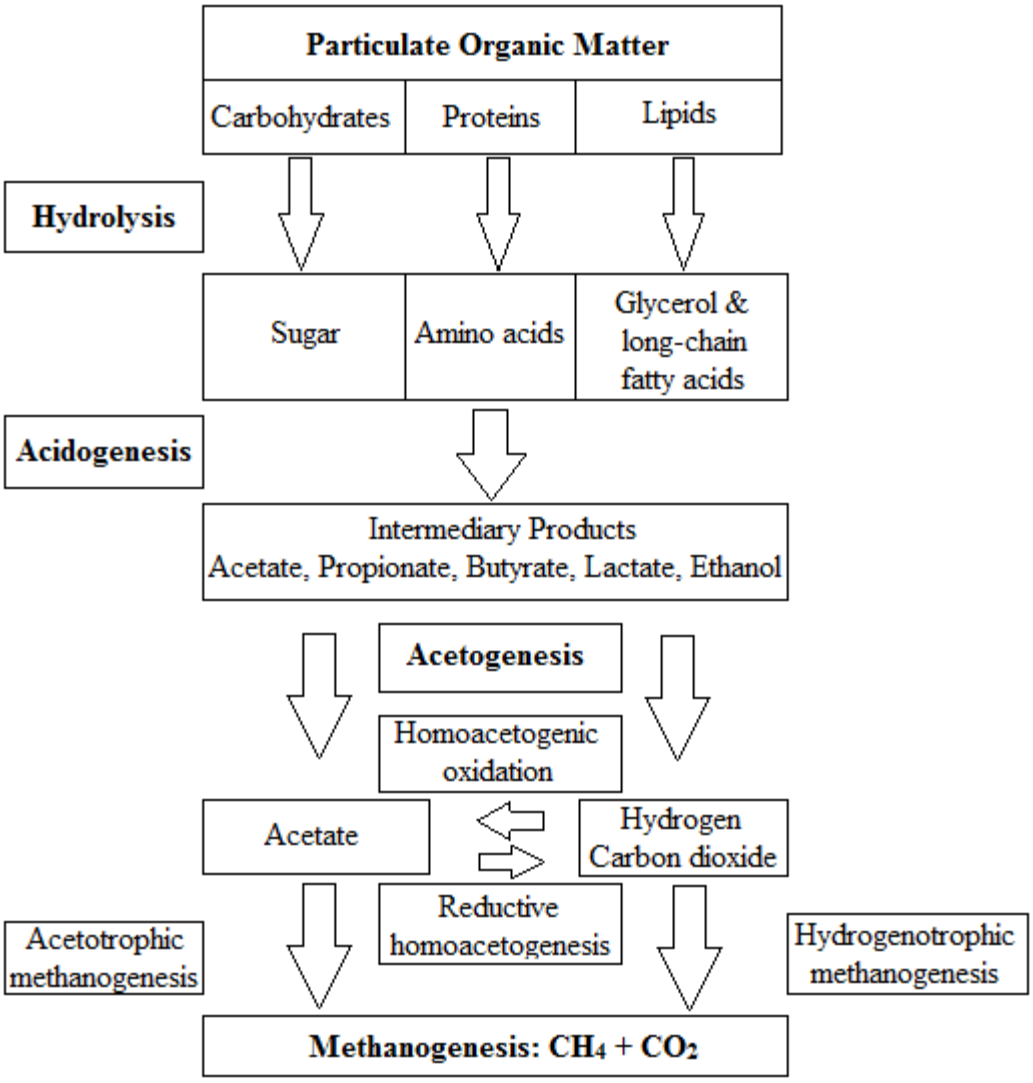
Table 1.3: Bacterial population from anaerobic digester*

Group	Cell/mL
Total hydrolytic bacteria	10^8-10^9
Proteolytic	10^7
Cellulolytic	10^5
Hemicellulolytic	10^6-10^7
Hydrogen-producing acetogenic bacteria	10^8-10^9
Homoacetogenic bacteria	10^6
Methanogens	10^5-10^6
Sulphate reducers	10^4

***Source: Khanna & Mohan, 1995; Henze, 2008**

1.2.1.1 Hydrolysis

Hydrolysis is defined by Gerardi, (2003) as 'the breaking of a large compound into small compounds by adding water.' This is normally the initial stage in AD and is more accurately termed as depolymerisation (Chynoweth & Pullammanappallil, 1996), Scheme 1.1. Hydrolytic bacteria are responsible for depolymerisation and are made up of both facultative and strict anaerobes. Macromolecules, such as carbohydrates, proteins and lipids are hydrolyzed by extra-cellular enzymes secreted by microorganisms into soluble products (Yadvika *et al.,* 2004; Parawira *et al.* 2005). The products of hydrolysis include simple sugars, alcohols, amino acids, fatty acids, carboxylic volatile acids, keto acids, hydroxy acids and ketone. These are simple small soluble molecules assimilated and metabolized in the microbial cells. The size of these soluble products must be small enough to allow their transport across the cell membrane of bacteria.



Scheme 1.1: Biomethanation stages (source: Demirel & Scherer, 2008)

Hydrolytic activity is fundamentally important in high organic waste and may become rate limiting. Previous studies have reported that hydrolysis is the rate-limiting step if the complex substrate molecules are large with a low surface-to-volume ratio (Sowers, 2000; Sowers *et al*., 2002; Henze, 2008). On the other hand, if the substrate is readily degradable, the rate-limiting step will be acetogenesis and methanogenesis (Björnsson *et al.,* 2001). The rate of hydrolysis is a function of factors, such as pH, temperature, composition and particle size of the substrate, and high concentration of intermediate products (Veeken *et al.,* 2000).

Hydrolysis occurs in two different ways. One the bacteria releases enzymes into the bulk liquid that are either adsorbed onto the particles or react with soluble substrates; and two the organisms engulf particles and release enzymes in their vicinity taking up the soluble products produced from the enzymatic reaction (Vavilin *et al*., 2008). The most common extra-cellular enzymes employed in hydrolysis are hydrolases and lyases. Hydrolases include lipases, glycosidases and peptases. Lipases and glycosidases hydrolyse ester bonds of lipids to produce fatty acids and glycerol, respectively. Peptases hydrolyse ester bonds of polysaccharide component of plant cell walls and peptide bonds in proteins. Lyases catalyse the non-hydrolytic removal of groups from substrates. Phosphodiesterases hydrolyse the ester bonds of modified polysaccharides that contain sugars with phosphohoryl, acyl or alkyl groups (Chynoweth & Pullammanappallil, 1996).

Manure solids consist of 40-50% biofibres as cellulose, hemicelluloses and lignin collectively known as lignocelluloses. Extracellular hydrolytic enzymes can break cellulose and hemicelluloses, whereas lignin is not readily degraded (Angelidaki & Ahring, 2000). According to Hobson and Wheatley (1993) the breakdown of fibres in stomachs of cows is achieved by bacteria attached to the exposed chewed fibre ends. Hydrolysis is affected by a couple of factors, for example, toxicity, substrate concentration, product concentration, temperature, and surface kinetics (Vavilin *et al*., 2008). For complex substrates, particle size is an important factor as reduced particle size increase available surface area for both enzymatic action and biomass colonisation.

1.2.1.2 Acidogenesis

Acidogenesis is the second stage in the four stages of AD. In this stage, products of the hydrolysis stage are the substrates for the acidogenic bacteria and are therefore readily accessible for acidogens (Henze, 2008). Schink (1997) describes acidogenic bacteria as both obligate and facultative anaerobes. In a stable anaerobic biogas digester, the soluble organic matter produced by hydrolytic bacteria is converted to simple organic compounds, for example volatile fatty acids (VFAs), alcohol, lactic acid and mineral

compounds, such as carbon dioxide, hydrogen, ammonia and hydrogen sulphide (Gerardi, 2003). The VFAs also referred to as volatile organic acids include components, such as acetate, propionate, butyrate, isobutyrate, valerate and isovalerate. This process is known as acidogenesis or fermentation. Earlier studies have reported that acidogenesis is the rapidest conversion step in AD of complex organic matter (Vavilin *et al.,* 1996; Henze, 2008). The most important of the organic acids is acetate since it can be used directly as a substrate by methanogenic bacteria (Myint *et al*., 2007). The primary reactions involved in this stage are shown in Equations 1.1, 1.2 and 1.3.

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \text{ (Acetic acid)} \quad (1.1)$$

$$C_6H_{12}O_6 + 2H_2 \longrightarrow 2CH_3CH_2COOH + 2H_2O \text{ (Propanoic acid)} \quad (1.2)$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \text{ (Butanoic acid)} \quad (1.3)$$

1.2.1.3 Acetogenesis

In the third stage, known as acetogenesis, obligate hydrogen generating acetogenic bacteria further convert the volatile organic acids to acetate, carbon dioxide and hydrogen which are direct substrates for methane producing methanogens (Henze, 2008). Acetogenic bacteria are syntrophic as they require the presence of hydrogen utilizing bacteria to maintain hydrogen concentration below 10^{-3} atm (Jorge-del-Real & Lopez-Lopez, 2012). This conversion process can only be thermodynamically preferred if the partial hydrogen pressure is kept low (Schink, 1997). High hydrogen concentrations result in increased long chain VFAs, low pH and inhibition of acetogenesis (Chen *et al.,* 2008). Hence, as a consequence of this, efficient removal of the produced hydrogen is necessary. Moreover, research shows that for vital functions of these bacteria that consume hydrogen, a steady temperature mode is crucial. Under anaerobic conditions, a rapid growth of acetogenic bacteria also occurs. They are active in a wide temperature range of 3 to 70 °C, with an optimum at around 30 °C. They require thorough direct contact with the substrates, meaning that the agitation of the substrate has positive effects (Gunaseelan, 1997). The residual compounds like alcohols, organic nitrogen compounds that methanogens cannot degrade are accumulated in the

digestate (Gerardi, 2003). Below are the primary reactions involved in the conversion of substrates to acetate (Equations 1.4-1.11):

Syntrophic acetogenic reactions:

$$CH_3CH_2COO^- + 3H_2O \longrightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \quad (1.4)$$

$$CH_3CH_2CH_2COO^- + 2H_2O \longrightarrow 2CH_3COO^- + H^+ + 2H_2 \quad (1.5)$$

$$CH_3CH_2COO^- + 2HCO_3^- \longrightarrow CH_3COO^- + 3HCOO^- + H^+ \quad (1.6)$$

$$CH_3CH_2CH_2COO^- + 2HCO_3^- \longrightarrow 2CH_3COO^- + 2HCOO^- + H^+ \quad (1.7)$$

Propanoate ion = $CH_3CH_2COO^-$; Butanoate ion = $CH_3CH_2CH_2COO^-$; Ethanoate ion = CH_3COO^-; Methanoate ion = $HCOO^-$; Hydrogen carbonate ion = HCO_3^-; Oxidane = H_2O; Molecular hydrogen = H_2; and Hydrogen cation = H^+

Homoacetogenic reactions:

$$CH_3CH(OH)COO^- \longrightarrow 1½CH_3COO^- + ½H^+ \quad (1.8)$$

$$C_2H_5OH + HCO_3^- \longrightarrow 1½CH_3COO^- + H_2O + ½H^+ \quad (1.9)$$

$$CH_3OH + ¼HCO_3^- \longrightarrow ¾CH_3COO^- + H_2O \quad (1.10)$$

$$4H_2 + 2HCO_3^- + H^+ \longrightarrow CH_3COO^- + 4H_2O \quad (1.11)$$

2-Hydroxypropanoate ion = $CH_3CH(OH)COO^-$; Ethanol = C_2H_5OH; and Methanol = CH_3OH

1.2.1.4 Methanogenesis

Lastly and most importantly, methane is produced by a wide array of bacteria called methane formers also known as methanogens from methanogenic substrates, namely acetic acid, hydrogen, carbon dioxide and methanol. In the ordinary anaerobic digesters, 90% of methane yield takes place at this stage, 70% from cleavage of acetic acid molecules to generate carbon dioxide and methane and 30% from carbon dioxide reduction with hydrogen by hydrogenotrophic bacteria (Klass, 1984; Vandevivere *et al.,* 2002). The acetic acid formation is the factor that defines the speed of methane formations. Methanogens are classified into two major groups. One is the acetate converting or acetoclastic methanogens, and the other is the hydrogen utilising or

hydrogenotrophic methanogens (Henze, 2008). Hydrogenotrophic methanogenesis (Equation 1.12) functions more efficiently at high hydrogen partial pressure, while acetoclastic methanogenesis (Equation 1.13) is independent of hydrogen partial pressure (Schink, 1997). Finally, it is important to note that at higher temperatures, the acetate oxidation pathway becomes more favourable (Schink, 1997). The primary reactions involved in the conversion of methanogenic substrates to methane are shown below in Equations 1.12, 1.13 and 1.14.

$$4H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + 3H_2O \quad (1.12)$$

$$CH_3COO^- + H_2O \longrightarrow CH_4 + HCO_3^- \quad (1.13)$$

$$CH_3OH \longrightarrow \tfrac{3}{4}CH_4 + \tfrac{1}{4}HCO_3^- + \tfrac{1}{4}H^+ + \tfrac{1}{4}H_2O \quad (1.14)$$

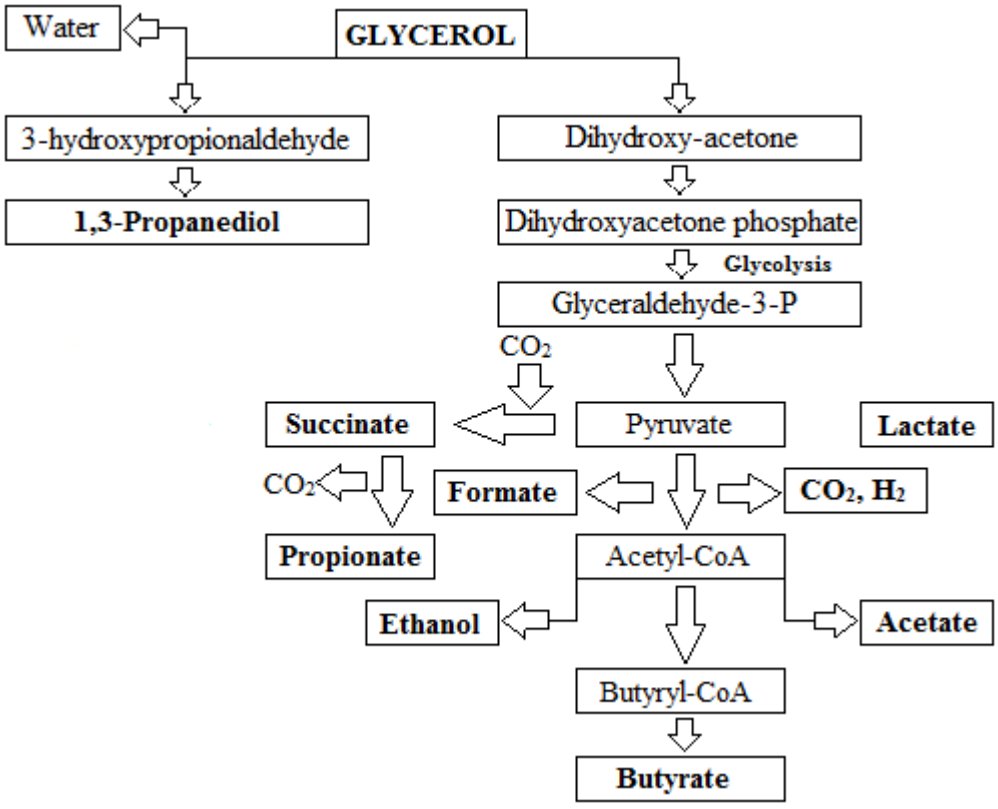
1.3 Glycerol Fermentation

Researchers have shown an increased interest in co-digestion of feedstocks with an organic carbon-rich substrate such as glycerol so as to enhance biogas yields. Literature report, (Schauder & Schink, 1989) describes glycerol as an essential constituent of the lipids in biomass. Glycerol is highly concentrated with organic carbon and very soluble in water. These properties make it a suitable co-substrate for improving digester efficiency. Besides, glycerol is highly biodegradable and most of the known glycerol-fermenting bacteria form 1,3-propanediol and acetate as reduced products. Only a handful of strict anaerobes ferment glycerol to 1,3-propanediol and 3-hydroxypropionate (Schink & Steib, 1983; Steib & Schink, 1984). *Citro bacter freundii* dismutate glycerol to mostly formate, acetate and ethanol (Schauder & Schink, 1989). Glycerol fermentation to propionate is accomplished by some classical propionic acid bacteria (Stjernholm & Wood, 1960), as well as by *Selenomonas ruminantium* and *Anaerovibrio lipolytica* (Hobson & Mann, 1961). Fermentative glycerol breakdown by rumen content yields acetate, propionate, butyrate and lactate (Schauder & Schink, 1989). *Anaerovibrio glycerini*, an anaerobic bacterium, is greatly specialized on glycerol utilization and forms propionate as the only organic fermentation product (Schauder & Schink, 1989).

Fermentation of glycerol to propionate releases a significant amount of energy; see Equation 1.15 (Thauer *et al.,* 1977).

$$C_3H_8O_3 \longrightarrow C_3H_5O_2^- + H^+ + H_2O; \triangle G^o = -150 \text{ kJ per mol} \qquad (1.15)$$

Diverse microorganisms ferment glycerol into various organic acids, solvents and 1,3-propanediol (Lengeler *et al.,* 1998). Scheme 1.2 below illustrates the most common metabolic pathway in the fermentation of glycerol by a mixed culture.



Scheme 1.2: Metabolic pathway in the fermentation of glycerol (source: Temudo *et al.*, 2008)

Whenever glycerol is used as a co-substrate in AD, it is important that a stabilized mixed culture of acetogens and methanogens is established, which can then actually convert the glycerol to methane (Drake, 1994). As shown in Equation 1.16, glycerol is first converted to acetic acid by acetogens.

$$C_3H_5(OH)_3 + 2CO_2 + H_2 \longrightarrow 2CH_3COOH + 2H_2O \qquad (1.16)$$

This is then followed by the conversion of acetic acid to methane by acetoclastic methanogens as shown in Equation 1.17.

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad (1.17)$$

Acetic acid can as well be formed from carbon dioxide and hydrogen in a different pathway, although different methanogenic bacteria can also utilize other carbon sources to generate methane (Gottschalk, 1986).

1.4 Substrate Physico-chemical Characteristics

Waste characterization is the process by which the physical and chemical properties of various waste streams are analyzed. Waste characterization plays a significant role in any treatment of waste that may arise. Developers of advanced waste technologies ought to take into account what precisely waste streams consist of in order to utterly treat the waste. Regardless of the approach taken in characterizing a waste, the aim is to widen the available knowledge that is needed to make the critical decisions. The biodegradable component of the waste stream is vitally important in the use of systems such as composting or AD. The composition of wastes affects the yield and biogas quality as well as the compost quality (Khalid *et al.,* 2007). Waste biodegradability can be evaluated based on characteristic features that are indicators of biomethanation potential of the substance (Esposito *et al.,* 2012). Essentially, there are many physical and chemical properties that play an important role in AD process; some of these are discussed in the following sections.

1.4.1 Total Solids

The Total Solids (TS) content of a sample is a measure of all organic and inorganic solids, both dissolved and suspended, per unit volume of the slurry. Total solids are measured as a percentage of the actual weight of the digested substance and it is only a part of the TS that is digested thus the importance of the physical property (Yavini *et al.,* 2014). Total solids are determined by weighing the digested material then it is dried up and re-weighed. Low solids AD systems take in less than 10% TS, medium solids

around 15-20% and high solids processes about 22% (Tchobanoglous *et al.,* 1993). An increase in TS in the digester brings about an equivalent decrease in the reactor volume (Nalo *et al.,* 2013). Fulford (1988) reported that the best biogas output occurs when TS is ranged from 8-12% so as to avoid solids settling down or clogging the flow of biogas formed at the bottom part of the anaerobic biogas digester. On that account, dilution of organic substrates or wastes with water to attain the desirable TS percentage is necessary.

1.4.2 Moisture Content

Water is the essential element for microorganisms' life and their activity. Bacterial movement and extracellular enzyme activity are highly determined by the water content in the anaerobic biogas digester (Nijaguna, 2002; Adelekan, 2012; Prabhu *et al.,* 2014). Optimum moisture content (MC) has to be maintained in the digester and the water content should be kept in the range of 60% to 80% (Bouallagui *et al.,* 2003; Khalid *et al.,* 2011; Gashaw, 2014). However, the optimum water content will probably differ with different feedstocks depending on the substrate physical and chemical properties as well as its biodegradation rate (Nijaguna, 2002; Prabhu *et al.,* 2014; Somashekar *et al.,* 2014).

1.4.3 Volatile Solids

When taking into consideration biogas production from slurry, the volatile solids (VS) content of the material is equally important as the TS content, for the reason that it represents the fraction of the solid material that may be converted into biogas. For this reason, VS content is an indicator of the potential of biogas and methane production from AD of organic wastes (Zhang *et al.,* 2007; Joy *et al.,* 2014). Total solids of any substance include all solid matter whether inorganic or organic. However, the VS content of a material is the organic portion of the solid matter. Besides, VS are those solids in water or other liquids that are lost on ignition of dry solids at 1020 °F (550 °C). Since the organic fraction of the solid material can be driven off at high temperatures, they are called VS. This is the actual part of the waste that is available for digestion and

for this reason; methane yield is reliant on the VS content in the waste (Ellis, 2004; Moller *et al.,* 2004; Kangle *et al.,* 2012; Begum & Nazri, 2013).

1.4.4 Total Carbon

Total carbon (TC) content is simply one valuable piece of information that is required by analysts interested in the carbon content of a sample. Total carbon is a measure of the amount of carbon-containing compounds in water. The measure includes both organic and inorganic forms of carbon in addition to compounds that are soluble and insoluble (Fulford, 1988). Having the knowledge of the source of carbon in the sample, whether it is derived from organic or inorganic material, is also of extreme importance. The capability to measure and characterize the carbon content of a sample is of utmost value in a diversity of different industries and research environments (Bernard *et al.,* 1995). The ordinary laboratory analysis involves the conversion of all forms of carbon to carbon dioxide and the subsequent measurement of the carbon dioxide produced. The parameter exemplifies an estimate of the strength of wastewater and the possible detriment that an effluent can cause to a receiving stream or other bodies of water as a result of the withdrawal of dissolved oxygen from the water (Khoiyangbam, 2010; Ezeoha & Ugwuishiwu, 2011).

1.4.5 pH

pH is a measure of the acidity or basicity of an aqueous solution; a solution in which the solvent is water. The pH value of the digester content is a significant indicator of the performance and the stability of an anaerobic biogas digester. In a well-balanced AD process, almost all products of a metabolic stage are continuously converted into the next breaking down product without any significant build-up of intermediary products such as different fatty acids which would result in a pH drop. Numerous aspects of the complex microbial metabolism are highly influenced by pH fluctuations in the digester. Although the sufficiently enough enzymatic activity of acid-forming bacteria can take place at pH 5.0, methanogenesis advances only at a high rate when the pH is kept in the neutral range. Various anaerobic bacteria including methane forming bacteria work

optimally in a pH range of 6.8 to 7.6 and the rate of methane production may drop if the pH is lower than 6.3 or greater than 7.8 (Stronach *et al.,* 1986; Fulford, 1988; Wen, 2006).

1.5 Parameters Influencing Anaerobic Digestion and Biogas Production

Biomethanation is brought about by bacteria employing several kinds of enzymes to catalyze reactions. For normal enzymatic activities, specific environmental conditions are required under which the reaction rates are optimum. The rate at which the microorganisms grow is of the utmost importance in the AD process. It can have an impact on the performance of the AD systems, either by process enhancement or inhibition. The operating parameters of the digester have to be controlled so as to improve the microbial activity and thus enhance the anaerobic degradation effectiveness of the system (Kangle *et al.,* 2012).

The rate and efficiency of the AD process are influenced to a great extent by a number of operational conditions inside and outside the anaerobic biogas digester. For the purpose of maintaining the appropriate conditions for bacterial activity and maximizing biogas/methane production, which in many cases is the eventual goal, there are certain operational parameters that need to be taken care of. This section will briefly discuss some of these operational parameters, namely: retention time, organic loading rate (OLR), temperature, pH and alkalinity, carbon to nitrogen ratio (C/N ratio), mixing, water content, gas pressure, air tightness and storage, and toxicity (Nijaguna, 2006). These parameters are interconnected; any rapid change in one parameter may affect the others positively or adversely eventually affecting the potential biogas yield or the methane content of the final gaseous product (Yadvika *et al.,* 2004). In accordance, therefore, they need to be taken into consideration throughout the bioreactor selection and design process, the choice of the substrate and operation and maintenance. Maintaining the system under optimum conditions can be challenging. The physico-chemical operating parameters have to be regularly analyzed to make certain that the

process is working correctly (Gerardi, 2003; Sakar *et al.,* 2009; Amani *et al.,* 2010). Some of the important physico-chemical factors are discussed below.

1.5.1 Retention Time

Retention time (RT) is understood to mean the theoretical time that a particular substrate resides in a digester (Kangle *et al.,* 2012; Ogur & Mbatia, 2013). In continuous systems, it is calculated as the volume of the digester divided by the volume of the slurry added per day and it is expressed as days (Dennis & Burke, 2001; Ogur & Mbatia, 2013). Retention time can be correctly distinct only in batch type facilities (Ezekoye *et al.,* 2011; Asgari & Gavanji, 2013). In an anaerobic digester, there are two types of retentions times that are significant design and process parameters. One is SRT and refers to the average time that the solids remain in the system (Gerardi, 2003). Solid retention time is determined by dividing the weight of VS in the system by the weight per unit time of VS leaving the system (Dennis & Burke, 2001). The other one is the HRT and is equal to the SRT in utterly mixed non-recycled digester systems (Nijaguna, 2006).

There is the least possible RT which enables the slowest growing bacteria to generate as well as the subsequent conversion of the organic material to biogas (Dennis & Burke, 2001). Besides, there is also a minimum RT required to bring about an adequate stabilization of the solid. If the RT is cut into half, gas production will decline and the process may break down owing to a condition called wash out where the bacterial cultures decrease to the point that they are no longer operative (Gerardi, 2003). Hydraulic retention time determines how much of the substrate will be degraded (Schnurer & Jarvis, 2010). If RT is more than ten (10) days at 35 °C, the gas production levels out and very little additional gas is produced for the supplementary time. It must, therefore, be noted that long RTs result in low efficiency of the AD process. More importantly, though, HRT is chosen so as to achieve a 70-80% of complete digestion (Nijaguna, 2006). The recommended average HRTs for mesophilic digestion are: cattle manure 12 to 25 days; cattle manure with straw bedding 15 to 35 days and pig manure

10 to 20 days (Sakar *et al.,* 2009; Kothari *et al.,* 2014). However, HRT values out of this recommended ranges have been reported (Sakar *et al.,* 2009).

1.5.1.1 Effect of Retention Time on Methane Production

The decomposition or digestion of organic substances under anaerobic conditions is slow and, therefore, these substrates have to be retained for varying lengths of time for digestion to end. Retention time describes the length of time the material is subjected to these reactions (Dennis & Burke, 2001). In the Indian-type digester, RT for both liquid and solid is the same because dung is mixed into homogeneous slurries prior to being charged into the digester. For this reason, HRT is equal to SRT (Muzenda, 2014). Further, together with the liquid of the digester, the bacterial cells are also let out in the digestate. Hence, in this case, HRT, SRT, and cell residue RT are equivalent. In few digester designs, the active cells in the effluents are recycled so as to increase the cell RT without increasing HRT or SRT (Nijaguna, 2006). The RT of different substrates is largely influenced by a whole range of factors that impact on biogas output, namely their rate of biodegradability, exposure to bacterial enzymes and physico-chemical properties of the substrates. Lastly, but most importantly, HRT is a design parameter that can be changed according to the size of the plant, temperature of fermentation, wash out time, among others (Sakar *et al.,* 2009).

1.5.2 Organic Loading Rate

Organic loading rate (OLR) is an important digester design parameter that determines the quantity of substrate per unit volume to be fed into the digester for a stabilized AD process as well as the part of it that eventually will be converted into biogas. The efficiency of the AD process is as well determined by the loading rate (Evans, 2001). The term ‘organic loading rate’ also referred to as ‘feedstock loading rate’ is used by Kangle *et al.* (2012) to refer to the rate at which feedstocks are loaded into the reactor per day. It is measured in the amount of VS added per day per digester volume.

A search of the literature reveals that; HRT, SRT, reactor volume, feeding and wasting rates and waste characteristics are some of the factors that influence OLR (Leitao *et al.,* 2006). Methane yield is directly proportional to OLR (Yadvika *et al.,* 2004). However, there is an optimum OLR for a system depending on the type of waste being treated. Some reports state that VS concentrations differ between different livestock wastes, leading to differences in OLR based on the waste (Hill & Bolte, 2000; Demirer & Chen, 2005). The effectiveness of the digester reduces drastically if OLR is increased beyond the optimum value owing to the accumulation of inhibiting substances such as fatty acids in the digester slurry (Demirer & Chen, 2008; Kangle *et al.,* 2012).

OLR is an important control parameter in continuous systems. Many plants have reported system failures as a result of overloading (Kangle *et al.,* 2012). Acetogens and methanogens are both affected by overloading. Given that digesters are overloaded, acetogens produce more acetate, which methanogens are not able to utilize as fast as it is produced. As a consequence, biogas production is lowered. Carbon dioxide and hydrogen, the other products of acetogenesis, also accumulate in the anaerobic digester. As more hydrogen is generated, the partial pressure of hydrogen rises to higher than 10^{-4} atm. The slow growing methanogens are not able to use this hydrogen, hence lower biogas yields and the lower methane content in the gas. The acidogens may also experience inhibition with increasing hydrogen partial pressure (Leitao *et al.,* 2006).

Typical loading rates for mesophilic and thermophilic processes are 2-3 kg VS/m^3·d and 4-5 kg VS/m^3·d, respectively. During reactor start-up, a low OLR should be applied so as to avoid overfeeding of the microorganisms. Immediately after the microorganisms have adapted, and the process is stable, the OLR can be increased gradually as the bacteria grow. It is important to keep the OLR as constant as possible over time (Schnurer & Jarvis, 2010; Kangle *et al.,* 2012). Anaerobic digestion systems treating pig waste have been operated at OLRs ranging from 0.9 to 15.5 g VS/L-day. However, as OLR increases, biogas production, methane content and VS removal decreases for both

mesophilic and thermophilic systems. The recommended OLR for pig waste is 3.0-3.5 kg VS/m^3-day (Burton & Turner, 2003).

1.5.3 Temperature

Owing to the heavy reliance of temperature on digestion rate, the temperature is the most critical parameter to maintain in a suitable range in order to optimize biogas production. Temperature has an impact on the rate of reaction and has secondary outcomes like for instance effects on the solubility of heavy toxic metals, solubility of carbon dioxide and consequently also on the buffering and composition of gas (Nijaguna, 2006). An increase in the ambient temperature increases the rate of reaction and also boosts the rate of biogas production (Bouallagui *et al.,* 2004). There are three temperature ranges under which anaerobic bacteria exhibit peak activity and these are: (a) Psychrophilic or Cryophilic (< 15 °C), (b) Mesophilic (15-45 °C), and (c) Thermophilic (45-65 °C) (Collins *et al.,* 2003; Evans & Furlong, 2003; Kangle *et al.,* 2012). Each of these ranges of temperature correlates to a different species of methanogens that will carry out the digestion (Bouallagui *et al.,* 2004).

The mesophilic and thermophilic are the most critical temperature ranges as anaerobic reactions necessarily cease below 10 °C (Amani *et al.,* 2010). Most of the methanogenic microorganisms are effective in these temperature ranges with optimal temperatures of around 35 °C and 40 °C for mesophilic and thermophilic, respectively (Labatut & Gooch, 2012). Mesophilic AD is by far the most widely used, especially because most of the methanogens are mesophiles. Only a few of these are thermophilic (Deublein & Steinhauser, 2008; Deublein & Steinhauser, 2011). Methanogens are sensitive to sudden changes of temperature. Small variations in temperature cause an appreciable decrease in activity. Thermophilic methanogens are more temperature-sensitive to fluctuation in temperature than mesophilic methanogens and rapid change of the order of 2 °C to 3 °C can upset the biogas production. To maintain a stable and functioning process, the digester temperature variation should be kept down to a level that will not threaten the

rate of biogas production and consequently the yield (Gerardi, 2003; Mukumba & Makaka, 2015). Therefore, the temperature should be kept exactly within a range of ± 2 °C. Considering the mesophilic and thermophilic temperature ranges, the energy balance is much better in the mesophilic range than in the thermophilic range (Deublein & Steinhauser, 2008).

However, it has been observed that higher temperatures in the thermophilic range reduce the required RT. Besides that, thermophilic digestion allows for better loading rates and achieves a higher rate of pathogen destruction, as well as a greater degradation of the substrate. In spite of that, thermophilic processes are sometimes regarded as less attractive from the energy point of view since they require more energy for heating. On the other hand, a mesophilic process requires longer RTs, although the stability of the process makes it highly attractive in current AD facilities (Zaher *et al.,* 2007). There is no significant difference in the ultimate yield in the temperature range of 30-60 °C though at 65 °C, yields drop. Concerning all methane gas generation systems, there is no kinetic advantage in carrying out digestion under thermophilic conditions (Nijaguna, 2006).

1.5.4 pH and Alkalinity

The pH of the digester liquid is necessary since microorganisms are considerably pH-dependent and sensitive to extreme variations. Specifically, the enzymatic activity of bacteria is mainly influenced by pH (Gerardi, 2013). The species associated with AD have different optimal pH growth ranges. These two interdependent parameters pH and alkalinity need to be adjusted so as to maintain the chemical conditions in the digester at an optimal state. It is however highly recommended to maintain the pH around neutrality (Christy *et al.,* 2014).

In AD, pH refers to the equilibrium between carbonic acid, bicarbonate alkalinity, and carbonate alkalinity, and also between ammonia and ammonium ions in anaerobic digesters (Ahring, 2003). The optimum pH range of an anaerobic digester is from 6.8 to

7.4 (Gerardi, 2003). Microorganisms and their enzymes are highly sensitive to pH deviations. Methanogens are more sensitive to pH variations than acidogens and are active only in the narrow pH range. The optimal pH for methanogens is between 6.8 and 8.5. Acidogens can survive at a pH as low as 5.5 (Khanal, 2008). pH values below or above this range may hinder the process in an anaerobic digester because microorganisms and their enzymes are sensitive to pH deviation (Yadvika *et al.,* 2004). Deviations from the optimum pH can give rise to the following changes in the enzymes and consequently reduce their activity; changes in the state of enzyme ionizable groups, alteration in the non-enzyme component of the system and denaturation of enzymes (Nijaguna, 2006).

pH in an anaerobic digester is a function of RT. Acid-forming bacteria grow at a much faster rate than methanogens. If acid forming bacteria grow too quickly, they may generate more acid than methanogens can utilize. Consequently, the pH drops, and the system may become unbalanced, inhibiting the activity of methane forming bacteria. As a result, methane production may stop altogether (Dennis & Burke, 2001). Ample alkalinity in a biogas digester is necessary for pH control (Kangle *et al.,* 2012). Alkalinity serves as a buffer that averts rapid pH change and maintains the stability of anaerobic systems (Gerardi, 2003). Buffer capacity represents the equilibrium of carbon dioxide and bicarbonate ions which offer resistance to significant and rapid changes in pH (Ward *et al.,* 2008). When organic matter is biodegraded in anaerobic digesters, organic acids such as acetate, butyrate and propionate are produced. High concentrations of organic acids in an anaerobic digester may cause a decrease in alkalinity below the usual operating level and as a result of this, almost assuredly, digester failure is imminent (Bjornsson *et al.,* 2001; Boe *et al.,* 2010). In a case where alkali compounds are inadequate in the feed substrate, alkalinity has to be balanced by adding chemicals such as sodium bicarbonate, potassium bicarbonate, sodium carbonate, calcium carbonate, calcium hydroxide or sodium nitrate to maintain stable operating conditions in the anaerobic digester (Gerardi, 2003).

As digestion reaches the methanogenesis stage, the concentration of ammonia rises and the pH value can increase to above 8. Once methane production is stabilized, the pH level stays between 7.2 and 8.2. Ammonium is an important parameter for the buffer capacity in an anaerobic reactor as it is non-toxic to anaerobic bacteria. With concentrations of up to 1000 mg/l, ammonium stabilizes the pH value (Fricke *et al.,* 2007). On the other hand, free ammonia of 100 ppm can be very toxic and cause digester failure (Gerardi, 2003).

1.5.5 Carbon to Nitrogen Ratio

The relationship between the amount of carbon and nitrogen available in organic materials is expressed in terms of the C/N ratio. For the successful operation of biogas digesters, the C/N ratio of the input substrate should be maintained within the appropriate range due to the fact that the nutrient composition has an effect on the optimal growth and activity of microorganisms (Nijaguna, 2002). It is important that the correct chemical form and concentration of nutrients are available for the optimal growth and activity of bacteria. Carbon in carbohydrates and nitrogen in proteins or nitrates are the primary nutrients for anaerobic bacteria. Carbon supplies energy and nitrogen are needed for building up the cell structure. Fermentative bacteria utilize carbon 25 to 30 times higher than nitrogen. Thus, for optimum functioning, microbes require 25-30:1 ratio of C to N with the largest part of the carbon being readily degradable. Deviation from this ratio slows down the process (Nijaguna, 2006).

For the various organic wastes used for biogas production, their C/N ratios differ from one another (Nijaguna, 2006). Co-digestion of different substrates can enhance the production of biogas since there is a supply of missing nutrients by the co-substrates. That being the case, waste materials with low carbon content can be mixed with other nitrogen-rich substrates so as to attain the required C/N ratio (Yadvika *et al.,* 2004) and therefore an optimum combination of the substrates is necessary in order to achieve the optimum C/N of 30. The C/N ratio of cow dung is around 16-25 (Nijaguna, 2002). Manure can also be co-digested with a different type of plant materials so as to increase

the production of biogas (Nijaguna, 2006). A high C/N ratio is a sign of rapid consumption of nitrogen by methanogens and consequently leads to a decrease in biogas production. However, a lower C/N ratio results in ammonia accumulation and pH values above 8.5, which is toxic to methanogens. Hence, optimum C/N ratios of substrates can be achieved by mixing feedstocks of high and low C/N ratios (Yadvika *et al.,* 2004).

1.5.6 Mixing

The substrate in an anaerobic digester is mixed intermittently ranging from several times a day to a number of times per hour (Lemmer *et al.,* 2013). Mixing plays an important role in the AD of solid waste. The degree of mixing varies depending on the feedstock and operating conditions. The close contact between microorganisms and the substrate material is necessary for an efficient digestion process (Yadvika *et al.,* 2004; Lemmer *et al.,* 2013).

Sufficient agitation of the reactor content is necessary for a well-functioning process in several aspects. Firstly, it is meant to achieve a homogeneous temperature in every part of the reactor and uniform distribution of the substrate. Mixing will prevent localized accumulation of inhibitory substances and deposition of large solid particles in the substrate. Secondly, it facilitates and makes easier the contact between microorganisms and the substrate as well as the close contact between the acetogens and methanogens. Stirring can, however, not be too vigorous as it can be detrimental to the aggregates between these microbial groups. Thirdly, good mixing reduces the risk of foam formation. Fourthly and lastly, it ensures an even release of biogas bubbles trapped in the substrate. Efficient mixing makes sure that the entire reactor volume is adequately utilized (Gerardi, 2003; Gray, 2004; Schnurer & Jarvis, 2010; Lemmer *et al.,* 2013).

Mechanical stirring equipment are used for agitating substrates. Some of the methods used may include: a circulation pump; gas compression through the substrate; or self-supporting equipment that uses the pressure of gas in the biogas digester are also used for agitating digester contents (Gerardi, 2003). Other ways may include, for example,

daily feeding of the substrate instead of long interval provides the preferred mixing effect. Installation of certain mixing devices such as propellers, scrapers, or pistons is also a mechanism for stirring (Yadvika *et al.,* 2004; Lemmer *et al.,* 2013).

1.5.7 Water Content

Water is the vital element for bacterial survival, their activity and movement. The hydration of biopolymers to facilitate easy breakdown of substrates and extracellular enzyme activity are highly dependent on the water content in the biogas digester. Optimum MC has to be maintained in the digester (Nijaguna, 2002; Nijaguna, 2006). Water content should be kept in the range of 60-80% (Bouallagui *et al.,* 2003; Khalid *et al.,* 2011; Gashaw, 2014). Waste characteristics can be altered by simple dilution. Water will reduce the concentration of certain constituents such as nitrogen and sulfur that generate products (ammonia and hydrogen sulfide) that are inhibitory to the AD process. High solids digestion creates high concentrations of end products that inhibit anaerobic decomposition of substrates. Therefore, some dilution can positively impact the process (Dennis & Burke, 2001).

In the case of cow dung based biogas plants in India, 9% TS in the digester has been established to be optimum. However, the optimum water content is likely to differ with different input materials depending on the substrates chemical characteristics and biodegradation rate (Nijaguna, 2002; Nijaguna, 2006). Production rates decrease with increasing concentration of TS. It is, therefore, imperative to determine the optimum solids concentrations for different feedstocks and digestion procedures. Further, if the water content is too high, the mean slurry temperature and hence the net biogas decreases and on the other hand, given that the water content is too low, active acids accumulate and impede fermentation process (Nijaguna, 2006).

1.5.8 Gas Pressure, Air Tightness, and Storage

Methane production is slowed down significantly if the gas pressure exceeds 1.2 bar absolute value (abs). A maximum gas pressure of 1.15-1.2 bar (abs) inside the digester is

ideal (Nijaguna, 2006). There is no marked difference between the performance of the conventional and vacuum fermenters at 4 x 10^{-2} bar. Hence, there is virtually no advantage in adopting costly and energy consuming vacuum fermentation systems. When designing a biogas plant, the gas pressure should be fixed in such a way that it doesn't affect biogas production or cause leakage problems or even reduce appliances efficiency. Excess pressure prohibits gas release from the digesting slurry and in the masonry; gas storage causes leakage through the micropores. Regular gas taps and piping joints start leaking owing to excess pressure. A separate gas holder and incorporation of non-return valves in the connecting pipes is required. This averts a return flow of the gas back to the digester as well as air being sucked into the digester or gas holder through the pipe. Methane bacteria are among the most strictly anaerobic microorganisms and quantities as low as 0.08 mg/l of dissolved oxygen completely inhibit their growth. It is therefore absolutely vital that biogas plants be leak proof (Nijaguna, 2006).

1.5.9 Toxicity

Many undesirable organic and inorganic substances might cause toxicity in biogas digesters. Substances may be acutely toxic, chronically toxic, or both. Acute toxicity results from the rapid exposure of an unacclimated population of bacteria to a relatively high concentration of a toxic waste. Chronic toxicity happens from the long exposure of an unacclimated population of bacteria to a toxic waste. Bacteria population may adapt to chronic toxicity by repairing their enzyme systems or growing a large population of bacteria that can degrade toxic organic compounds (Gerardi, 2003).

Mineral ions, heavy metals, and detergents are some of the toxic materials that inhibit the normal growth of pathogens in the digester. However, low concentrations of the mineral ions, such as sodium, potassium, calcium, magnesium, ammonium and sulphur, are needed for stimulation of bacterial growth. Moreover and by contrast, if the concentration of these ions were too high, it would lead to toxification. Addition of substances including soap, antibiotics, organic solvents, etc. should be avoided, since

this would lead to inhibition of the activity of methane-producing bacteria (Dennis & Burke, 2001; Nijaguna, 2006; Chen *et al.,* 2008).

1.6 Feeding Modes in Biogas Digesters

There are two feeding modes used in AD of solid wastes, namely the batch process and the continuous process.

1.6.1 Batch Process

In the batch type digester, the airtight digester tank is charged once with fresh feedstock, with or without the addition of an inoculum and in some cases a chemical to maintain the digester pH and sealed for the complete RT, after which it is opened and the effluent removed (Nijaguna, 2002; Vandevivere *et al.,* 2002; Vandevivere *et al.,* 2003). The daily biogas yield is built up to the maximum level and then drops after some retention days (Nijaguna, 2002). Substrate management is uncomplicated with this method in spite of the fact that there is a significant difference in the production of biogas both in quality and quantity (Rajendran *et al.,* 2012; Obiukwu & Grema, 2013). The erratic biogas production in the batch process can be balanced out by running three to four digesters in parallel but charging them at different times. The batch process provides the highest biodegradation of the feedstock, and all biodegradable material can be converted to biogas if the RT is sufficient enough.

Regardless of the fact that batch systems have not succeeded in taking a considerable market share, particularly in more developed countries, the systems are attractive to developing countries. Perhaps this is because the process offers a number of advantages on account of the fact that it does not require: (a) fine shredding of waste; (b) complex and expensive mixing or agitation equipment; and (c) high-pressure vessels. Consequently, this significantly reduces the investment costs (Vandevivere *et al.,* 2002; Koppar & Pullammanappallil, 2008).

1.6.2 Continuous Process

In this process, the substrate material is regularly pumped into the digester and an equal volume of the digested material is displaced and hence the volume of the digester remains constant. Continuous feeding of the substrate is possible with this kind of process which eventually gives a steady and considerable biogas yield as compared to the batch process. For smaller digesters, the feeding of substrate material is commonly done once or twice a day. However, larger digesters are operated more continuously with feeding intervals of less than one hour (Vandevivere *et al.,* 2002; Vandevivere *et al.,* 2003).

1.7 General Aspects of Co-digestion

Organic wastes can be degraded and stabilized through AD; an appropriate technique; before their final disposal. In recent years, a lot of efforts have been geared towards improving digester biogas production, so as to upgrade their role in stabilizing organic wastes and also to produce a feasible bioenergy power plant. Co-digestion is an appealing option for improving methane yield in digesters. Also termed as co-fermentation, co-digestion is achieved by digesting two or more substrates one of them acting as a co-substrate in a digester.

Co-digestion of certain substrates can produce synergistic or antagonistic effects. Synergism would be seen as an additional methane yield for co-digestion samples. Similarly, evidence of antagonism would be translated into a lower methane yield in the co-digestion samples. Synergistic effects may arise from the contribution of additional alkalinity, trace elements, nutrients, enzymes, or any other amendment which a substrate by itself may lack, and could result in an increase in substrate biodegradability, and, therefore, biomethane potential. Antagonistic effects can come from several factors, such as pH inhibition, ammonia toxicity, high volatile acid concentration, among others (Chen *et al.,* 2008; Labatut & Scott, 2008).

Co-digestion is mostly advantageous for the adjustment of the C/N ratio of waste (Wang *et al.,* 2014). By co-digestion in most cases, biogas yield is improved due to synergism developed in the digester and also as a result of the supply of the nutrients missing in the digestion medium by some of the co-substrates (Gupta *et al.,* 2012). Moreover, through sharing of equipment during co-digestion, significant economic benefits are also realized. Further, adjustment of MC or even TS of feedstocks is also accomplished from co-digestion. Easier and better management of mixed wastes is another advantage of co-digestion (Andriani *et al.,* 2014). The productivity of anaerobic digesters can be improved by supplementing with readily digestible co-substrates (Angelidaki *et al.,* 1997). Glycerol is a readily digestible substance, which can also be easily stored for an extended period. These advantages make glycerol an ideal co-substrate for the AD process.

Glycerol is currently underutilized as a co-digestion feedstock. Recent experiments with co-digestion, applying glycerol to mixtures of slaughterhouse wastewater, municipal solid waste, olive mill wastewater, pig manure, maize silage and rapeseed meal, have shown a significant increase in the methane yield. However, in order to maintain a stable digestion process the amount of glycerol added had a limiting concentration level (Amon *et al.,* 2006; Holm-Nielsen *et al.,* 2007; Fountoulakis *et al.,* 2009). These results demonstrate that glycerol can be applied advantageously, but a strict control strategy is necessary to regulate the amount added, to avoid the risk of organic overloading. This process is well known, especially in Denmark, resulting in much higher methane yields when food waste and similar types of organic waste were combined with cow and pig slurries at biogas plants (Kuusik *et al.,* 2014).

A balanced nutrient supply and a stable pH are prerequisites for reliable process performance. An optimized C/N ratio during co-digestion, for instance was reported to be beneficial for the gas yield (Sonowski *et al.,* 2003). Mshandete *et al.* (2004) reported an improvement of the pH stability as an advantage of co-digestion. However, a careless decision on the type of wastes for co-digestion and the ratio of biowaste; co-substrate in

full-scale anaerobic digesters often lead to a significant reduction in the biogas amount or even to failure of the biogas process (Murto *et al.,* 2004; Zaher *et al.,* 2009).

There are a number of studies on co-digestion of glycerol with various substrates. For example, in an upflow anaerobic sludge blanket (UASB) reactor treating potato processing wastewater, Ma *et al.,* (2008) found that the biogas production increased by 0.74 l biogas per mL glycerol added. Furthermore, a better biomass yield was observed for the supplemented reactor compared to the control.

Fountoulakis and Manios (2009) examined the effect of crude glycerol on the performance of single-stage anaerobic digester treating different types of organic waste. Their objectives were to evaluate glycerol use as a co-substrate in improving biogas and hydrogen production during anaerobic treatment of organic fraction of municipal solid waste (OFMSW) and a mixture of olive mill with slaughterhouse waste water at a ratio of 1:4. OFMSW Feedstock was supplemented with 1% volume by volume (v/v) crude glycerol and the rate of methane production observed. Feed supplementation with crude glycerol had a significant positive effect in all cases given total biodegradation of glycerol. A mixture of olive mill and slaughterhouse waste water at a ratio of 1:4 was supplemented with 1% v/v crude glycerol. The methane production rate increased from 479 mL/d to 1210 mL/d.

Fountoulakis *et al.* (2010) co-digested sewage sludge with glycerol and their primary objectives were to evaluate the use of glycerol as a co-substrate to boost biogas production on AD of sewage sludge. Kinetic removal of glycerol, the effect of glycerol supplementation on methane yield and glycerol limiting concentration during AD were also examined. Methane gas production without glycerol was 1106 mL/d and with glycerol addition was 2353 mL/d. Hence, glycerol addition at 1% v/v boosted methane production tremendously by about 1247 mL/d.

1.8 Statement of the Problem

Agricultural wastes contribute significantly to air, soil and water pollution if inappropriately managed. Given that such wastes are properly managed, they constitute an enormous potential for bioenergy production. These wastes are regarded as having little or no value and are disposed of in landfills. However, landfilling of energy-rich agricultural wastes should be avoided or kept to a minimum, mainly because of its low recovery of resources (Eriksson *et al.,* 2005).

The technology for biogas production is up until now developing and not yet optimized and, as a consequence not fully cost-effective. Oftentimes, biogas digesters are operated based on an assumption that the conditions they are working under are utterly suitable. Consequently, many a time the applied dilution ratios of substrates to water in biogas digesters by farmers, as well as researchers for specific substrates, are hypothetical and ambiguous. In his study, Babatola (2008) points out that too much or little substrate or water have adverse effects on biogas yields and may even lead to stalling of the process before the minimum required solid retention time (SRT) or hydraulic retention time (HRT) is achieved. A search of literature does not give information on the subject of the optimal substrates to water dilution ratios specific to different agricultural wastes under which biogas digesters can optimally be operated with to yield greater biogas output.

The most commonly used types of animal manure for biogas production through AD are cattle and pig manure (Agro Products, 2008). For the two most common substrates, the present literature does not provide optimal substrates to water dilution ratios for each with which farmers or even researchers can work with to further scale up biogas yields in their biogas digesters. Thus, biogas digester owners and operators haphazardly apply substrates to water dilution ratios and end up very likely obtaining the least biogas yield from their biogas digesters. Although there are reports on co-digestion of glycerol with various substrates (Ma *et al.,* 2008; Fountoulakis & Manios, 2009; Fountoulakis *et al.,* 2010), there were no literature reports addressing the feasibility of co-digesting agricultural residues mainly cattle and pig manure with another high-energy feedstock

like glycerol based on optimal mesophilic temperature for biogas production and the optimum substrates to water dilution ratios as this research seeks to do.

1.9 Justification

As part of agricultural waste management, AD could be employed to increase the value of agricultural wastes and avoid unneeded disposal costs through the production of biogas. Provided that anaerobic processes are implemented in engineered biogas systems, methane the main constituent of biogas, which can be utilized for energy production, may be recovered from a variety of feedstocks (Ahring, 2003). However, for the technology to be commercially competitive with other types of fuels, efficiency improvements of the process are necessary (Thorin *et al.,* 2012). In view of this, much more attention has primarily been focused on the improvement of digester biogas production.

There is a gap of information about how an optimization of biogas plants could be accomplished. The biogas industries, as well as the academic institutions, are all well-focused on finding solutions to gradually increase the productivity of the process (Lindmark*,* 2012). Even so, optimization of the process in terms of biogas digester temperature and substrates to water dilution ratios has been lacking. Many a time researchers have often hypothesized the dilution ratios of substrates to water applied in biogas digesters (Babatola, 2008; Adelekan, 2012). The optimal temperature of AD and the substrates to water dilution ratios for production of biogas varies with different substrates, that is, may vary depending on feedstock composition as well as operational conditions and the type of digester. The dilution ratio of substrates to water depends on the substrate in use at that particular moment in time given that one is to obtain optimal biogas yield.

Further, the process parameters such as load, temperature and retention time have a considerable influence on how efficiently a given substrate is biodegraded. The extent to which organic materials are biodegraded in an AD process also depends on, one pre-

treatment of the substrate, two whether it is the sole substrate and three if it is co-digested with other organic material. In any case, co-digestion of two or more substrates together is equally a likely way of enhancing biogas yields (Schnurer & Jarvis, 2010). High biogas production is positively correlated with the addition of high concentrate organic by-products. On top of that, co-digestion of organic wastes offers colossal advantages such as increased process stability and biogas yield as well as a better management of mixed waste streams. Besides, co-digestion is useful if there is limited availability of one single substrate (Schnurer & Jarvis, 2010).

The biogas industries together with scientists have well-focused on further enhancing biogas digesters with the intent of making the process much better and economically more viable (Fayyaz *et al.,* 2014). It is therefore particularly important that biogas digesters are operated at optimal digestion temperatures, and the optimum substrate is to water dilution ratios specific to the various agricultural wastes with a view to optimizing biogas yields. In view of this, the research set out with the aim of further enhancing the AD process to make biogas digesters economically more feasible by focusing attention on some key issues in the process, namely the optimal mesophilic temperature and the optimum substrates to water dilution ratios for cattle and pig manure as well as the individual co-digestion of the two substrates with glycerol using the established optimal mesophilic temperature and substrates to water dilution ratios for each substrate to further boost biogas yields.

1.10 Hypotheses

i. Optimal mesophilic temperatures for AD of cattle and pig manure substrates are unascertainable.
ii. Optimum substrates to water dilution ratios for AD of cattle and pig manure substrates are indeterminable.
iii. Biogas production from cattle and pig manure substrate cannot be scaled up by the addition of glycerol.

1.11 Objectives

1.11.1 General Objective

To investigate the effect of glycerol supplementation on biogas yield under the appropriate optimal mesophilic temperatures and the optimum substrates to water dilution ratios of cattle and pig manure substrates.

1.11.2 Specific Objectives

i. To determine the physico-chemical characteristics of cattle and pig manure substrates, glycerol and the inoculum.
ii. To determine the optimal mesophilic temperatures for AD of cattle and pig manure substrates.
iii. To determine the appropriate optimum substrates to water dilution ratios for AD of cattle and pig manure substrates.
iv. To determine the effect of glycerol supplementation on biogas yield under the appropriate optimal mesophilic temperatures and the optimum substrates to water dilution ratios of cattle and pig manure substrates.

1.12 Thesis Overview

This thesis consists of three chapters in addition to this introductory chapter (1), which provides a broad overview of key issues and relevant previous works specific to biogas production. Methods applied in this thesis are presented in chapter 2. The results of the study are presented, interpreted and discussed in detail in chapter 3. Chapter 3 also presents the study's conclusions and recommendations.

CHAPTER TWO

MATERIALS AND METHODS

Described in this chapter is the research design that includes materials and procedures used to examine biogas production from cattle and pig manure substrates. Further, the chapter covers the method used in determining glycerol supplementation effect on the two manure substrates. Besides, the experimental procedures followed to measure the quality of biomass and parameter levels for different variables are also presented.

2.1 Materials

2.1.1 Reagents

All the reagents used in the study were of analytical grade and were used without further purification. Glycerol purchased from Sigma-Aldrich (Seelze, Germany) was used in supplementing cattle and pig manure substrates at the established respective optimum conditions of temperature and dilution. pH 4, 7 and 10 buffer solutions purchased from BDH (Middle East L.L.C - Dubai, UAE) were used in calibrating the pH meter prior to use.

2.1.2 Equipment

A muffle furnace (mrc: Carbolite-CWF 12/23, London, UK) for dry ashing was used to ash samples for the analyses of percentage volatile solids (%VS) ignited at 550 °C. An analytical balance (mrc: BPS-1000-C2, London, UK) was used to weigh accurately the required amount of cattle and pig manure substrates, as well as glycerol. A Magnetic stirrer and stir bars (mrc: HS-4, London, UK) were used to stir cattle and pig manure substrates during the analyses of percentage total solids (%TS) dried at 105 °C. A thermostatically controlled shaking water bath (mrc: WBT-400, London, UK) was used to heat and maintain the biogas digesters at the required experimentation temperatures. An incubator (mrc: DN-50, London, UK) was used to store the inoculum at 40 °C before use in the experiments. An oven (mrc: DNO-30, London, UK) was used in the analyses

of cattle, and pig manure substrates for %TS dried at 105 °C. The pH of samples was measured using a Hanna HI-8915 (Michigan, USA) pH meter.

2.1.3 Cleaning of Glassware and Sample Containers

All glassware and other materials used in this work were thoroughly cleaned with hot distilled water and liquid detergent. They were then rinsed with distilled water before drying them in a dust-free cabinet. Immediately before use they were rinsed thoroughly several times with hot deionized water and dried in the oven for about 2 hours at 150 °C.

2.1.4 Manure substrates and Inoculum Collection and Storage

Fresh cattle and pig manure substrates utilized in this research were randomly collected from animal holding pen units on a farm at Dedan Kimathi University of Technology (DeKUT) in Nyeri, Kenya. The samples were kept refrigerated at 4 °C until used. Manure substrates used in carrying out different experiments for comparison purposes did not differ in any material respect from each other achieved by the use of identical homogenized samples for every run.

The inoculum used in the experiment was collected from a biogas plant at Wambugu Farm in Nyeri. The plant operates under the mesophilic condition and treats cattle manure. The inoculum was stored at 40 °C in an incubator for three days before use and was used as starter seed in all experiments.

2.2 Methods

2.2.1 Analytical Methods

To characterize the fresh manure, the digestate, the inoculum and glycerol and also evaluate the performance of the biogas digesters, several parameters outlined below were measured and determined, mostly following APHA *et al.* (2005) - Standard Methods for the Examination of Water and Wastewater.

2.2.1.1 Total Solids Dried at 105 °C

Total Solids (TS) of the samples were determined in line with APHA - Standard Method set out in section 2540 B (APHA *et al.,* 2005). An appropriate number of crucibles were placed in an oven at 105 ºC for one (1) hour, cooled and stored in desiccators. All crucibles were weighed before use on an analytical balance. Samples were stirred with a magnetic stirrer before transfer, and 10 g of each homogenous sample weighed and placed in pre-weighed crucibles. The crucibles with the samples were dried at 105 ºC for one (1) hour, cooled in desiccators and weighed. The cycle of drying, cooling, desiccating and weighing was repeated until weight change was less than 4% of previous weights. All TS analyses were carried out in triplicate. Mean values and standard deviations were calculated. The mathematical formula (Equation 2.1) needed to calculate %TS is shown below;

$$TS\% = \frac{(A - B)}{(C - B)} \times 100\% \qquad (2.1)$$

where,

A is Weight of dried residue + crucible after drying at 105 °C;

B is Weight of pre-dried crucible; and

C is Weight of pre-dried crucible + sample before drying.

2.2.1.2 Moisture Content

Moisture Content (MC) was determined in accordance with the method provided in section 2540 E of APHA - Standard Methods (APHA *et al.*, 2005) for analysis of TS. Percentage moisture content (%MC) was then calculated based on the formula below (Equation 2.2);

$$MC\% = \frac{Ws - A}{Ws} \times 100\% \qquad (2.2)$$

where,

A is weight of sample after drying; and

Ws are the wet sample weight in g, which, in this case, is 10 g.

Along this line, %TS can also be calculated by subtracting the %MC from 100% as shown in Equation 2.3;

$$\%TS = 100\% - \%MC \qquad (2.3)$$

2.2.1.3 Volatile Solids Ignited at 550 °C

Volatile Solids (VS) were determined conforming to the method given in section 2540 E of APHA - Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2005). The residues produced by Method 2540 B to constant weights were ignited in a muffle furnace at a temperature of 550 °C and maintained there for three (3) hours. Samples were partially cooled in the air till most of the heat had dissipated then transferred to desiccators for final cooling in a dry atmosphere. Crucibles with samples were cooled in desiccators and weights obtained on cooling. The cycle of igniting, cooling, desiccating and weighing was repeated until weight change was less than 4% of previous weights. All VS analyses were carried out in triplicate. Mean values and standard deviations were calculated. The mathematical formula (Equation 2.4) used to calculate %VS is shown below;

$$VS\% = \frac{(A - B)}{(A - C)} \times 100\% \qquad (2.4)$$

where,

A is Weight of dried residue + crucible after drying at 105 °C;

B is Weight of dried residue + dish after igniting at 550 °C; and

C is Weight of pre-dried dish.

2.2.1.4 Total Carbon

Total Carbon (TC) includes both inorganic and organic sample constituents. Total carbon content is determined in dried sediments. The empirical equation (2.5) below (Badger *et al.,* 1979; Haug, 1993; Jigar, 2011) was applied to obtain the %TC in both fresh manure substrates and digestate waste samples.

$$\%TC = \frac{\%VS}{1.8} \qquad (2.5)$$

2.2.1.5 pH Value

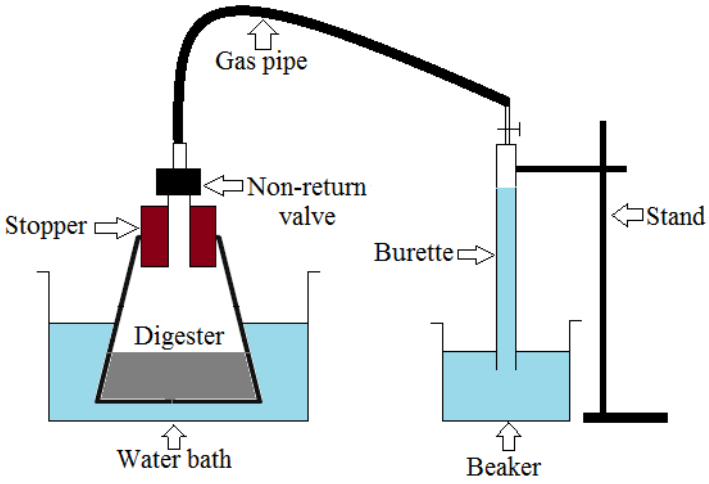
The pH values of cattle and pig manure substrates, inoculum and digestates of the batch experiments were determined electronically using Hanna pH meter, Figure 2.1. The meter was calibrated prior to use using pH 4, 7 and 10 buffer solutions. As the check reference, pH paper was also used to determine the pH values.

Figure 2.1: Plate of the Hanna pH meter (HI-8915) used in the experimental work

2.2.2 Reactor Design and Operation

The experimental set-up was made up of inverted 50 mL borosilicate burettes filled with water and immersed in beakers to measure biogas volume, Figure 2.2. The digesters were a 100 mL capacity pyrex conical flasks with a working volume of 25 mL immersed in a water bath maintained at the mesophilic temperature of 40 °C. Polyvinyl chloride tubes connected the digesters to the burettes. Non-return valves were placed on the stoppers at the top of the digesters to avert flow of biogas back to the digesters. The joints were sealed with candle wax to preclude any form of leakage. The biogas produced from the digesters was collected by downward delivery of gas, achieved by filling the burettes with water and inverting them in beakers containing water with some

allowance between the burettes and the base of the beakers. Owing to the equilibrium between the atmospheric pressure and the water in the beakers, the level of water in the burettes remained unchanged. As biogas is produced in the digesters, it pushed the water in the burettes downwards by displacement. The change in volume in the burettes indicates the amount of biogas produced. The anaerobic digesters were operated under atmospheric pressure. The biogas was stored at room temperature.



(a)

(b)

Figure 2.2: Diagram (a) and plate (b) of the experimental set-ups of bench scale batch biogas digesters in a water bath at 40 ºC

2.2.3 Experimental Designs and Processes

2.2.3.1 Determining the Optimum Mesophilic Temperature for Optimal AD of Cattle and Pig Manure Substrates

Analyses of cattle and pig manure substrates for the vital physico-chemical characteristics and properties were first carried out. This was then followed by a study of the optimum mesophilic temperature for AD of cattle and pig manure substrates using the experimental set-up described in section 2.2.2 and shown in Figure 2.2. Cattle and pig manure substrate to water dilution ratios of 12.5:12.5 (mass-*g* to volume-*mL* ratio - m/v) and 4 g of inoculum were used for all the temperature experimental runs for each manure substrate. Four biogas digesters were used; two containing cattle manure substrate and the other two had pig manure substrate for each of the experimentation temperatures. Biogas production was closely monitored and the daily biogas volumes recorded under the mesophilic temperatures of 30 °C, 35 °C, 40 °C and an increase to the thermophilic temperatures of 45 °C and 50 °C. The biogas digesters were batch operated with RTs of 30 and 25 days for cattle and pig manure substrates, respectively. The volume of biogas produced was recorded every 24 hours using the downward displacement of air over water for all the temperatures.

2.2.3.2 Determining the Optimum Manure Substrate to Water Dilution Ratio (m/v) for Optimal AD of Cattle and Pig Manure Substrates

Cattle and pig manure substrates of various masses were separately loaded into biogas digesters (100 mL conical flasks) each with varying amounts of deionized water (manure substrate to water dilution ratios - g/mL) as follows: 22.5:2.5, 20:5, 17.5:7.5, 15:10, 12.5:12.5, 10:15, 7.5:17.5, 5:20, and 2.5:22.5. From the dilution ratio of manure substrate to water of 12.5:12.5, the manure substrates were increased at an interval of 2.5 g to the left while shrinking that of water by 2.5 mL up to a ratio of manure substrate to water dilution ratio of 22.5:2.5. Equivalently, from the dilution ratio of 12.5:12.5, water amount was added on in intervals of 2.5 mL to the right while lowering manure substrate amount by an interval of 2.5 g up to a dilution ratio of substrate to water of 5:20. Each

dilution ratio was then inoculated with 4 g of inoculum. All dilution ratio experimental runs for each manure substrate; cattle and pig manure substrates; were carried out in duplicate. The biogas digesters were batch operated at the established optimum mesophilic temperature for each manure substrate. The RT was 30 and 25 days for cattle and pig manure substrate, respectively, which were the duration for maximum biogas production. Biogas yields were closely monitored by measuring the respective volumes and regularly recording at intervals of 24 hours.

2.2.3.3 Determining the Effect of Supplementing Cattle and Pig Manure Substrates with Glycerol

The established respective optimum manure substrate to water dilution ratios (m/v) for cattle and pig manure substrates were then supplemented with 0.5%, 1% and 1.5% glycerol (m/m). The amounts of glycerol supplement added to each of the biogas digesters was calculated as a percentage of the mass of the manure substrates fed to each of the biogas digesters. The respective amounts of glycerol supplement added to the biogas digesters containing cattle manure substrate were 0.1 g, 0.2 g and 0.3 g and for pig manure substrate the amounts were 0.03 g, 0.05 g and 0.08 g. The biogas digesters were batch operated at the established optimum mesophilic temperature with RTs of 30 and 25 days for cattle and pig manure substrates, respectively. All experimental runs for each manure substrate were carried out in duplicate. Biogas production rate (mL biogas/day) was carefully monitored by measuring the generated biogas volumes for each biogas digester and recording the amounts as close to 24 hours, after the previous recording, as possible.

CHAPTER THREE

RESULTS AND DISCUSSIONS

In this chapter, the results of the study are presented and discussed. Various physico-chemical characteristics of cattle and pig manure substrates and their respective digestates, glycerol and inoculum are presented in this chapter. The chapter also includes the optimum mesophilic temperature for optimal AD of cattle and pig manure substrates. Besides, the respective optimum manure substrate to water dilution ratios (m/v) for optimal AD of cattle and pig manure substrates are also presented. Further, the chapter contains the results of supplementing cattle and pig manure substrates with glycerol at the established optimum mesophilic temperature and the respective optimum manure substrate to water dilution ratios.

Biogas digesters operated under atmospheric pressure, and biogases collected and stored at room temperature.

The performance of the anaerobic digesters treating cattle and pig manure substrates were examined based mainly on the results obtained from the process monitoring of biogas production. The results were analyzed using Microsoft Office Excel 2007 for mean values for both n = 2, and n = 3 and standard deviations for n = 3, where n is the number of measurements. The main findings of the study are presented in the form of tables and figures. They have been used to discuss and describe the results.

3.1 Cattle Manure Substrate

3.1.1 Physico-chemical Characteristics of Cattle Manure Substrate, Glycerol, and Inoculum

Cattle manure substrate, glycerol, and inoculum were analyzed for various physico-chemical characteristics. All determinations were carried out in triplicate. Results were

expressed as mean values ± standard deviations (n = 3) for each of the five characteristics presented in Table 3.1.

The results of the physico-chemical characteristics obtained from the analysis of cattle manure substrate show that the manure substrate had percentage total solids (%TS) of 14.49 ± 0.05 percent, Table 3.1. The obtained %TS is almost within the reported range. This is in accordance with Fulford (1988) who reported that the total solid content of cow dung varies between 16 percent and 20 percent, while the recommended value for slurry is between 8 percent and 12 percent. This consequently means that dung must be diluted with water before it is used in a biogas plant. The %TS concentration of the organic waste influences the pH, temperature and the effectiveness of the microorganisms in the decomposition process (Joy *et al.,* 2014). The amount of biogas produced is a power function of the %TS concentration (Igoni *et al.,* 2008; Mohapatro *et al.,* 2014). Besides, the biodegradability of manure substrates is indicated by biogas production or methane yield and the percentage of solids (TS or total VS) that are destroyed in the AD process (Joy *et al.,* 2014).

Cattle manure substrate had 85.52 ± 0.02 percent moisture content, Table 3.1. The percentage moisture content (%MC) recorded falls within the expected range of 72-85% (Fulford, 1988). The inside %MC of a digester should normally be around 90% of the mass of the total digester contents (Adelekan, 2012). There must be suitable %MC of the feedstock as the microorganisms' excretive and other metabolic processes require water (Adelekan, 2012; Prabhu *et al.,* 2014). However, the %MC to be maintained for degradation depends on the type of organic waste utilized (Somashekar *et al.,* 2014). Both over-dilution and under-dilution with water are harmful, with too much water; the rate of biogas production per unit volume in the digester will fall, consequently preventing the optimum use of the digester. If the %MC is too low, acetic acids will accumulate inhibiting the AD process and hence biogas production. Furthermore, a rather thick scum will form on the surface of the substrate. This scum may prevent

efficient mixing of the charge in the digester. Hence, the optimum water content has to be maintained within the anaerobic digester (Prabhu *et al.,* 2014).

Cattle manure substrate had sufficient percentage volatile solids (%VS) of 78.68 ± 0.04 percent to effect reasonable biogas production, Table 3.1. The %VS are within the reported range. According to literature reports (Fulford, 1988), the %VS of cow dung are usually around 80 percent of the TS. The high proportion of VS to TS (5.43:1) in the manure substrate depicts that; a large fraction of the substrate was biodegradable and could serve as an important feedstock for biogas production (Jha *et al.,* 2013; Li & Jha, 2014). Biogas or methane yield is measured by the volumetric amount of biogas or methane that can be produced per unit of VS contained in the substrate after subjecting it to AD for a sufficient amount of time at a particular temperature (Zhang *et al.,* 2007; Joy *et al.,* 2014).

As detailed in Table 3.1, cattle manure substrate had percentage total carbon (%TC) of around 43.70 ± 0.04 percent in the VS that can be converted into biogas. The result was slightly above the reported range. Literature report, (Fulford, 1988) point out that the %TC content of cow dung ranges between 35% and 40%. Organic carbon can be removed in anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or biogas production (Gerardi, 2003; Somashekar *et al.,* 2014). Some of the carbon is bound up in indigestible lignin (Fulford, 1988). Therefore, the carbon is partially removed from the digested material, reducing the C/N ratio (Kirchmann & Witter, 1992; Moller *et al.,* 2008).

Further, the pH 7.18 ± 0.05 of the fresh cattle manure substrate was primarily within the acceptable limit for AD, Table 3.1. The pH value was near neutral (≈ 7); optimum for biogas production. The optimal pH for methanogens is between 6.8 and 8.5 (Lay *et al.,* 1997; Senturk *et al.,* 2014). pH should be close to 7; near neutral; for optimal microbial activity. This implies average buffering capacity of the manure substrate, meaning, therefore, that the substrate can withstand rapid pH fluctuations (Khanal, 2008). When a

biogas plant is newly started, acidogens become active first, reducing the pH to below 7, thus increasing the acid concentration. Methanogens then start utilizing these acids, increasing the pH back to neutral. A working biogas plant is buffered, in other words, the acid level is controlled by the process itself (Fulford, 1988). The pH of the substrate remains relatively neutral throughout digestion maintaining microbial stability within the anaerobic digester (Wen, 2006). However, if the pH of digester remains acidic, the reduction in pH can be controlled by the addition of lime or recycled filtrate obtained during residue treatment (Kangle *et al.,* 2012).

Table 3.1: Physico-chemical characteristics of substrates and inoculum

Substrates and Inoculum	TS (%)	MC (%)	VS (%)	TC (%)	pH
Cattle manure substrate	14.49 ±0.05	85.52 ±0.02	78.68 ±0.01	43.70 ±0.04	7.18 ±0.05
Glycerol	94.48 ±0.04	5.35 ±0.03	1.15 ±0.05	0.64 ±0.08	7.12 ±0.09
Inoculum (Starter seed)	9.24 ±0.08	90.79 ±0.07	59.68 ±0.06	33.15 ±0.02	7.97 ±0.01

Values are mean ± standard deviations, n = 3

Key: %TS = percentage total solids, %MC = percentage moisture content, %VS = percentage volatile content, %TC = percentage total carbon and pH = potential hydrogen.

3.1.2 The Optimum Mesophilic Temperature for Optimal AD of Cattle Manure Substrate

To determine the optimum mesophilic temperature for biogas production from cattle manure substrate, biogas digesters were batch operated in duplicate with a 30 days RT at 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. The first three temperatures are mesophilic, and the other two are thermophilic. A dilution ratio of cattle manure substrate to water of 12.5:12.5 (m/v) and 4 g of inoculum were charged into the anaerobic digesters and batch

operated at the various experimentation temperatures. Biogas production was closely monitored for 30 days. Biogas yields were regularly recorded at intervals of 24 hours.

In Figure 3.1, biogas production commenced within 24 hours of charging the digesters. This can be attributed notably to the optimum composition of the cattle manure substrate and the effect of the added inoculum. Cow dung used as starter seed has been acclaimed to contain bacteria that kick-starts the AD process (Chukwuma & Orakwe, 2014). The five experimented temperatures shown in Figure 3.1 showed almost similar trends of biogas production. Between the first and the eighth day of the retention period, biogas productions increased more steeply from the initial day onward up until the eighth day of observation. The rapid initial biogas production could possibly be associated with the shorter lag phase growth, the availability of readily biodegradable organic matter in the cattle manure substrate, and the high presence of methanogenic archaea (Aragaw *et al.,* 2013; Chukwuma & Orakwe, 2014). Biogas production levels peaked between the eighth and the eleventh day of the experiment for all studied temperatures. Optimum biogas production is attained when methanogenesis dominates the AD process (Obiukwu & Grema, 2013). On the eleventh day onward up until the last day of the retention period, the performance of the digester systems remained unsteady with the fluctuating production of biogases. In some days, biogas yields were zero, most likely due to the slow metabolism of the methanogenic bacteria (Obiukwu & Grema, 2013).

In Figure 3.1, it can also be observed that, trends of daily biogas production kept increasing until reaching the peak and then began to decline. The reason for this trend can be attributed in particular to biogas production rate in batch condition which directly corresponds to the specific growth rate of methanogenic bacteria in the biogas digester (Gupta *et al.,* 2009; Budiyono *et al.,* 2014). Further, on the twenty-second and the twenty-sixth day of observation, biogas yields were zero onward up to the thirtieth day for 50 °C and 45 °C, respectively. This can mainly be attributed to the excessive high thermophilic temperatures that harm and ultimately kill off anaerobic bacteria in the

biogas digester (Chukwuma & Orakwe, 2014). Higher temperature AD systems are therefore considered to be less stable (Obiukwu & Grema, 2013).

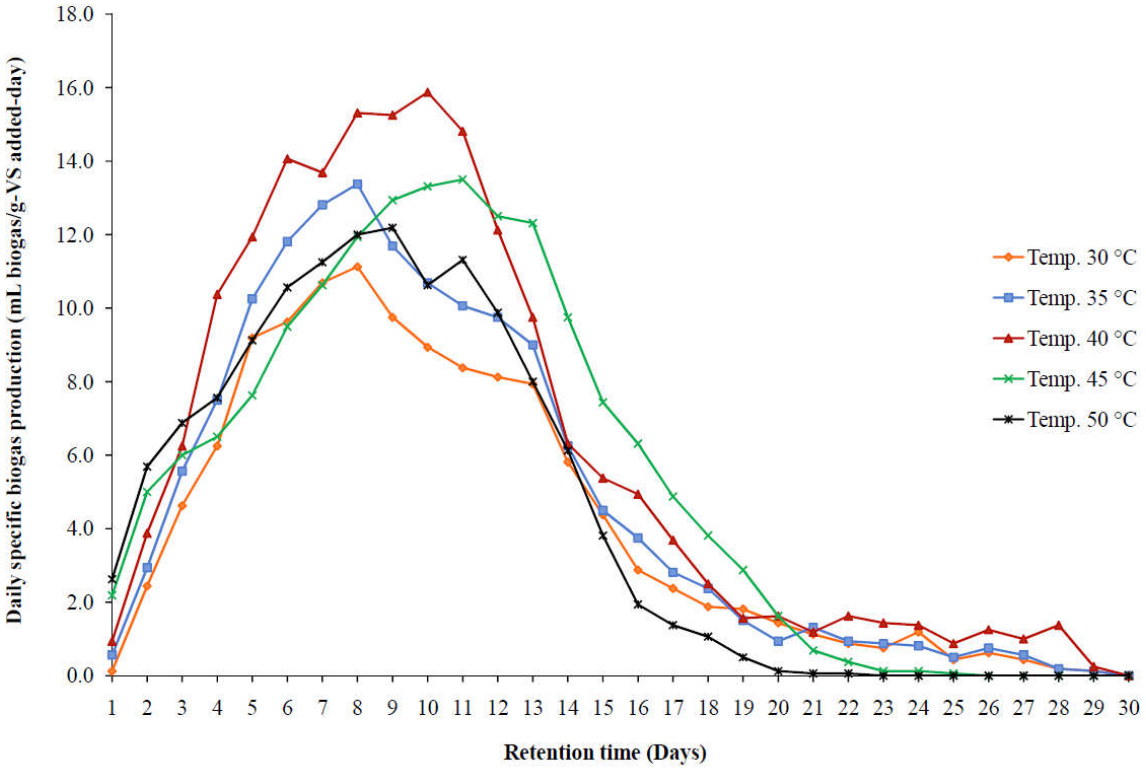


Figure 3.1: Specific daily biogas productions (mL biogas/g-VS added-day) from cattle manure substrate on a 30 days' RT at the various selected temperatures (°C)

The plot of the total biogas yields at the various experimented temperatures is shown in Figure 3.2. The maximum total biogas yield was observed at 40 °C with a total biogas yield of 180.6 mL biogas/g-VS added. These results indicate that increased and steady biogas production can probably be largely achieved, particularly under the optimum mesophilic temperature of 40 °C when cattle manure substrate is digested in the batch digestion process. Obiukwu and Grema (2013) and Uzodinma *et al.* (2007) both studied the optimum temperature for biogas production from blends of animal-based wastes and reported similar findings, where plots showed optimum increases in biogas production at 40 °C. Therefore, the upper limit of the mesophilic range (30-40 °C) produces a

higher biogas yield. However, biogas yields were obtained at all the five experimented temperatures. This can largely be explained by the fact that different species of methanogenic bacteria can survive at different temperature ranges (Obiukwu & Grema, 2013).

It is apparent from Figure 3.2 that, below and above 40 °C, there were comparatively lower total biogas yields. At 40 °C, the total biogas yield was 180.6 mL biogas/g-VS added and at 30 °C and 35 °C, total biogas yields were 123.5 and 144.2 mL biogas/g-VS added, respectively. Total biogas yield was least at 30 °C, higher at 35 °C and highest at 40 °C in that ascending order. It is evident, therefore, that the rate of bacteriological methane production increases with temperature. A plausible explanation for this low biogas yields at 30 °C is that methanogens are not sufficiently activated for enhanced biogas production. This would consequently lead to little biodegradation of organic wastes followed by poor biogas yield (Uzodinma *et al.,* 2007). The highest biogas yield at 40 °C was probably due to the fast metabolism of methanogenic bacteria caused by favorable temperature and optimal pH conditions. Besides, increases in the ambient temperature facilitate faster reaction rates by increasing the activation energy of the bacteria and, as a consequence, more biogas yields are realized (Nijaguna, 2006; Uzodinma *et al.,* 2007; Raja and Lee, 2012; Obiukwu & Grema, 2013).

For temperatures 45 °C and 50 °C the total biogas yields were 162.0 and 132.8 mL biogas/g-VS added, respectively. Total biogas production was least at 50 °C, higher at 45 °C and highest at 40 °C in that ascending order based on yields. It is apparent that, when the temperature was further increased from 40 °C to 45 °C and finally to 50 °C, biogas productions decreased; indicating that yields were adversely affected by increased temperatures. An increase in temperature higher than the optimal 40 °C leads to a reduction in metabolic rate and consequently a drop in biogas production. According to Chukwuma and Orakwe (2014) together with Obiukwu and Grema (2013), this can probably be ascribed to the fact that exceeding the optimal temperature; 40 °C;

causes the destruction, and ultimately, death of bacterial strains even though biogas yields could be noted. Subsequently, the biogas digesters biologically collapse.

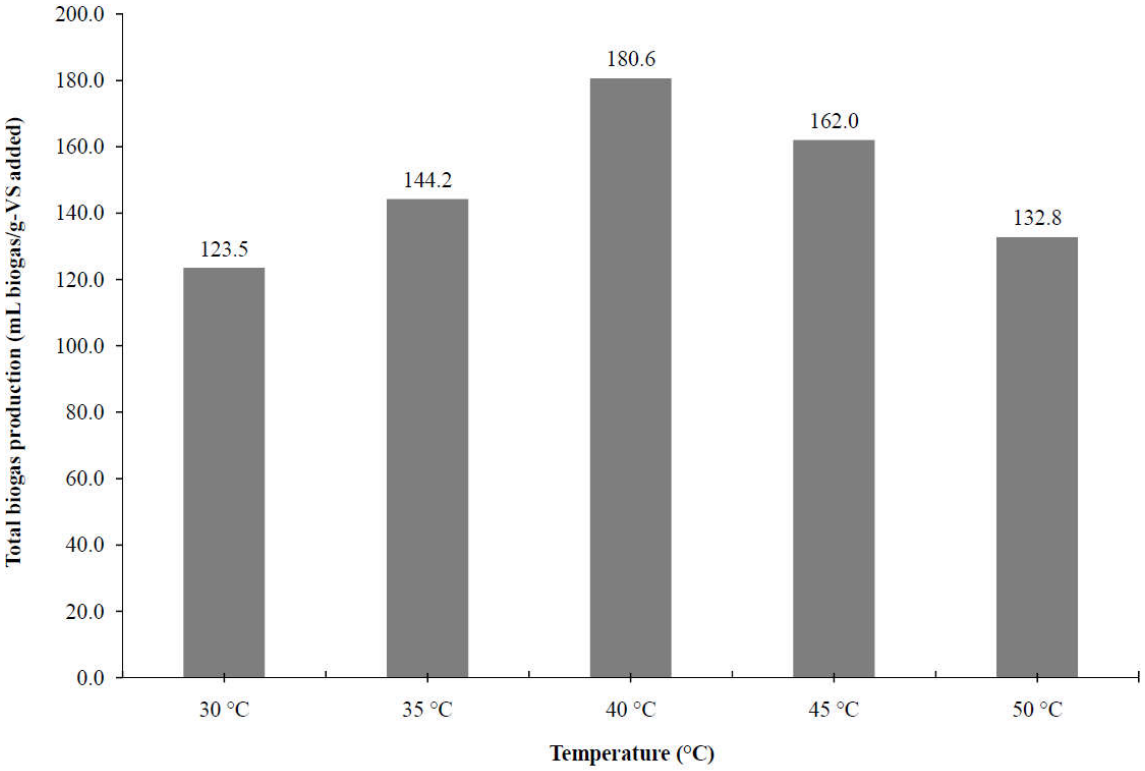


Figure 3.2: Total biogas productions (mL biogas/g-VS added) from cattle manure substrate on a 30 days' RT at the various selected temperatures (°C)

Figure 3.3 shows the plot of cumulative volumes of biogas generated at the five experimented temperatures. The highest cumulative biogas yield of 180.6 mL biogas/g-VS added was collected at 40 °C. This also suggests that the optimal temperature for AD of cattle manure substrate is 40 °C. At 40 °C, large amounts of biogas were produced between the first and the seventeenth day of the retention period; indicating that methanogenesis had dominated the AD process. Therefore, it seems likely that there was high methanogenic activity caused by the optimal temperature and pH conditions, both of which are major factors in biogas production (Chukwuma & Orakwe, 2014; Obiukwu & Grema, 2013). Biogas production then slowed down between the seventeenth and the

thirtieth day of the retention period. This is an indication of the slow metabolism of the methanogenic bacteria. In the batch AD process, the rate of biogas production is fast in the beginning and slows down as the process goes on. Besides, this is further corroborated by the fact that, biogas production in a batch AD process is directly proportional to the specific growth rate of the methanogenic bacteria (Budiyono *et al.,* 2014; Chukwuma & Orakwe, 2014). Similar trends were observed at 30 °C and 35 °C, where the rates of biogas production were high between the first and the fourteenth day of observation, Figure 3.3. Both slowed down after the fourteenth day onward up until the thirtieth day of the experiment. On the other hand, the two thermophilic temperatures; 45 °C and 50 °C; had almost similar biogas production rates, Figure 3.3. At 45 °C and 50 °C biogas production rates were high from the first day onward to the twentieth and the eighteenth day of observation, respectively. The results, therefore, indicate that there was an elevated activity of methanogenic bacteria owing to the optimum temperature and pH conditions of the biogas digester. Biogas production virtually stopped for both temperatures after the twentieth and the eighteenth day of observation for temperatures 45 °C and 50 °C, respectively. In this respect, the results further emphasizes the fact that exceeding the optimal temperature; 40 °C; causes the destruction of anaerobes with time even though initial high biogas yields had been realized, leading eventually to the biological collapse of the system as the bacteria finally died off slowly (Obiukwu & Grema 2013; Chukwuma & Orakwe, 2014).

All bacteria involved in biogas production are active only within a limited temperature range (Raja & Lee 2012). Zupancic and Grilc (2012) reported that mesophilic microorganisms can operate up to 47 °C above the mesophilic temperature range, thermophilic microorganisms can already operate as low as 45 °C below the thermophilic temperature range. However, the rate of reaction is low, and it may happen that the two groups of microorganisms may exclude each other and compete in the overlapping range. These results in reduced efficiency of the process, and, therefore, these temperatures are rarely applied. Indeed, this is simply in concurrence with the

findings in Figures 3.2 and 3.3, where above and beyond 40 °C; with a corresponding biogas yield of 180.6 mL biogas/g-VS added, there were notable decreases in total biogases generated for digesters operated at 45 °C and 50 °C with respective biogas yields of 162.0 and 132.8 mL biogas/g-VS added. Although some variation is considered normal, digester temperature should always be maintained between 35 °C and 40 °C. Operating anaerobic digesters at temperatures outside the normal range will result in decreased biogas yield. In addition, long periods of time under these conditions may eventually stop biogas production and cause digester failure. In general, the process will be more affected at higher temperatures than at lower ones (Labatut & Gooch, 2012).

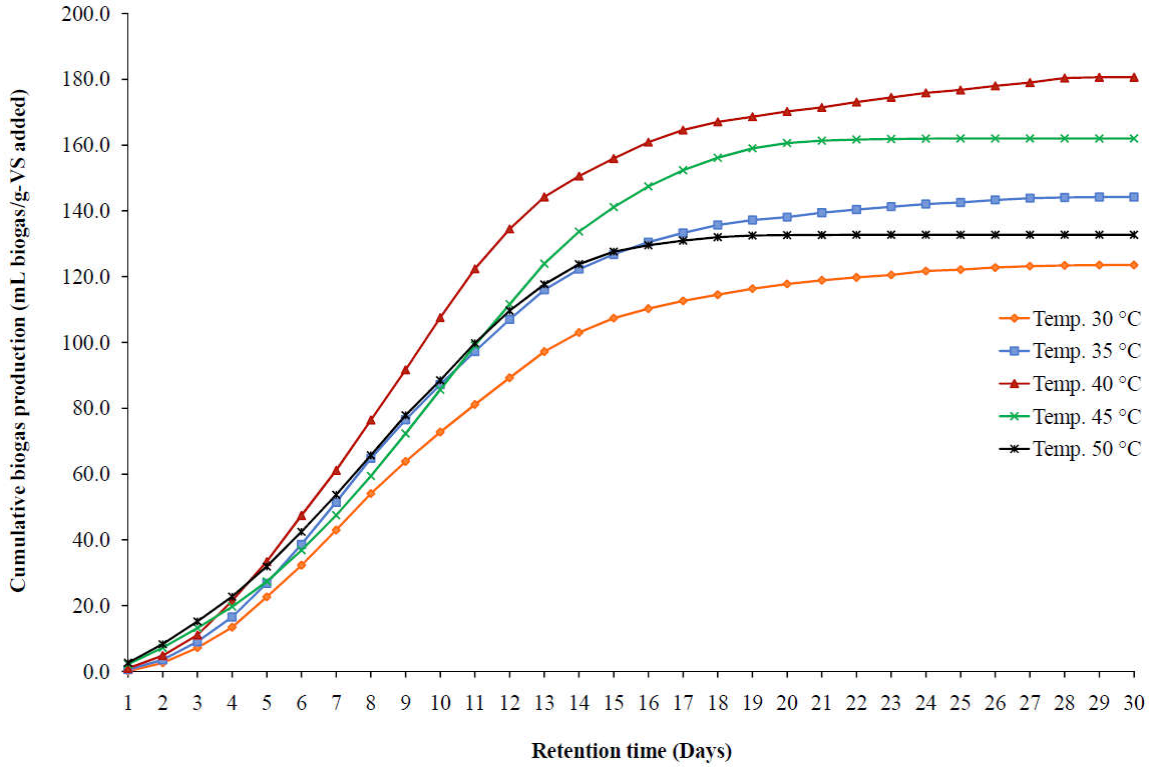


Figure 3.3: Cumulative biogas productions (mL biogas/g-VS added) from cattle manure substrate on a 30 days' RT at the various selected temperatures (°C)

3.1.3 The Optimum Cattle Manure Substrate to Water Dilution Ratio for Optimal AD of the Manure Substrate

One of the crucial factors that will enhance AD is the presence of water. Too much, too little, or a lack of water will adversely affect the rate of AD of the manure substrate on account of the fact that the microorganisms' excretive and other metabolic processes require water (Babatola, 2008; Adelekan, 2012). Drawing from related scientific literature, it has further been established that the amount of water present in biodegradable waste has a potentially significant influence on biogas yield (Babatola, 2008; Adelekan, 2012). In consequence, much time was dedicated to this part of the work to investigate the actual optimum water content needed for AD of cattle manure substrate.

Cattle manure substrates of various masses were loaded into several anaerobic digesters with varying amounts of deionized water and their respective patterns of biogas production over a period of 30 days carefully monitored to establish the highest cumulative biogas yield. Cattle manure substrate to water dilution ratio was varied so as to achieve the optimum dilution ratio. Biogas digesters were batch operated at the established optimum mesophilic temperature of 40 °C. Biogas production commenced within 24 hours of charging the digester systems.

Figure 3.4 shows the trend of total biogas yields from the nine cattle manure substrate to water percents (%m/v). The cattle manure substrate to water percents are also equivalent to the corresponding mass to volume (m/v) ratios listed below Figure 3.4. Cattle manure substrate to water percents of 900.0% and 400.0% which were the least dilute with water produced the least quantity of total biogas volumes with yields of 33.8 and 64.2 biogas/g-VS added, respectively. These findings may perhaps be because they were the least diluted biogas digesters, therefore, meaning that there was a lack of adequate water dilution that is reasonably likely to influence adversely the biogas yields. There are two overriding reasons why water content is an important parameter affecting the biomethanation of organic wastes. These are; water makes possible the movement and

growth of bacteria facilitating the dissolution and transport of nutrient, and also water reduces the limitation of the mass transfer of non-homogenous or particulate substrate. Normally, if adequate amount of water is not added, biomass would not be soaked enough to go through the degradation process efficiently and, consequently, less biogas is produced (Patil *et al.,* 2011). Besides, if the MC is too low, acetic acids will accumulate inhibiting the digestion process and, hence, biogas production. Moreover, a rather thick scum will form on the surface of the manure substrate inside the digester. This scum may prevent efficient mixing of the manure substrate in the digester (Adelekan, 2012). Efficient mixing ensures an even release of biogas bubbles trapped in the manure substrate in addition to making sure that the entire biogas digester is adequately utilized (Gerardi, 2003; Gray, 2004; Schnurer & Jarvis, 2010; Lemmer *et al.,* 2013).

However, for a retention period of 30 days, a key finding was that the cattle manure substrate to water percent of 900% also equivalent to the 17.5:7.5 (g/mL) had the highest yield of biogas with a total biogas volume of 210.6 mL biogas/g-VS added, Figure 3.4. The most plausible explanation for this finding is the exponential growth of methanogenic bacteria, possibly as a result of the combined effect of the applied optimum temperature; 40 °C; and an almost neutral pH; near 7; as well as the optimum cattle manure substrate to water dilution ratio established to be 17.5:7.5. Additionally, the high biogas yield could indeed be attributed to the applied optimum %TS of between 8.86 ± 0.06 and 9.18 ± 0.04 (Table 3.5), corresponding to the optimum cattle manure substrate to water dilution ratio of 17.5:7.5. The recommended %TS for slurries is between 8% and 12% for highest biogas production (Fulford, 1988). This emphasizes the fact that the %TS directly correspond to water content (Patil *et al.,* 2012), in addition to the fact that the amount of biogas produced is a power function of the %TS concentration (Igoni *et al.,* 2008; Mohapatro *et al.,* 2014).

Accordingly, if 7.5 mL of deionized water dilutes 17.5 g of cattle manure substrate optimally, then at a standard volume of 1 litre (water), 2.33 kg of cattle manure substrate can be added to yield the optimum cattle manure substrate to water dilution ratio.

Figure 3.4 also shows that, between the cattle manure substrate to water percents of 150.0% and 11.1%, there were declines in total biogases with the most dilute ratio of 2.5:22.5 (11.1%) producing the least volume of biogas of 113.2 mL biogas/g-VS among the six dilution ratios. This perhaps indicates that further dilution beyond the optimum cattle manure substrate to water dilution ratio of 17.5:7.5 has a detrimental impact on the rate of biogas production and is therefore indeed an over dilution. The study results also suggest that, too much water adversely affects the rate of digestion of the manure substrate, which in addition substantiates the fact that the amount of water present in the manure substrate has a significant impact on the rate of biogas production (Babatola, 2008; Adelekan, 2012). Besides, if too much water is added to the slurry, especially if the purpose is dilution, the rate of biogas production per unit volume in the anaerobic digester will fall, consequently preventing the optimum use of the biogas digester (Adelekan, 2012).

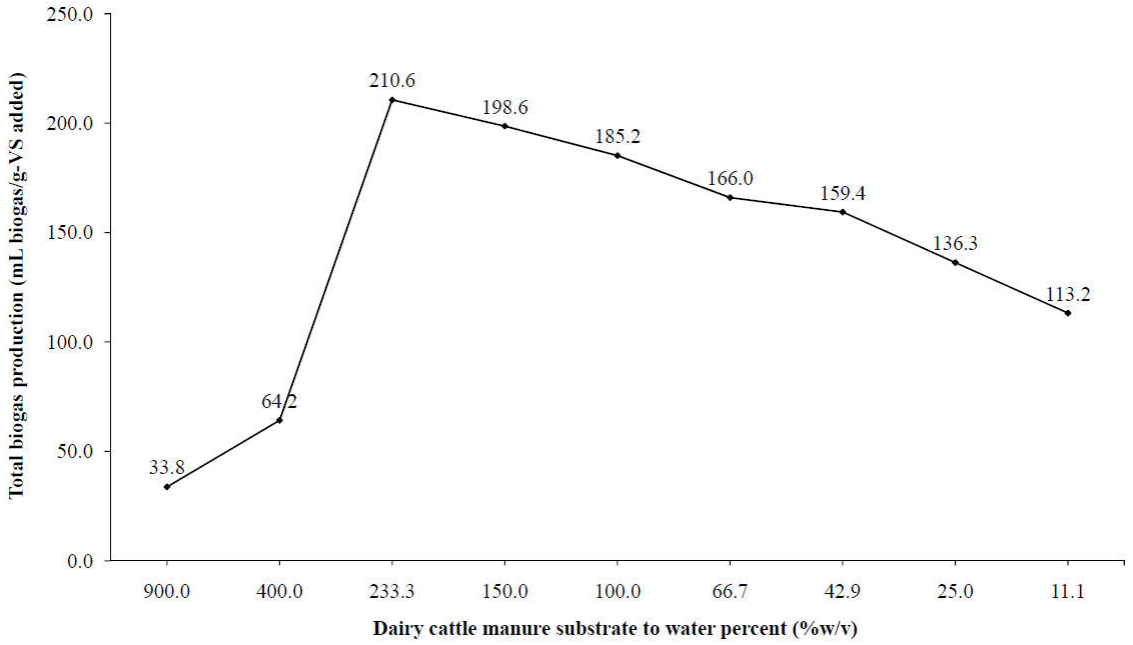


Figure 3.4: Total biogas productions (mL biogas/g-VS added) from the various cattle manure substrate to water percents (%m/v) on a 31 days' RT at the optimum mesophilic temperature of 40 °C

The values on the x-axis in Figure 3.4 represent cattle manure substrate to water percents (%m/v) also equivalent to the corresponding mass to volume ratios (m/v): 900.0% = 22.5:2.5, 400.0% = 20:5, 233.3% = 17.5:7.5, 150.0% = 15:10, 100.0% = 12.5:12.5, 66.7% = 10:15, 42.9% = 7.5:17.5, 25.0% = 5:20 and 11.1% = 2.5:22.5.

Figure 3.5 presents the specific daily biogas production trend from the optimum cattle manure substrate to water dilution ratio of 17.5:7.5 also equal to 233.3%. Biogas production began on the first day of observation. This may simply be attributed to the combined effect of the added inoculum as well as the stable pH (≈ 7) and optimum temperature (40 °C) and, in addition, the optimum cattle manure substrate to water dilution ratio of 17.5:7.5. A drop in the biogas production was observed between the first and the second day of observation. The observed decline in biogas production might be due to the production of VFAs by the microorganism that hinders the production of

biogas (Al Mamun & Torii, 2014). Further, biogas production was lower in the beginning and towards the end of the retention period; this finding underscores the fact that biogas production in batch condition corresponds to the specific growth rate of methanogenic bacteria (Nopharatana, 2007; Budiyono, 2010; Patil *et al.,* 2011).

Biogas production significantly increased from the second day through to the fifth day of observation, mainly because of the exponential growth of methanogens brought about by the optimum conditions of pH, temperature and dilution ratio. Biogas production almost reached its peak on the fifth day of the retention period; suggesting that methanogenesis had dominated the AD processes. After the fifth day of observation onward to the seventh day, there was an observed decrease in biogas production rate; conceivably due to the stationary phase of microbial growth (Ntengwe *et al.,* 2010; Chinwendu *et al.,* 2013; Ogunwande *et al.,* 2013).

Between the seventh and the eighth day, there was an abrupt increase in biogas production rate that slowed down between the eighth and the tenth day then declined after that up until the eleventh day of the retention period. Daily biogas yield increased sharply with a steep slope between the eleventh and the twelfth day followed by a slow down between the twelfth and thirteenth day of observation. The sudden increases in biogas production rates imply increased methanogenic activity, while the slowing down or even the observed declines could be associated with the slow metabolism of the anaerobic methanogenic bacteria or the lag phase of microbial growth. Production reached its peak on the twelfth day of the retention period, likely due to the control of the AD processes by methanogens. After the thirteenth day onward to the twenty-first day of observation, there was a rapid drop in biogas yield rates with slight rises between the seventeenth and the eighteenth day as well as between the nineteenth and the twentieth day of the retention period. From the twenty-first day onwards to the last day of observation, biogas production essentially slowed down. The stationary phase of microbial growth, and/or even the slow metabolism of methanogenic bacteria, as well as pH fluctuations as a result of high VFAs formation, could conceivably have led to the

decline in biogases. Primarily, biogas production kept increasing until reaching the peak on the fifth and the twelfth day of the experiment, slowed down and declined up until the last day of observation. This trend may be attributed to the fact that biogas production rate in batch anaerobic conditions directly corresponds to the specific growth rate of methanogens in the digester (Budiyono *et al.,* 2010; Patil *et al.,* 2012).

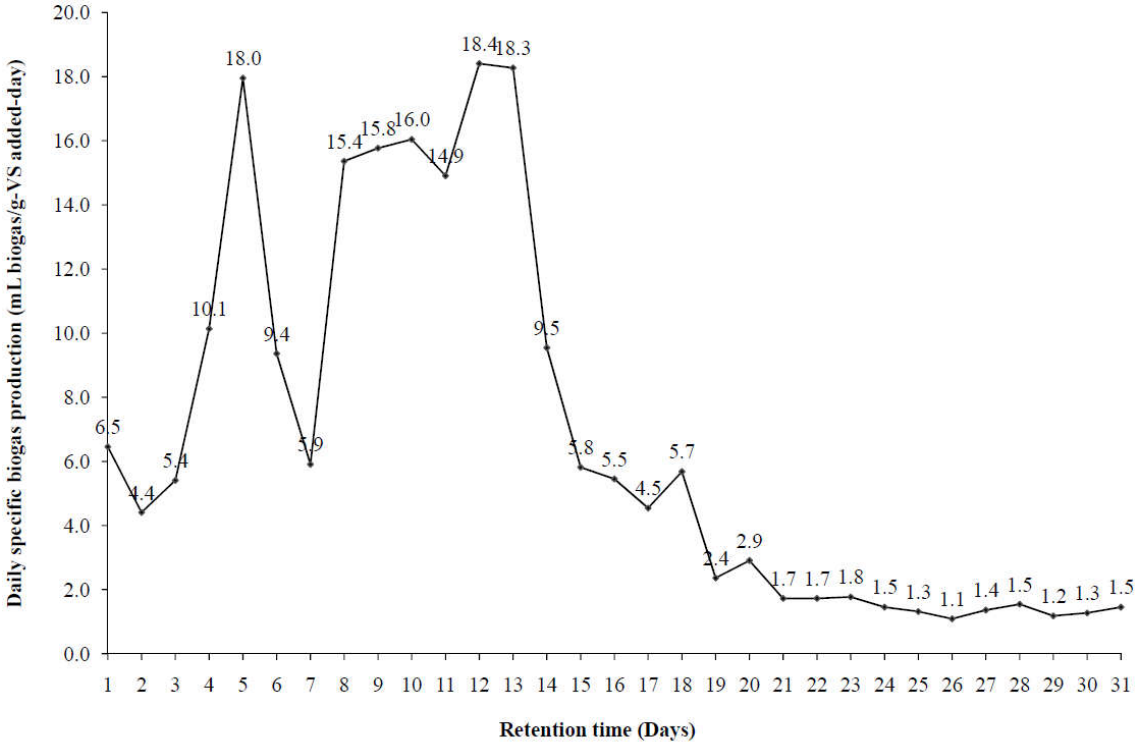


Figure 3.5: Specific daily biogas production (mL biogas/g-VS added-day) from the optimum cattle manure substrate to water percent (%m/v) of 900.0% on a 31 days' RT at the optimum mesophilic temperature of 40 °C

Figure 3.6 presents the trends of specific daily biogas production with time from the nine cattle manure substrate to water dilution ratios. The first and the second least dilute ratios of 22.5:2.5 and 20:5 had the least biogas yields though 20:5 had a relatively high total biogas yield between the two dilution ratios. The trends of biogas yields were pretty much the same for the two dilution ratios both having produced the least specific daily biogas yields in subsequent days up until the fourteenth day of observation. The

observed least biogas yields from these digesters can be ascribed to a lag phase of microbial growth during these periods of the run. The long lag of fourteen days for the two dilution ratios; 22.5:2.5 and 20:5; may be because of the complexity of biodegradation involving lignin. Anaerobic bacteria may either very slowly or even not at all degrade lignin and some other hydrocarbons. In other words, higher lignin content lowers biodegradability of waste. Cattle manure contains 40-50% lignocelluloses and, therefore, anaerobic degradation is rather un-optimum (Nielsen *et al.,* 2004; Nielsen & Angelidaki, 2008). Still, the low biogas yields can also be ascribed mainly to the sub-optimum manure substrate to water dilution ratios. The lack of adequate dilution of the manure substrates means, therefore, that the biomass is not soaked enough to go through the degradation process efficiently and, consequently, less biogas is produced (Patil *et al.,* 2011). Besides, the low biogas yields could also have been caused by the low MC, leading to accumulation of VFAs thereby inhibiting the AD process and, hence, biogas production (Adelekan, 2012). This is evident from the low pH values in Table 3.2, which show that the first two dilution ratios, that is, 22.5:2.5 and 20:5 had acidic pH values of 5.08 ± 0.07 and 5.33 ± 0.03, respectively. This could be the plausible explanation of the low biogas production for the two dilutions.

After the fourteenth day of observation onward, the dilution ratio of 20:5 produced notable daily biogas yields' rapidly reaching peak biogas production on the twentieth day before declining and stopping biogas production. This implies that methanogens had started utilizing the produced organic acids by acid formers, increasing the pH back to neutral mainly attributable to high buffering capacity of the manure substrate (Fulford, 1988; Gashaw & Teshita, 2014). The specific daily biogas evolution rate for the two was very low, and the digesters choked after retention periods of seventeen and twenty-three days, respectively. Low pH leads to increased concentration of ammonium nitrogen that might be assumed to inhibit the process (Abubakar & Ismail, 2012). Chen *et al.* (2008) described the high concentration of ammonium nitrogen as toxic to anaerobic bacteria. It decreases digester efficiency and eventually upsets the entire process. In consequence,

this could have lead to the formation and accumulation of VFAs that decreased the pH of the slurry to about 5. The decrease in pH is more than likely to have diminished the growth of methanogenic bacteria and methanogenesis hence the low biogas yield (Patil *et al.,* 2011). All the above can probably be ascribed to the fact that the two digesters were the least diluted. Taken together, these results would seem to suggest that too little water will detrimentally affect biogas production.

The effect of pH on the production of biogas was investigated, Table 3.2.

Table 3.2: Cattle manure substrate digestate pH values for all the dilution ratios

	Dilution ratios of cattle manure substrate to water (m/v)								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
pH	5.08 ±0.07	5.33 ±0.03	7.19 ±0.02	7.20 ±0.09	7.25 ±0.04	7.04 ±0.07	7.18 ±0.06	7.32 ±0.01	7.16 ±0.04

Values are mean ± standard deviation, n = 3

Key: The letters A-I represent the corresponding dilution ratios of cattle manure substrate to water (g/mL), where A = 22.5:2.5, B = 20:5, C = 17.5:7.5, D = 15:10, E = 12.5:12.5, F = 10:15, G = 7.5:17.5, H = 5:20 and I = 2.5:22.5.

The other cattle manure substrate to water dilution ratios, which were; 17.5:7.5, 15:10, 12.5:12.5, 10:15 and 7.5:17.5 had almost similar trends of biogas production rates, Figure 3.6. In this respect, rapid increases and decreases in the amounts of biogas produced were observed from the first day through to the twenty-first day of observation, as seen in Figure 3.6, with biogas productions reaching a peak on the fifth day and between the tenth and the twelfth day of observation. A plausible explanation for the observed marked increases in biogases could be credited to the fact that there was exponential growth of methanogens resulting from the combined effect of the applied optimum temperature of 40 °C and a stable pH close to neutral (Omar *et al.,* 2008). They can be said to have reached their optimum biogas productions on the fifth and between the tenth and the twelfth day of the experiment followed by either a slowdown and or

decline in biogas production. In their detailed study of biogas production from cattle manure, Omar *et al.* (2008) observed a nearly similar biogas production trend. After the thirteenth day of observation, biogas production tended to decline. The decline is as a result of the stationary phase of microbial growth and pH fluctuations. More importantly, the present study shows that biogas production was slow in the beginning, but steadily increased, after which it reached a maximum followed by the subsequent decline in biogas production. Literature reports by Omar *et al.* (2008) and Abubakar & Ismail (2012) corroborate these observations. Similar observations were made by Abubakar and Ismail (2012) in their study on biogas production from cow dung. Biogas production was very slow at the beginning and the end period of observation. A possible explanation for this is the direct proportionality between biogas production rate in a batch operation mode and the specific growth rate of methanogenic bacteria in the anaerobic digester (Nopharatana, 2007; Al Mamun & Torii, 2014).

The two most dilute ratios had nearly similar biogas production trends. Dilution ratio 5:20 reached a peak and had optimum biogas production on the second day of observation but then declined in daily biogases onward to the last day of the retention period. Further, the ratio 2.5:22.5 had no production between the first and second day; concurring with the first phase of biomass decomposition via acetogenesis process and mainly due to the lag phase of microbial growth (Omar *et al.,* 2008). The ratio then rapidly produced biogas to peak and give optimum biogas yield on the third day of observation. It thereafter declined in daily biogas yields up until the last day of observation. These results, therefore, indicate that over dilution indeed negatively influences the rate of biogas production (Babatola, 2008; Adelekan, 2012). Besides, the results underline the very important point that, if too much water is added to the digester, the rate of biogas production per unit volume of the digester falls, consequently preventing the optimum use of the biogas digester (Adelekan, 2012).

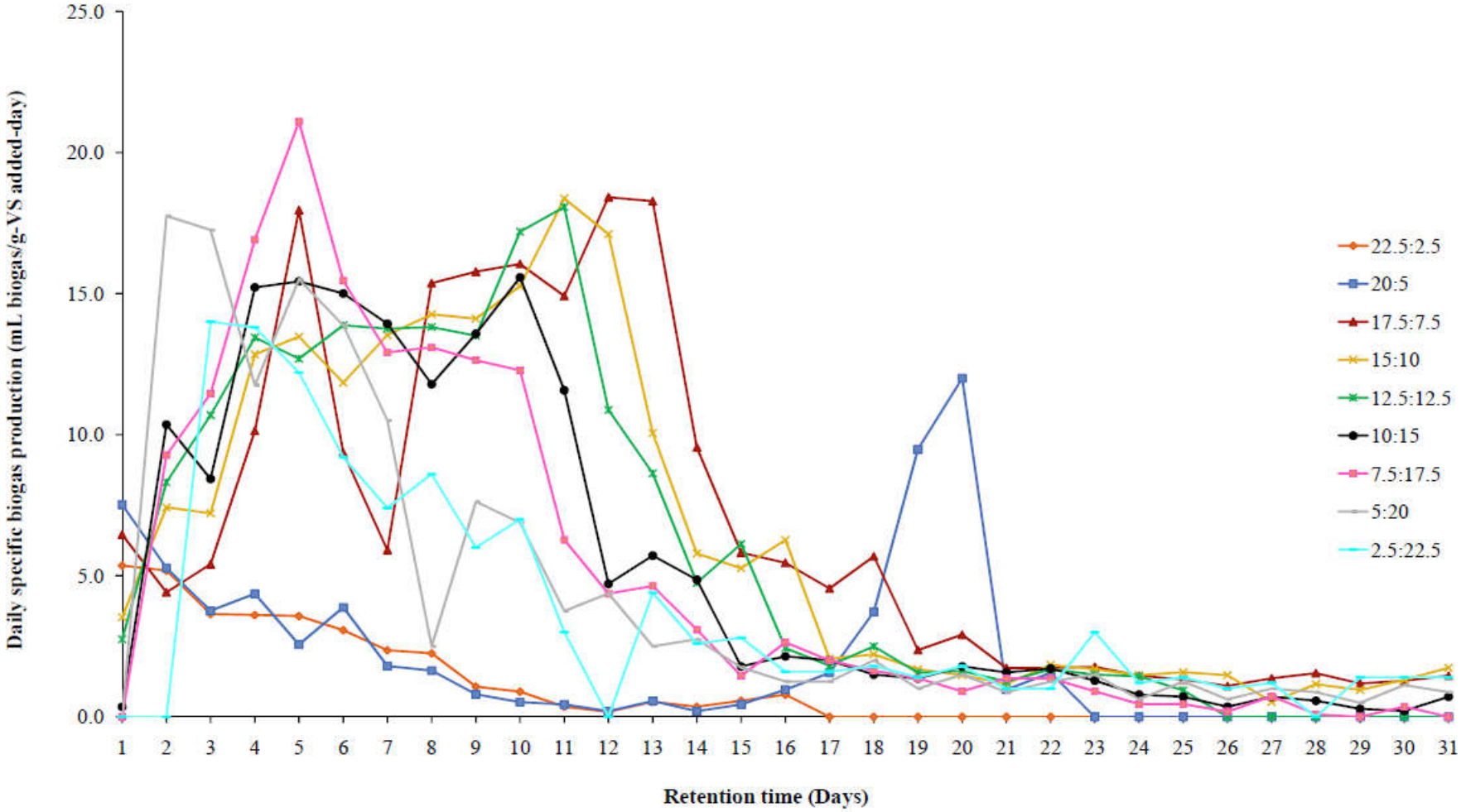


Figure 3.6: Specific daily biogas productions (mL biogas/g-VS added-day) from the various cattle manure substrate to water dilution ratios (m/v) on a 31 days' RT at the optimum mesophilic temperature of 40 °C

As is the usual practice, the cumulative change in biogas produced per total VS added, that is, specific biogas production was plotted against the cumulative change in time elapsed, and Figure 3.7 below shows the trends of biogas production from the nine cattle manure substrate to water dilution ratios. Figure 3.7 reveals that the cumulative biogas yields from the dilution ratios of 22.5:2.5, 20:5, 5:20 and 2.5:22.5 cattle manure substrate to water were the least in cumulative volumes of biogas generated among the nine biogas digesters. The respective biogas yields for the four dilution ratios averaged 33.8, 64.2, 136.3 and 113.2 mL biogas/g-VS added. The first two sets of the nine dilution ratios, that is, 22.5:2.5 and 20:5 were the least dilute with water and the other two sets, that is, 5:20 and 2.5:22.5 were the most dilute ratios with water. The results further emphasize the fact that under-diluting or over-diluting manure substrates with water impacts negatively on the rate of biogas production. The trend of biogas production from the ratio of 22.5:2.5 which is the least dilute among the nine ratios shows that it had a slow start of biogas production from the first day onward to the sixteenth day of observation. This gradual start of biogas production can be attributed to the slow metabolism of methanogenic bacteria caused by inadequate water content in the digester, which could have potentially led to the accumulation of VFAs, thereby suppressing biogas production. After that, it stopped producing biogas probably due to the observed acidic pH value of 5.08 ±0.07 (Table 3.2) in the digester, which eventually choked off biogas production. The dilution ratio of 20:5 had an almost similar trend of biogas production, where it had a slow start but managed to generate biogas between the fifteenth and the twenty-second day, where it had already slowed down before stopping biogas production onward up until the last day of observation. Biogas production tapered off gradually and abruptly stopped possibly owing to the observed low pH value of 5.33 ±0.03 (Table 3.2) from the biogas digester set.

The two most dilute ratios with water, that is, 5:20 and 2.5:22.5 commenced producing biogas on the first and second day of observation, respectively, up until the thirteenth day when they gradually slowed down up to the last day of the experiment. The highest

cumulative biogas yield of 210.6 mL biogas/g-VS added was collected from the dilution ratio of 17.5:7.5; indicating, therefore that this was the optimum cattle manure substrate to water dilution ratio for AD of the manure substrate. The biogas production trend for the dilution ratio of 17.5:7.5 was particularly notable in Figure 3.7 as it cut through all the other dilution ratios to emerge the highest in biogas yield. This is attributable mainly to the optimum dilution ratio of 17.5:7.5 and, in addition, the combined effect of the applied optimum temperature of 40 °C and a stable pH; 7.19 ± 0.02; which was near neutral (≈ 7). Between the first and the eighteenth day of observation, there was rapid and high yield biogas production from the dilution ratio of 17.5:7.5; indicating that methanogenic microorganisms had dominated the anaerobic decomposition process. This means, therefore, that there was high methanogenic activity caused by the optimum conditions of temperature, pH and dilution ratio. Biogas production then slowed down progressively between the eighteenth and the thirtieth day of the retention period. This is an indication of the slow metabolism of the methanogenic bacteria at the beginning and towards the end of the retention period in a batch anaerobic process which is directly proportional to the specific growth rate of the methanogenic bacteria (Obiukwu & Grema, 2013; Hassan, 2014). Similar trends were observed in the other dilution ratios, including 15:10, 12.5:12.5, 10:15 and 7.5:17.5. Biogas production was slow at the beginning and the end period of observation.

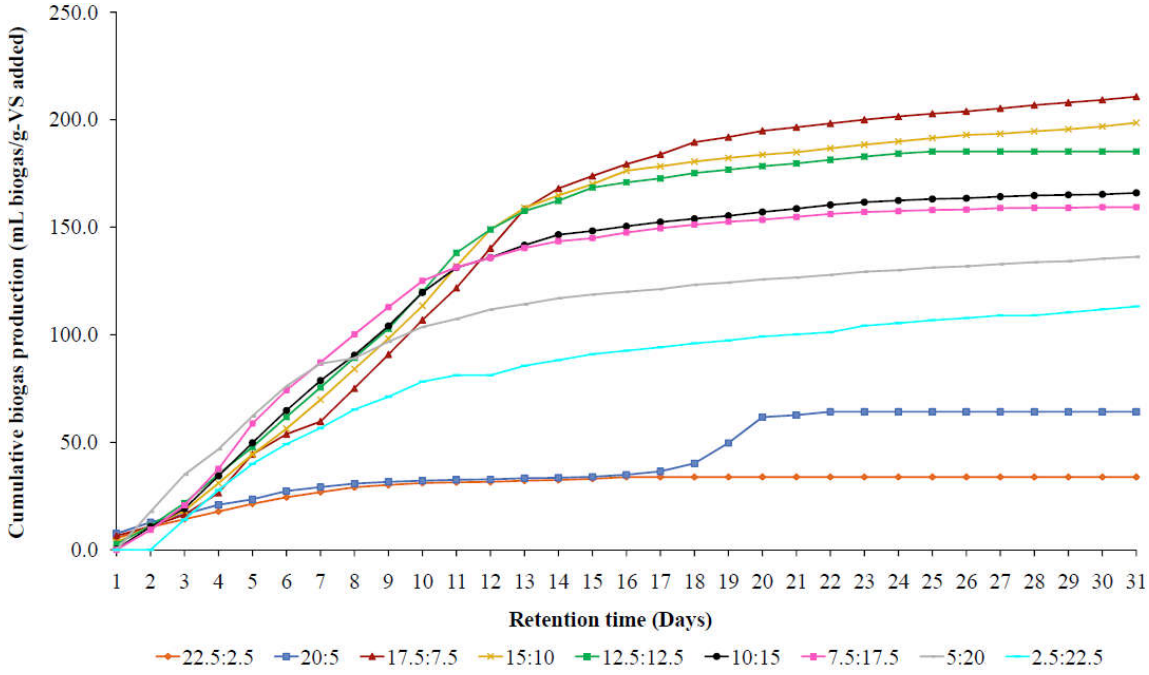


Figure 3.7: Cumulative biogas productions (mL biogas/g-VS added) from the various cattle manure substrate to water dilution ratios (m/v) on a 31 days' RT at the optimum mesophilic temperature of 40 °C

3.1.4 Supplementing Cattle Manure Substrate with Glycerol Supplement while Applying the Established Optimum Temperature and Dilution Ratio

The use of glycerol as a supplement for AD of livestock manure has been proven to increase both biogas and methane production for the reason that glycerol has a high amount of readily biodegradable soluble chemical oxygen demand (COD), which can be with greater ease utilized by anaerobic bacteria (Wohlgemut *et al.,* 2011). It is precisely for this reason that the effect of addition of propane-1,2,3-triol (glycerol); in this case, glycerol was investigated using three sets of anaerobic digesters in the experimental runs under the established optimum mesophilic temperature of 40 °C and cattle manure substrate to water dilution ratio of 17.5:7.5 (g/mL).

Table 3.3 presents the portions of glycerol used in supplementing the optimum dilution ratio, which is split into three sets of ratios, that is, 17.5:7.5:4.0:0.1, 17.5:7.5:4.0:0.2 and 17.5:7.5:4.0:0.3.

Table 3.3: Optimum dilution ratio and the added amounts of glycerol

Cattle manure substrate (g)	Water (mL)	Inoculum (g)	Mass (g) of glycerol	Percentage of glycerol
17.5	7.5	4.0	0.1	0.5%
17.5	7.5	4.0	0.2	1.0%
17.5	7.5	4.0	0.3	1.5%

The physico-chemical characteristics of the used glycerol are summarized in Table 3.4. The percentage TS, VS and TC values of 94.48±0.09, 1.15±0.02 and 0.64±0.07 percent were high enough to bring about biogas production from the glycerol. Biogas production directly corresponds to the percentage of solids; TS or VS; that are destroyed in the AD process (Joy *et al.,* 2014). The pH value of 7.12±0.06 fell within the favourable range for biogas production (Iortyer *et al.,* 2012). The optimum pH for high yield biogas production is between 6.8 and 8.5 (Lay *et al.,* 1997; Senturk *et al.,* 2014).

Table 3.4: Physico-chemical characteristics of the glycerol used

TS (%)	MC (%)	VS (%)	TC (%)	pH	Appearance	Odour
94.48 ±0.09	5.35 ±0.04	1.15 ±0.02	0.64 ±0.07	7.12 ±0.06	Clear	Odourless

Values are mean ± standard deviation, n = 3

Further, Table 3.5 shows the evaluated physico-chemical characteristics of the digestate. The digestate characteristics, which were optimum, appear to be giving a sufficiently clear view of the anaerobic digesters contents. The %TS for the three ratios was within the expected optimum range for a slurry of 8-12 percent for high yield biogas production (Fulford, 1988). The digesters had optimum MC, as depicted by the measured %MC

values, Table 3.5. The inside %MC of a digester should normally be around 90% of the mass of the total digester contents (Adelekan, 2012). Fresh cattle manure substrate had %VS of 78.68 ±0.01percent. The %VS of cow dung are usually around 80 percent of the TS (Fulford, 1988). Biogas yield is measured by the volumetric amount of biogas that is produced per unit of VS contained in the substrate after subjecting it to AD for a particular RT (Joy *et al.,* 2014). The digestate had %VS of about 57-59% meaning, therefore, that there was a reduction of the initial VS of the fresh cattle manure substrate. This therefore suggests that a portion of the %VS (≈ 21%) was converted to biogas. The initial %TC for the fresh cattle manure substrate was 43.70 ±0.04 percent. The %TC of cow dung ranges between 35% and 40% (Fulford, 1988). The digestate had %TC of around 33-34%. This also means, therefore, that there was a reduction of the initial %TC of the fresh cattle manure substrate; implying that a portion of the %TC (≈ 10%) was converted to biogas. However, some of the carbon is bound up in indigestible lignin, meaning that the carbon is partially removed from the digested material (Fulford, 1988; Kirchmann & Witter, 1992; Moller *et al.,* 2008). The pH values of the digestate fell precisely into the desired range for optimal biogas production (Iortyer *et al.,* 2012). The optimum pH in the digester for high yield biogas production is between 6.8 and 8.5 (Lay *et al.,* 1997; Senturk *et al.,* 2014).

Table 3.5: Physico-chemical characteristics of the digestate bearing the glycerol supplement

Ratio	TS (%)	MC (%)	VS (%)	TC (%)	pH
17.5:7.5:4.0:0.1	8.86	91.13	58.56	33.87	7.31
	±0.06	±0.05	±0.03	±0.08	±0.04
17.5:7.5:4.0:0.2	9.36	90.65	57.76	33.53	7.05
	±0.09	±0.07	±0.09	±0.03	±0.10
17.5:7.5:4.0:0.3	9.18	90.81	59.97	34.20	7.97
	±0.04	±0.05	±0.01	±0.07	±0.03

Values are mean ± standard deviation, n = 3

Figure 3.8 presents the specific daily biogas productions from the three biogas digester sets supplemented with glycerol. Before the addition of glycerol supplement into the biogas digesters, stable daily biogas productions were observed from the control, Figures 3.5 and 3.8. Once glycerol supplement was added to the three treatment biogas digesters at varying amounts of the supplement; 0.5%, 1% and 1.5%; stable biogas production was still observed in all the three sets of anaerobic digesters.

The biogas digester set 17.5:7.5:4:0.1 had been added with the least amount of glycerol of about 0.1 g. As can be seen in Figure 3.8, the digester set had a unique biogas production trend among the four biogas digester sets including the control where between the first and the ninth day of observation, gradual biogas production was observed from almost the first day of the experiment. As from the eighth day of observation onward, the biogas digester set exhibited a nearly identical trend of biogas production with the rest of the biogas digester sets. Biogas production commenced on the first day of observation; possibly indicating that methanogenic bacteria were able to utilize quickly the added glycerol. The observed initial biogas production was also likely due to the readily biodegradable organic matter in the substrates and the high presence of methanogens from the inoculum. Biogas production then slowed down between the second and the third day; indicating, therefore, that the bacteria could switch to feeding on the manure substrate once the glycerol was used up. Rapid biogas production was then observed between the third and the ninth day of observation before reaching peak biogas production on the twelfth day followed by a drop in daily biogases onward to the last day of observation. Biogas production rate is significantly increased during the period considered, mainly because of the exponential growth of microorganisms and then tends to decrease due to the stationary phase of microbial growth.

The two biogas digesters fed with 1% (17.5:7.5:4:0.2) and 1.5% (17.5:7.5:4:0.3) glycerol supplement revealed a comparable trend of biogas production, where between the third and the fifth day of observation, there was a sharp rise in biogas production. This increase in biogas production rate could be as a result of the added amount of

glycerol that was readily available for degradation by anaerobic bacteria. A decline was then observed on subsequent days between the fifth and the seventh day. This decline may be attributed primarily to the fact that normal hydrolysis was taking place in the two days after which a sharp increase was observed. Besides, digester sets 17.5:7.5:4:0.2 and 17.5:7.5:4:0.3 showed an initially higher biogas production as the bacteria utilized the glycerol, and then gas production rate slowed down to a very similar one as the control; indicating that the anaerobic bacteria could probably reverse to feeding on the manure substrate once they have exhausted glycerol. From the seventh through to the ninth day of observation, a steady rise in biogas production was evident as seen in Figure 3.8. This could possibly be explained by the fact that on the microorganisms exhausting the readily biodegradable soluble COD provided by the glycerol supplement, they embarked on degrading the actual substrate, which is cattle manure substrate that had already undergone hydrolysis.

It was then observed that after achieving peak values biogas production began to decline, that is, daily biogas generation kept increasing until reaching the peak, and then began to fall. This is primarily attributable to the slow biogas production at the beginning and the end period of observation, owing to biogas production rate in a batch system which directly corresponds to the specific growth rate of methanogenic bacteria in the biogas digester (Patil *et al.,* 2011; Al Mamun & Torii, 2014).

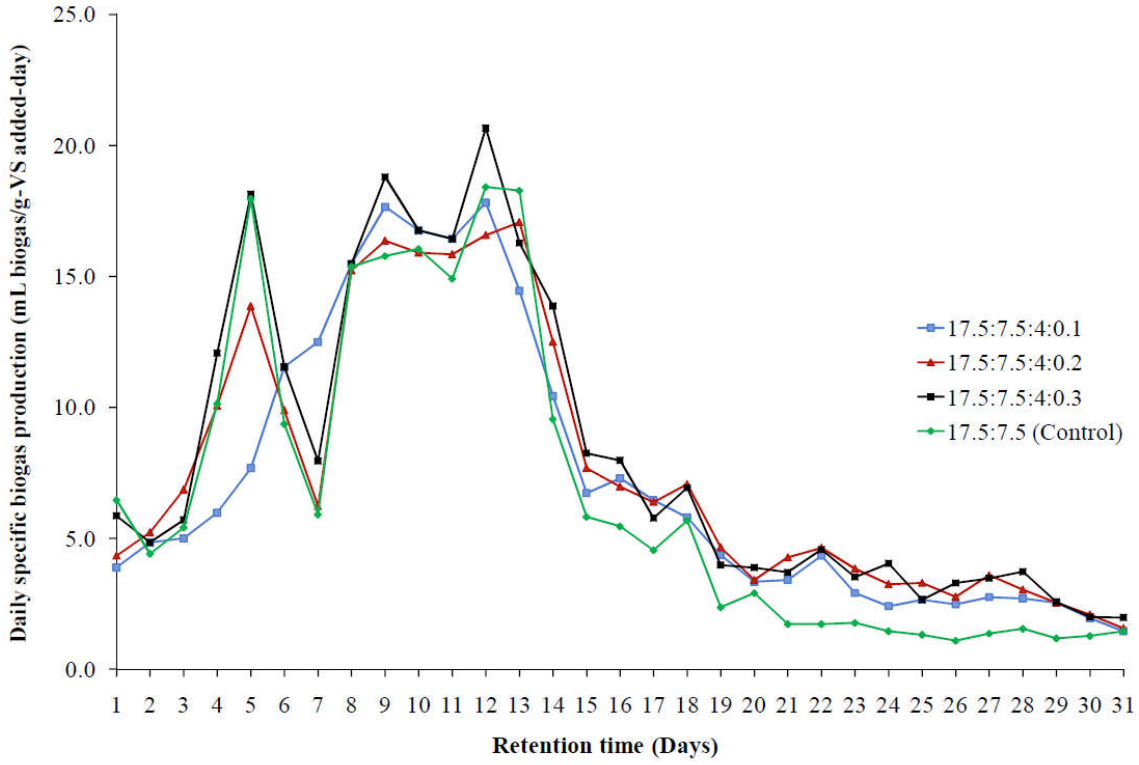


Figure 3.8: Specific daily biogas productions (mL biogas/g-VS added-day) from the optimum dilution ratio of 17.5:7.5 (g/mL) supplemented with varying amounts of glycerol at the optimum mesophilic temperature of 40 °C for 31 days' RT

Figure 3.9 shows the collected total biogas volumes from the three biogas digester sets supplemented with glycerol. The biogas digester set 17.5:7.5:4:0.3 supplemented with 1.5% glycerol generated the highest total biogas yield of 256.7 mL biogas/g-VS added. The least total biogas yield was collected from 17.5:7.5:4:0.1 supplemented with 0.5% glycerol with a total biogas yield of 224.0 mL biogas/g-VS added. The digester sets in order of the total biogas collected: 17.5:7.5:4:0.3 > 17.5:7.5:4:0.2 > 17.5:7.5:4:0.1 > 17.5:7.5:4. The response in biogas production in the biogas digester set 17.5:7.5:4:0.3 supplemented with 1.5% glycerol was most likely as a result of the breakdown of the readily biodegradable soluble COD in the glycerol that was relatively more in quantity compared to the other biogas digester sets 17.5:7.5:4:0.1 and 17.5:7.5:4:0.2 supplemented with 0.5% and 1% glycerol, respectively. The results obtained, therefore,

seem to indicate that biogas yields increased with increasing glycerol amounts. There is a need, however, to maintain a stable digestion process, and therefore the amount of glycerol added has a limiting concentration level (Amon *et al.,* 2006; Holm-Nielsen *et al.,* 2007; Fountoulakis *et al.,* 2009).

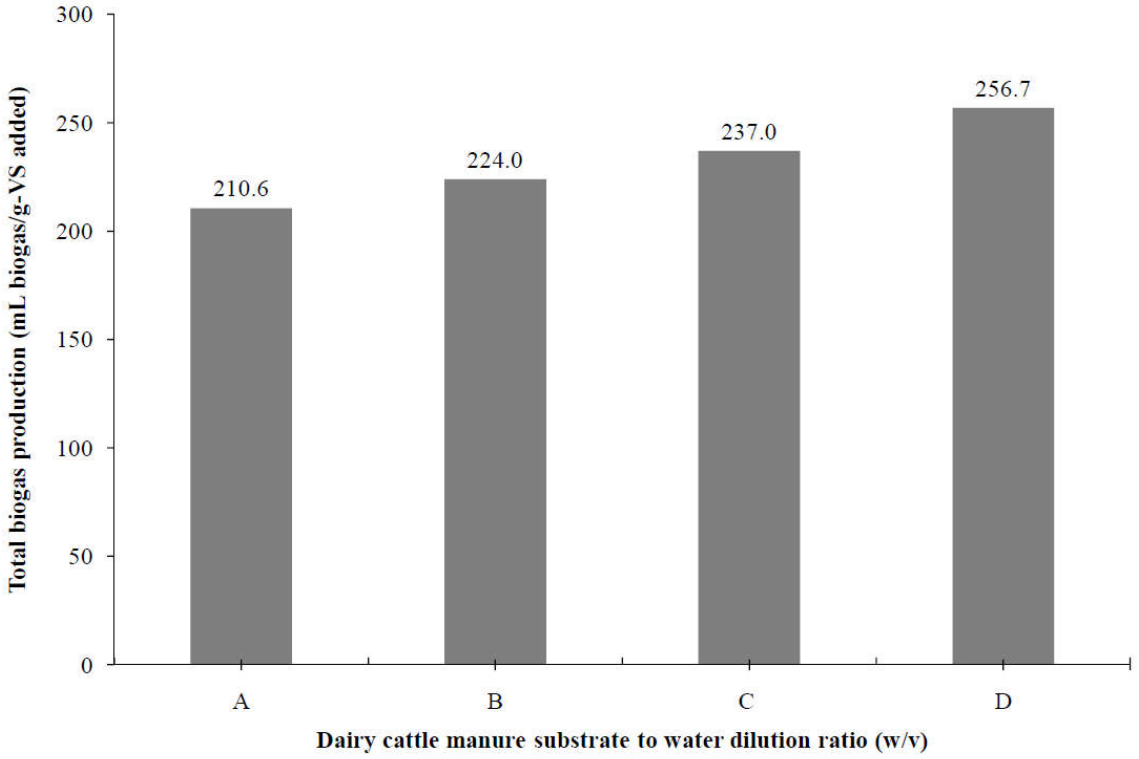


Figure 3.9: Total biogas productions (mL biogas/g-VS added) from the optimum dilution ratio of 17.5:7.5 (g/mL) supplemented with varying amounts of glycerol at the optimum mesophilic temperature of 40 °C for 31 days' RT

The letters in Figure 3.9 represent cattle manure substrate to water ratios (m/v) to inoculum to glycerol ratios as follows: A = 17.5:7.5:4 (control; without glycerol supplement), B = 17.5:7.5:4:0.1, C = 17.5:7.5:4:0.2 and D = 17.5:7.5:4:0.3.

Figure 3.10 shows the cumulative volumes of biogas generated from the three sets of biogas digesters supplemented with glycerol. Trends of cumulative biogas production with time in the three digester sets were very similar. It can be observed from Figure

3.10 that biogas production rate tends to obey the sigmoid function (S-curve) as it generally occurs in the batch growth curve. In a batch system, cumulative biogas production usually follows a sigmoidal curve (Beuvink & Kogut, 1993) with three distinguishable phases: an initial or lag phase with slow or no biogas production, a second or exponential phase with rapid biogas production, and a final or asymptotic phase where biogas production slows down and finally reaches zero. This is basically attributable to the biogas production rate in batch operation conditions which corresponds directly to the specific growth rate of methanogenic bacteria in the biogas digester (Gupta *et al.,* 2009; Hassan, 2014). In the first three days of observation, biogas productions were low; this is probably because of the lag phase of microbial growth. Between the third and eighteenth day of the retention period, biogas was very rapidly produced and achieved significantly increased yields owing to the exponential growth of microorganisms. After the eighteenth day of observation, biogas production decreased, probably caused by the stationary phase of microbial growth.

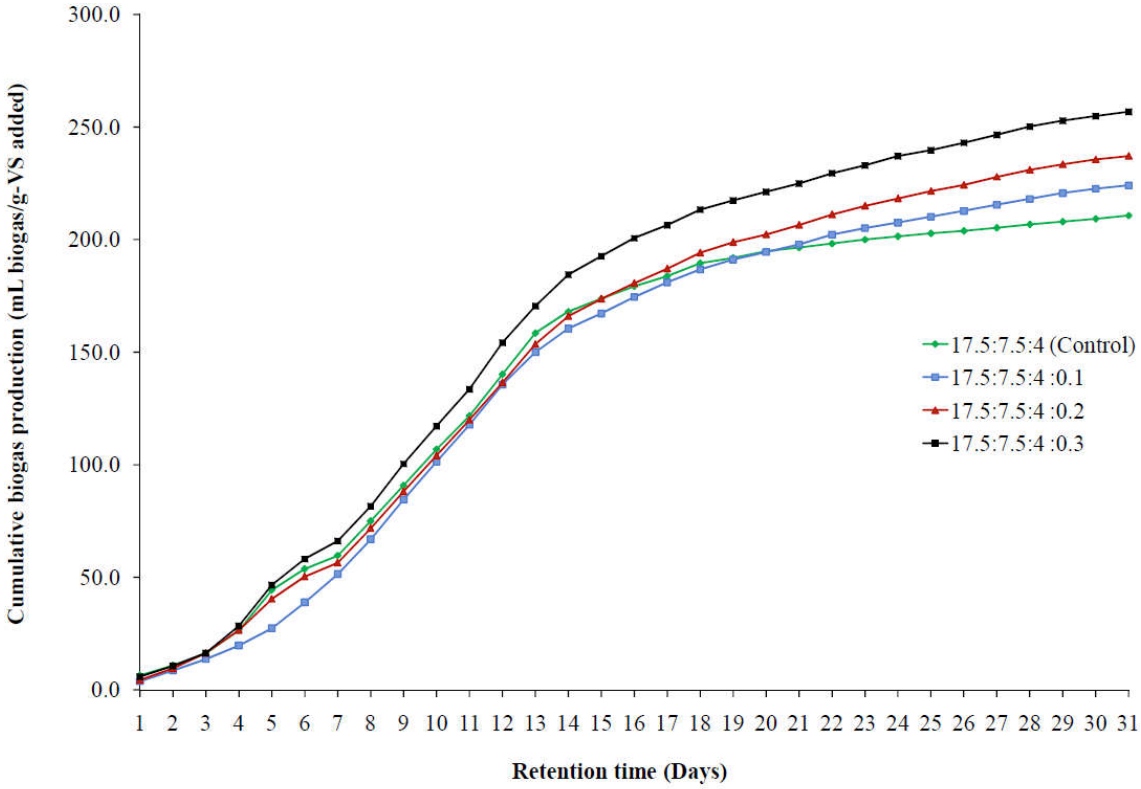


Figure 3.10: Cumulative biogas productions (mL biogas/g-VS added-day) from the optimum dilution ratio of 17.5:7.5 (g/mL) supplemented with varying amounts of glycerol at the optimum mesophilic temperature of 40 °C for 31 days' RT

Comparing the specific, total and cumulative biogas productions from the control (without glycerol addition) which had a total biogas yield of 210.6 mL biogas/g-VS added with biogas yields from the glycerol supplemented biogas digester sets, it can be seen from Figures 3.8, 3.9 and 3.10 that there were substantial increases in biogas yields. From the control; 17.5:7.5:4; with a total biogas yield of 210.6 mL biogas/g-VS added, based on the total biogas yields, the percentage increases in biogas yields were 6.363% for; 17.5:7.5:4:0.1; 0.5% (m/m) glycerol supplementing, 12.54% for; 17.5:7.5:4:0.2; 1% (m/m) glycerol supplementing and 21.89% for; 17.5:7.5:4:0.3; 1.5% (m/m) glycerol supplementing.

The theoretical methane potential from glycerol alone, based on the Buswell's Formula and the Ideal Gas Law, is 0.47 litres (470 mL) of methane per gram of glycerol (Ma *et al.,* 2008). The percentage methane gas available in biogas is about 56-60%. On average 57.5% of methane gas is available in biogas generated from organic wastes (Karthick *et al.,* 2014; Santhosh & Revathi, 2014). Based on the control's total biogas volume of 210 mL Biogas/g-VS added and a normalization factor of 2.2, the expected total biogas yields for 0.1, 0.2 and 0.3 g of added glycerol are 247.87, 285.15 and 322.42 mL Biogas/g-VS added, respectively. The expected respective percentage increases in biogas yields were 17.7%, 35.4% and 53.1% (v/v) for 0.1, 0.2 and 0.3 g of added glycerol. The actual total biogas yields were lower (Figure 3.9) compared to the expected volumes. The actual biogas yield should always be lower than the theoretical value, because part of the substrate feed will always be used for cell growth, and some substrate will leave the digester without being degraded (Poulsen, 2003). Besides, the exact biogas yield will also depend on the various environmental conditions, such as feedstock, temperature and microbial populations (Lusk, 1998).

3.2 Pig Manure Substrate

3.2.1 Physico-chemical Characteristics of Pig Manure Substrate and Inoculum

Pig manure substrate and inoculum were analyzed for five important physico-chemical characteristics pursuant to APHA *et al.* (2005) - Standard Methods for the Examination of Water and Wastewater. Results of the analyses obtained as mean values ± standard deviations (n = 3) for each of the five physico-chemical characteristics are listed in Table 3.6.

As shown in Table 3.6, pig manure substrate had %TS, %MC, %VS, and %TC of 25.35 ± 0.09, 74.65 ± 0.12, 74.05 ± 0.08, and 41.14 ± 0.1 percent, respectively. The respective reported values for %TS, %MC, %VS, and %TC by Fulford (1988) are 25, 82, 80, and 53 percent and are very close to the measured once. The fresh pig manure pH value of 8.37 ± 0.09 fell within the optimum pH range of between 6.8 and 8.5 for high yield biogas production. The optimum pH value of 8.37 ± 0.09 means, therefore, that pig manure substrate could effect reasonable biogas production as depicted by the physico-chemical characteristic, Table 3.6.

Table 3.6: Physico-chemical characteristics of fresh pig manure substrate and inoculum

Substrate and Inoculum	TS (%)	MC (%)	VS (%)	TC (%)	pH
Pig manure substrate	25.35	74.65	74.05	41.14	8.37
	±0.09	±0.12	±0.08	±0.11	±0.09
Inoculum (Starter seed)	9.41	90.60	58.85	32.70	7.64
	±0.07	±0.05	±0.02	±0.06	±0.01

Values are mean ± standard deviation, n = 3

3.2.2 The Optimum Mesophilic Temperature for Optimal AD of Pig Manure Substrate

The study primarily set out to establish the optimum mesophilic temperature for optimal AD of pig manure substrate. Figure 3.11 shows the specific daily biogas yields collected from the various experimentation temperatures. Biogas production commenced within 24 hours of loading the anaerobic digesters. This quick biogas generation can primarily be attributed to the optimum composition of the pig manure substrate (Table 3.6) and the influence of the added inoculum. Further, pig manure may have brought an abundant microflora to create a favorable environment that aided faster digestion and an average yield of biogas (Kasisira & Muyiiya, 2012; Chinwendu *et al.,* 2013). From Figure 3.11, there are both sharp and progressive increases in biogas production at the various experimented temperatures. Moreover, Figure 3.11 shows that, temperatures 30 °C and 35 °C had closely similar trends of biogas production. The patterns depict that the two sets had gradual biogas productions, reaching the maximum output on the eleventh day of the experiment for both sets. Firstly, a plausible explanation for this low biogas yields at 30 °C and 35 °C is that, methane forming bacteria were inadequately activated for enhanced and high yield biogas production. Consequently, this would lead to a slow hydrolyzing reaction caused by low biodegradation of pig manure followed by minimal biogas yields (Ntengwe *et al.,* 2010). Secondly, the observed least biogas yields from these anaerobic digesters can also be ascribed to a lag phase of microbial growth during the retention period due to the complexity of biodegradation involving lignin. Pig manure contains a lot of cellulose, semi-celluloses lignin and pectin. Anaerobic bacteria may either very slowly or even not at all degrade lignin and some other hydrocarbons. Thus, higher lignin content lowers biodegradability of waste (Chinwendu *et al.,* 2013; Oparaku, 2014). Thirdly and lastly, methanogens undergoing a metamorphic growth process by consuming methane precursors produced from the initial activity as suggested by Lalitha *et al.* (1994) could also have affected the onset and/or the volume of biogas produced (Aremu & Agarry, 2012; Ogunwande *et al.,* 2013).

Optimum biogas productions are attained when methanogenesis dominates the AD process due to the exponential growth of methanogens meaning, therefore, that acetogenic methanogenic bacteria required for methane production are active (Chinwendu *et al.,* 2013). Biogas generation in the two anaerobic digester sets dropped after reaching optimum biogas productions, that is, after the eleventh day onwards. Besides, biogas production was very slow at the beginning and the end period of observation, owing to biogas production rate in a batch system which directly corresponds to the specific growth rate of methanogenic bacteria in anaerobic digesters (Chinwendu *et al.,* 2013).

Comparably, temperatures 40 °C, 45 °C and 50 °C had more or less identical biogas production trends. A steep rise in biogas production was observed in the three sets 24 hours post charging the anaerobic digesters. The rapid high yield biogas production could be as a result of the shorter lag phase growth, and the strong presence of methanogenic archaea. Optimum biogas productions were observed for the three sets on the fifth day of the experiment for 40 °C and on the fourth day of observation for both 45 °C and 50 °C, which was earlier compared to the other two sets ran at temperatures 30 °C and 35 °C. Peak biogas production suggests that, methanogens have dominated the entire AD process. Biogas production decreased immensely immediately after attaining the optimum biogas production onwards and tapered off on the twenty-fifth, twenty-first and the nineteenth day of observation for temperatures 40 °C, 45 °C, and 50 °C, respectively. This trend may also be attributed to the fact that biogas production rate in batch anaerobic conditions directly corresponds to the specific growth rate of methanogens in the anaerobic digester (Chinwendu *et al.,* 2013).

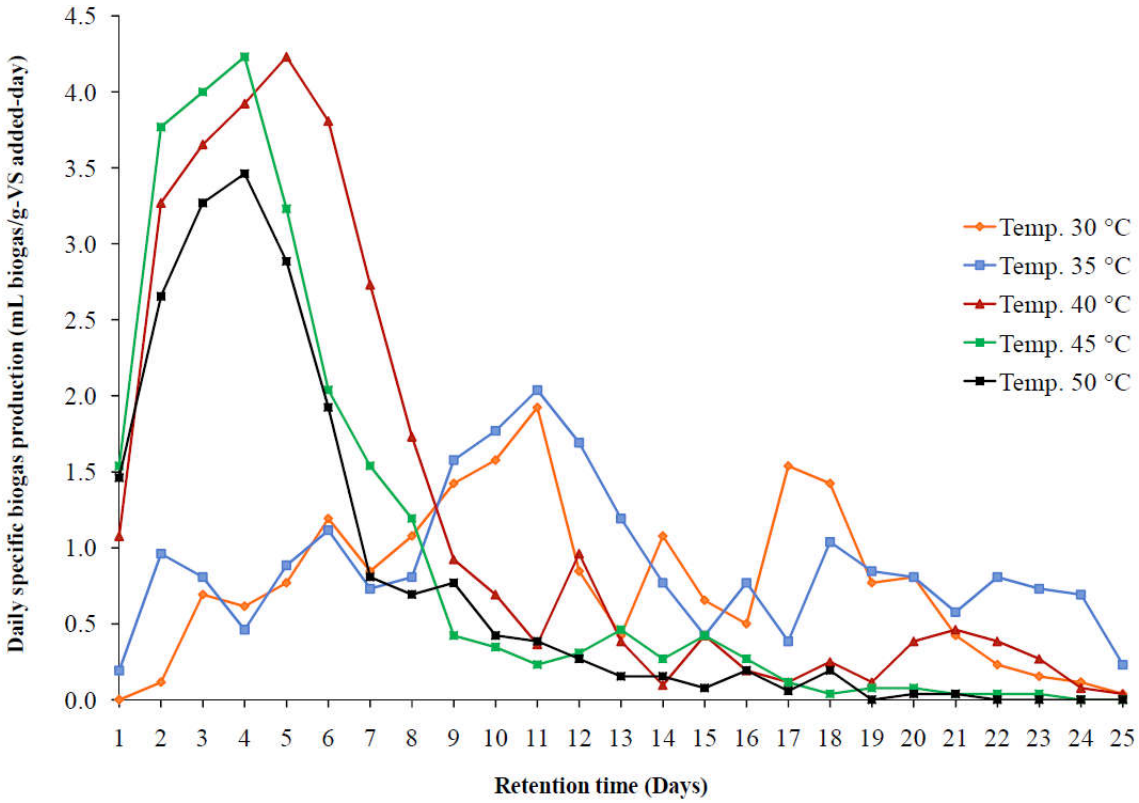


Figure 3.11: Specific daily biogas productions (mL biogas/g-VS added-day) from pig manure substrate on a 25 days' RT at the various selected temperatures (°C)

The plot of the total biogas yields at the various experimented temperatures is shown in Figure 3.12. An observation similar to that in Figure 3.2 was indeed observed in Figure 3.12, where the trends of biogas production in the two figures were quite comparable. The maximum total biogas volume was collected at 40 °C from pig manure substrate on a 25 days' RT, where the gas output totalled 30.6 mL biogas/g-VS added; indicating that the upper limit of the mesophilic range (30-40 °C) gives a higher biogas yield. Besides, biogases were collected at all the five experimented temperatures; substantiating the fact that different species of methanogenic bacteria can survive at different temperature ranges (Dhanariya *et al.,* 2014). Below and above 40 °C, there were relatively lower total biogases, where at 40 °C, the total biogas yield was 30.6 mL biogas/g-VS added and at 30 °C and 35 °C, total biogas yields were 19.2 and 22.3 mL biogas/g-VS added,

respectively. Total biogas yield was least at 30 °C, higher at 35 °C and highest at 40 °C in that ascending order; indicating that the rate of bacteriological methane production increases with temperature (Fulford, 1988; Adelekan, 2012). At 30 °C, anaerobes are inadequately activated for high yield biogas production inevitably resulting in low biodegradation of pig manure and hence the little biogas yields. The combined effect of optimum conditions of temperature and pH may have contributed to the observed high biogas yields at 40 °C possibly due to the increased microbial activity hence, higher rates of biological degradation of pig manure substrate (Fulford, 1988; Ntengwe *et al.,* 2010; Adelekan, 2012; Ogunwande *et al.,* 2013; Oparaku, 2014).

Temperatures 45 °C and 50 °C had total biogas yields of 24.7 and 19.9 mL biogas/g-VS added, respectively. Total biogas production was least at 50 °C, higher at 45 °C and highest at 40 °C in that ascending order based on biogas yields. It is therefore apparent that, a further increase in temperature from 40 °C to 45 °C and finally to 50 °C, caused a decrease in biogases; indicating that biogas yields were adversely affected by increased temperatures towards the thermophilic range. An increase in temperature above the optimum 40 °C causes a reduction in metabolic rate and consequently a drop in biogases (Adelekan, 2012). This can probably be attributed to the fact that exceeding the optimum temperature; 40 °C; causes the destruction, and ultimately, death of anaerobic bacteria, which subsequently leads to the biological collapse of the anaerobic digesters. At 50 °C, the relatively small biogas yield could also have been due to the overlap of the mesophilic and thermophilic ranges, where the microorganisms including mesophiles and thermophiles exclude each other and compete in the overlapping range, which results in reduced efficiency of the process (Zupancic & Grilc, 2012).

In the event of an increase in the ambient temperature, typically the rate of reaction increases. On this account, a greater rate of biogas production is realized (Nijaguna, 2006; Raja & Lee, 2012). On the whole, the process will be more affected at higher temperatures than at lower ones (Labatut & Gooch, 2012). Labatut and Gooch (2012)

point out that it is vitally important that we keep optimum digester temperature at 35 °C - 40 °C for mesophilic digesters this because it is the optimum temperature range for all of the bacteria groups.

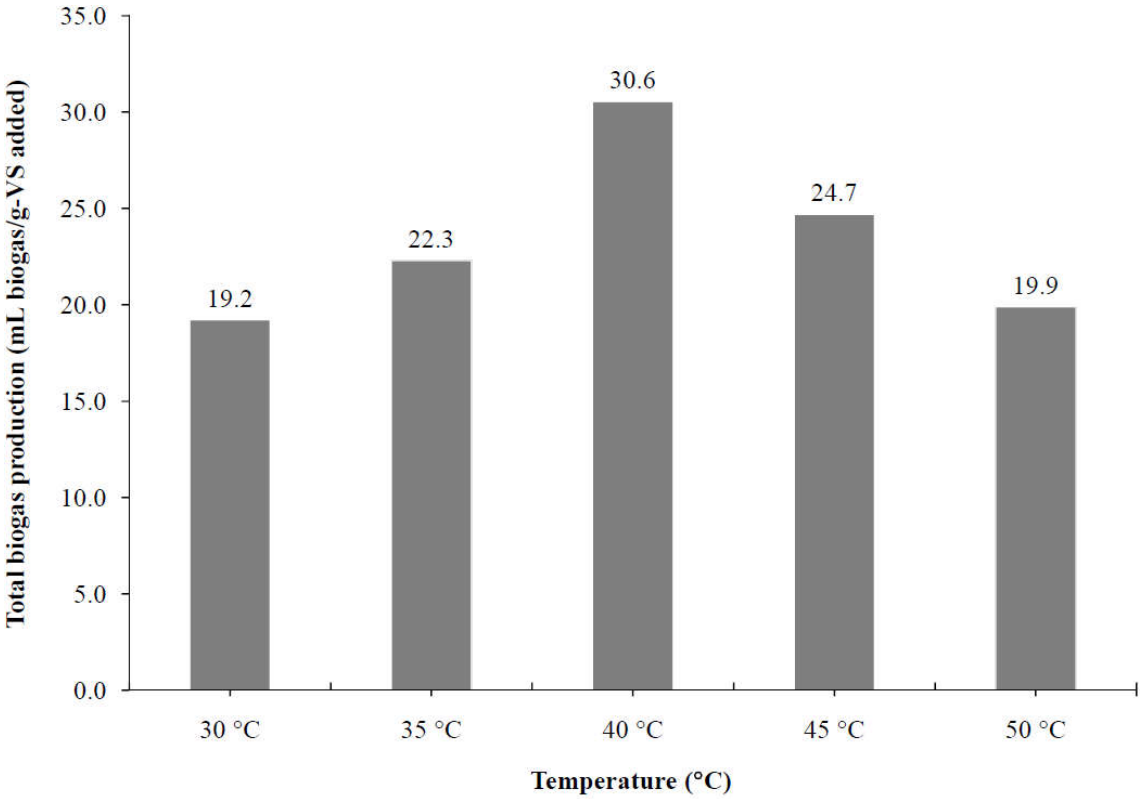


Figure 3.12: Total biogas productions (mL biogas/g-VS added) from pig manure substrate on a 25 days' RT at the various selected temperatures (°C)

Figure 3.13 shows the plot of cumulative biogas yields for the five experimented temperatures. The plot shows the highest performance at 40 °C; indicating 40 °C as the optimum temperature for AD of pig manure. Further, the study findings revealed that the upper limit of the mesophilic range gives a higher biogas yield (Adelekan, 2012). It can be seen from Figure 3.13 that, biogas production rate does obey the sigmoid function (S-curve) as it occurs in the batch growth curve. In a batch system, cumulative biogas production typically follows a sigmoidal curve (Beuvink & Kogut, 1993) with three distinct phases: an initial or lag phase with slow or no biogas production, a second or

exponential phase with fast biogas production, and a final or asymptotic phase in which biogas production slows up and eventually reaches zero. Batch growth curves are attributable to the biogas production rate in the batch process which corresponds directly to the specific growth rate of methanogenic bacteria in the anaerobic digester (Chinwendu *et al.,* 2013). Temperature 30 °C and 35 °C had nearly similar patterns of biogas production. They both had gradual but steady biogas production up until the eleventh day of observation. There was a slow up from the twelfth day until the twenty-fifth day, which was the final day of the experiment. At 40 °C, there was an observed increased biogas production between the first and the fifth day of observation. There was a slow up from the sixth day onward to the last day of the experiment. At 45 °C and 50 °C, rapid biogas production was observed between the first and the fourth day of the retention period. There was also a slow up in biogas production rate at 40 °C from the fifth day onward up until the twenty-fourth day. Biogas production stopped onwards until the final day. Biogas production slowed down at 50 °C between the fifth and the eighteenth day of observation, stopped on the nineteenth and the twenty-second day up until the last day of the retention period. The initial rapid biogas production is as a result of the exponential growth of methanogens, whereas the slow up is due to a lag phase of microbial growth due to the delay in the bacteria to multiply effectively in order to break up the pig manure substrate. Stalled biogas productions are associated with the stationary phase of microbial growth. In addition, the stoppage of biogas production suggests completion of the AD process or process breakdown possibly as a result of methane inhibitors in the manure substrate (Ntengwe *et al.,* 2010; Chinwendu *et al.,* 2013; Ogunwande *et al.,* 2013).

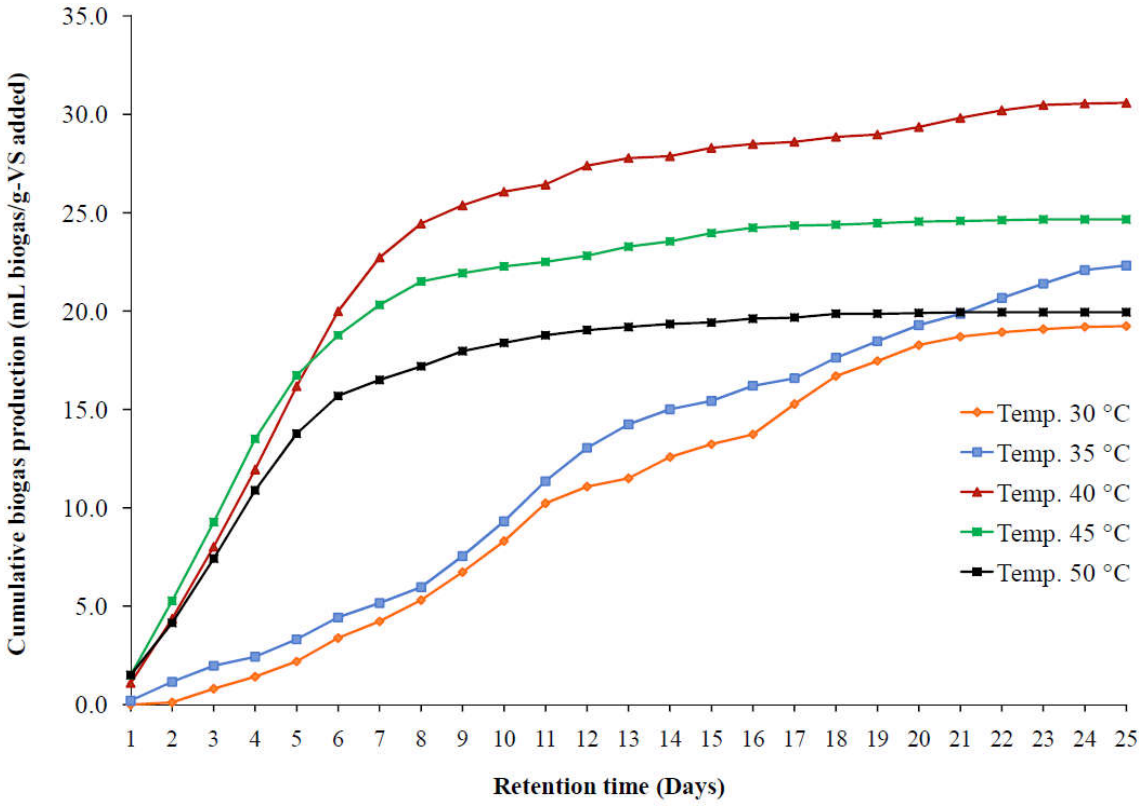


Figure 3.13: Cumulative biogas productions (mL biogas/g-VS added) from pig manure substrate on a 25 days' RT at the various selected temperatures (°C)

3.2.3 The Optimum Pig Manure Substrate to Water Dilution Ratio for Optimal AD of the Manure Substrate

The second part of the study investigates the optimum pig manure substrate to water dilution ratio that would yield the most amount of biogas. The biogas digesters were batch operated at atmospheric pressure and also ran at the established optimum mesophilic temperature of 40 °C. Biogas productions were closely monitored to determine the specific daily biogases, highest total and cumulative biogas yields.

Figure 3.14 shows the pattern of total biogas yields from the various pig manure substrate to water percents (%m/v). The pig manure substrates to water percents are also equivalent to the corresponding mass to volume (m/v) ratios listed below Figure 3.14. Total biogas yields increased with increased water dilution from 900.0% to 25% with

the first and the latter giving the least and the highest biogas yields of 20.1 and 102.0 mL biogas/g-VS added, respectively. Biogas yields dropped after that on further water dilution, Figure 3.14. These findings contradict the fact pointed out earlier that too much or too little water will adversely affect biogas yield (Adelekan, 2012). However, Masse *et al.* (2003) reported that pig production units handling pig manure in a more dilute state produce significantly more methane than those keeping manure in a more concentrated state, even though the effect of dilution may depend on temperature. Further, Masse *et al.* (2003) evaluated methane production from pig manure on a per kg of VS basis and established that there were significantly higher yields at low than high TS contents. Therefore, an adequate amount of water has to be added to anaerobic digesters for biomass to be soaked enough to go through the degradation process efficiently for high yield biogas production (Masse *et al.,* 2003; Chinwendu *et al.,* 2013). Besides, sufficient water in anaerobic digesters ensures efficient substrate mixing and consequently an even release of biogas bubbles trapped in the manure substrate in addition to making sure that the entire biogas digester is adequately utilized (Gerardi, 2003; Gray, 2004; Schnurer & Jarvis, 2010; Lemmer *et al.,* 2013). The high biogas yield of 102.0 mL biogas/g-VS added from the dilution ratio of 5:20 also equal to 25% can be ascribed to the fact that the dilution ratio was optimum for high yield biogas production and, in addition, the combined effect of the applied optimum conditions of temperature and pH probably allowed the growth of a more active methanogen population.

However, if 20 mL of deionized water dilutes 5 g of pig manure substrate optimally, then at a standard volume of 1 litre, 0.25 kg of pig manure substrate can be added to yield the optimum pig manure substrate to water dilution ratio.

The observed decrease in total biogas yields shown in Figure 3.14 for 11.1% (2.5:22.5) with the gas output totaling 96.0 mL biogas/g-VS added; indicates that further dilution beyond the optimum dilution ratio has detrimental effects on the rate of biogas production and is therefore indeed over dilution. Besides, if too much water is added to the substrate, especially if the purpose is dilution, the rate of biogas production per unit

volume in the biogas digester will fall, consequently preventing the optimum use of the anaerobic digester (Babatola, 2008; Adelekan, 2012).

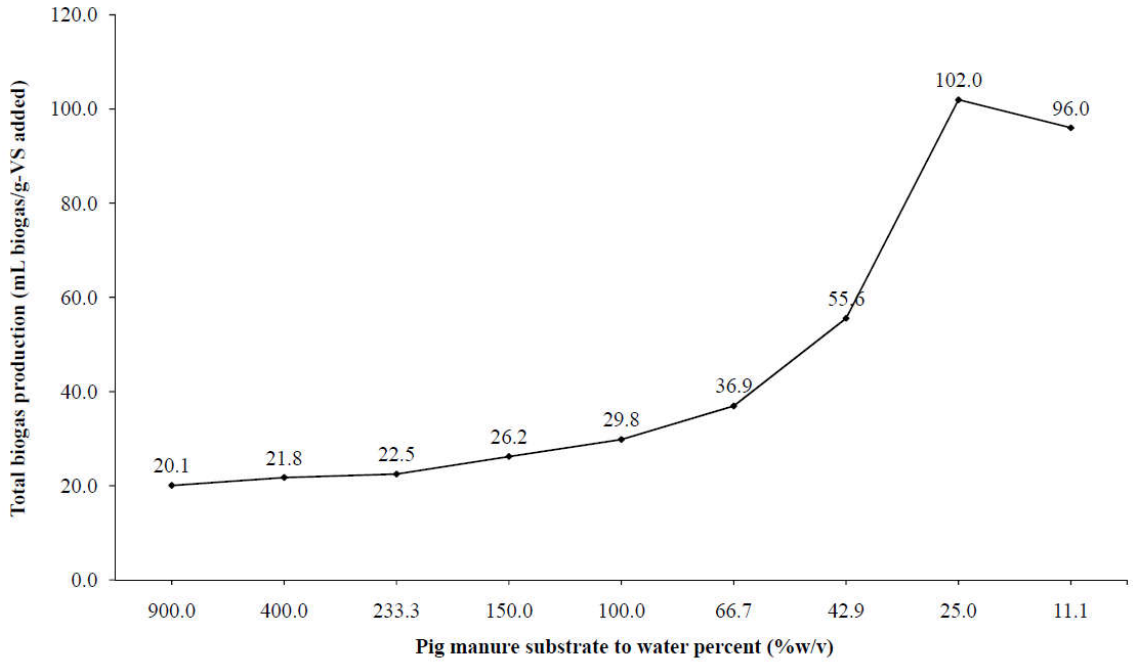


Figure 3.14: Total biogas productions (mL biogas/g-VS added) from the various pig manure substrate to water percents (%m/v) on a 25 days' RT at the optimum mesophilic temperature of 40 °C

The values on the x-axis in Figure 16 represent pig manure substrate to water percent each mass to volume percent (%m/v) being equivalent to the following mass to volume ratios (m/v): 900.0% = 22.5:2.5, 400.0% = 20:5, 233.3% = 17.5:7.5, 150.0% = 15:10, 100.0% = 12.5:12.5, 66.7% = 10:15, 42.9% = 7.5:17.5, 25.0% = 5:20 and 11.1% = 2.5:22.5.

Figure 3.15 presents the specific daily biogas production trend from the optimum pig manure substrate to water dilution ratio of 5:20 also equal to 25.0%. Biogas production began on the first day and rapidly rose to the second day of the retention period. This may be attributed to the combined influence of the added inoculum, the presence of native microflora in the pig dung, a proper nutrient balance as well as the optimum

conditions of temperature (40 °C) and a stable pH (≈ 7) able to buffer its self and, in addition, the optimum dilution ratio of pig manure substrate to water of 5:20 (Kasisira & Muyiiya, 2009). A drop in biogas production was observed between the second and the twelfth day of the experiment with a few increases and drops afterwards between the twelfth and the eighteenth day of retention. The observed decline in biogas production might have been due to either a lag phase of microbial growth or the methanogens were undergoing a metamorphic growth process by consuming methane precursors produced from the initial activity. Besides, this may also be attributed to the fact that acid forming bacteria produce VFAs resulting in declining pH and diminishing growth of methanogenic bacteria and methanogens (Aremu & Agarry, 2012; Chinwendu *et al.,* 2013; Ogunwande *et al.,* 2013). Biogas production significantly increased from the eighteenth day through to the twenty-fourth day of the retention period; indicating that the applied optimum conditions of pH, temperature and dilution ratio brought about the exponential growth of methanogens. Biogas production reached its peak on the twenty-fourth day of the retention period; suggesting that methanogenesis had dominated the AD process due to exponential growth and microbial activity of acetogenic methanogenic bacteria (Chinwendu *et al.,* 2013). After the twenty-fourth day of observation onward to the twenty-fifth day, no appreciable production of biogas occurred but at a reducing rate; conceivably due to the stationary phase of microbial growth; suggesting completion of the digestion process or process breakdown possibly as a result of methane inhibitors in the manure substrate (Ogunwande *et al.,* 2013). Biogas production rate in batch systems directly corresponds to the specific growth rate of methanogenic bacteria and, therefore, increases until it reaches peak production then, slows down and declines up until the last day of observation (Chinwendu *et al.,* 2013; Ogunwande *et al.,* 2013).

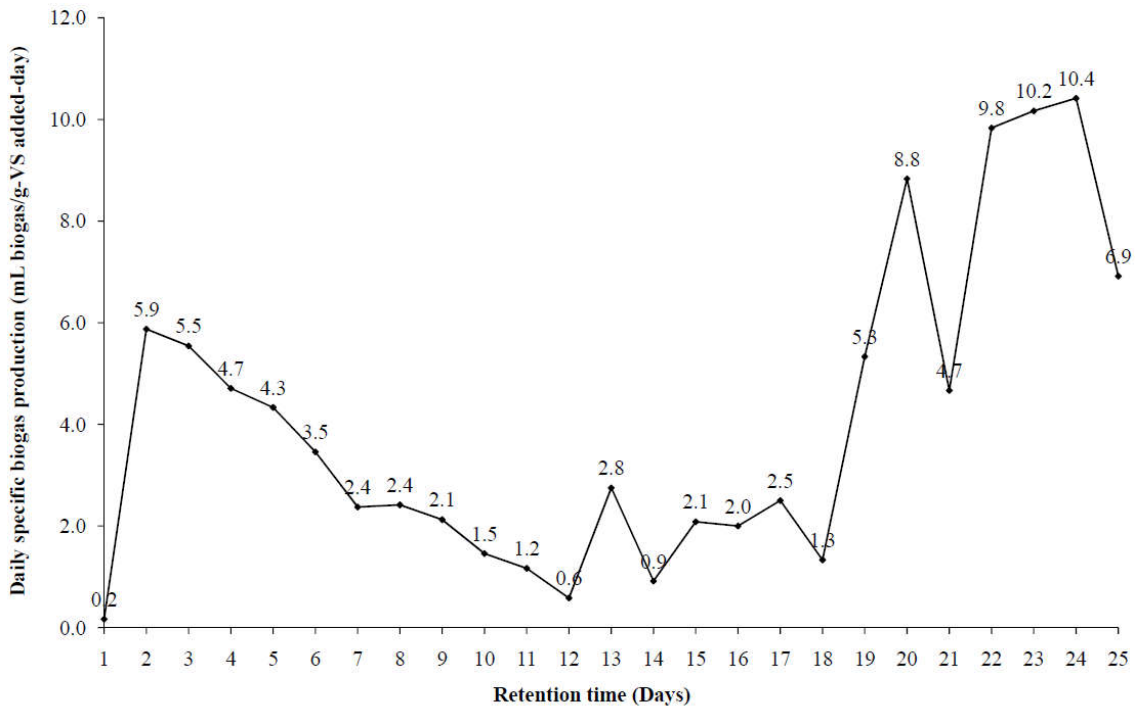


Figure 3.15: Specific biogas production (mL biogas/g-VS added-day) from the optimum pig manure substrate to water percent (%m/v) of 25.0% on a 25 days' RT at the optimum mesophilic temperature of 40 °C

Figure 3.16 presents the trends of specific daily biogas production with time from pig manure substrate to water dilution ratios. The first to the fifth least dilute ratios of 22.5:2.5, 20:5, 17.5:7.5, 15:10 and 12.5:12.5 had nearly similar biogas production patterns. They were among the least diluted ratios that had least biogas yields. The trends of biogas yields were pretty much the same for the five dilution ratios all having produced the least specific daily biogas yields in subsequent days up until the final day of observation. The observed least biogas yields from these anaerobic digesters can be ascribed to the slow hydrolyzing reactions due to a lag phase of microbial growth during these periods of the run. There could also be delayed growth of methane forming bacteria which depends on the level of solids in the slurries and in this case the water content in the ratios was relatively small (Ntengwe *et al.,* 2010). Still, the low biogas yields can also be attributed mainly to the sub-optimum manure substrate to water

dilution ratios. The lack of adequate dilution of the manure substrates means, therefore, that the biomass is not soaked enough to go through the degradation process efficiently and, consequently, less biogas is produced (Chinwendu *et al.,* 2013).

The trends of biogas yields were pretty much the same for 10:15, 7.5:17.5, 5:20 and 2.5:22.5. Dilution ratios of 10:15 and 7.5:17.5 began biogas production on the first day and reached peak biogas productions on the second day. Both declined in specific daily biogas yields from the second day onward until the nineteenth day of observation, where they recovered, and gradual increases in biogas productions observed up until the twenty-fourth day. Decline in biogases was after that observed. Dilution ratios of 5:20 and 2.5:22.5 had almost similar trends of biogas production. Biogas production began on the first day for the two dilution ratios but abruptly declined for the dilution ratio of 5:20 on the second day presumably due to the pH fluctuations in the anaerobic digester. Both ratios had increases and decreases in biogases in subsequent days possibly also due to pH changes. The dilution ratios of 5:20 and 2.5:22.5 reached peak production on the twenty-fourth and the twenty-second day of observation, respectively. The increase in biogas production could be attributed to the exponential growth of methanogens. None of the anaerobic digesters choked throughout the entire retention period of twenty-five days. This is attributable to the optimum pH observed in the biogas digesters (Table 3.7), implying high buffering capacity of the manure substrate and hence reduced pH fluctuations (Fulford, 1988; Pereira *et al.,* 2010). Various anaerobic bacteria work optimally in a pH range of 6.8 to 8.5 (Ntengwe *et al.,* 2010; Adelekan, 2012).

Table 3.7: Pig manure substrate digestate pH values for all the dilution ratios

	Pig manure substrate to water dilution ratio (m/v)								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
pH	6.93 ±0.11	7.47 ±0.09	6.96 ±0.07	6.96 ±0.09	7.07 ±0.14	7.03 ±0.01	7.28 ±0.04	7.13 ±0.11	7.17 ±0.03

Values are mean ± standard deviation, n = 3

Key: The letters A-I represent the corresponding dilution ratios of pig manure substrate to water, where A = 22.5:2.5, B = 20:5, C = 17.5:7.5, D = 15:10, E = 12.5:12.5, F = 10:15, G = 7.5:17.5, H = 5:20 and I = 2.5:22.5.

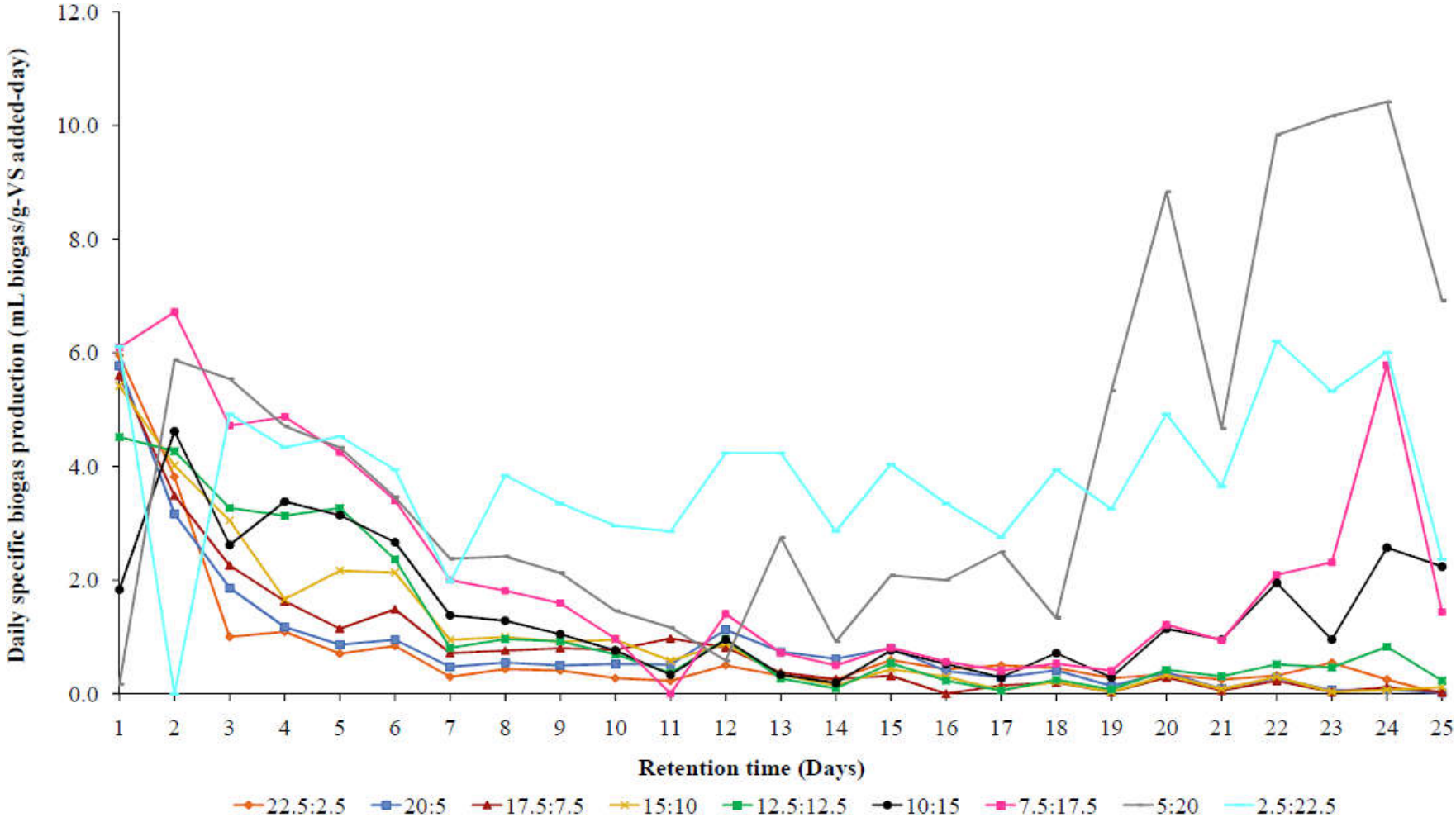


Figure 3.16: Specific biogas productions (mL biogas/g-VS added-day) from the various pig manure substrate to water dilution ratios (m/v) on a 25 days' RT at the optimum mesophilic temperature of 40 °C

The cumulative change in the biogas produced per total VS added was plotted versus the cumulative change in time elapsed. Figure 3.17 shows the biogas production trends for the dilution ratios. The least dilute ratios of 22.5:2.5, 20:5, 17.5:7.5, 15:10 and 12.5:12.5 had almost similar cumulative biogas production trends that were synonymous with a batch growth curve, Figure 3.17. The most dilute ratios of 10:15, 7.5:17.5, 5:20 and 2.5:22.5 had similar looking trends of biogas production obeying the sigmoidal function. Biogas production rate follows the sigmoid function (S-curve) as it occurs in a batch growth curve, where the rate of biogas production directly corresponds to the specific growth rate of methanogens (Beuvink & Kogut, 1993; Chinwendu *et al.,* 2013). According to Beuvink and Kogut (1993), cumulative biogas production in a batch system follows a sigmoidal curve with three distinguishable phases: a lag phase with slow or no biogas production, an exponential phase with quick biogas production, and an asymptotic phase in which biogas production slows down and subsequently reaches zero.

The dilution ratio 5:20 pig manure substrate to water began with a low cumulative biogas yield to a point of even being overtaken in cumulative biogas yield by the most dilute ratio, that is, 2.5:22.5 on the seventh day of observation, Figure 3.17. However, on the second day of observation onwards, it cuts through all the other dilution ratios to emerge as the highest in cumulative biogas production with a yield of 102.0 mL biogas/g-VS added. From Figures 3.14 and 3.17, biogas production trends predict that the most dilute the sample ratios are, the more biogas is produced. The least dilute ratio of 22.5:2.5 had the least biogas yield while one of the most dilute ratios; 5:20 had the highest biogas yield.

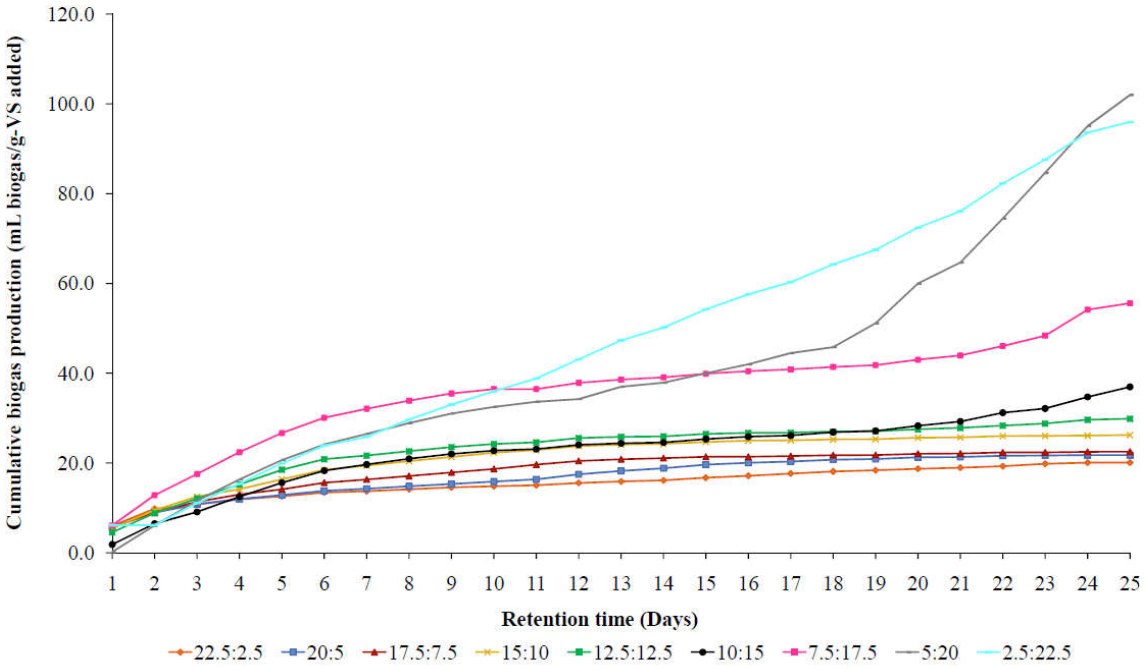


Figure 3.17: Cumulative biogas productions (mL biogas/g-VS added) from the various pig manure substrate to water dilution ratios (m/v) on a 25 days' RT at the optimum mesophilic temperature of 40 °C

3.2.4 Supplementing Pig Manure Substrate with Glycerol Supplement while Applying the Established Optimum Temperature and Dilution Ratio

Glycerol was used to supplement the three sets of biogas digesters while applying the established optimum mesophilic temperature of 40 °C and pig manure substrate dilution ratio (m/v) of 5:20 also equivalent to 25.0% (%m/v). Tables 3.4 and 3.8 present the physico-chemical characteristics and portions, respectively, of glycerol used in supplementing the optimum dilution ratio, which is split into three sets of ratios, namely 5:20:4:0.03, 5:20:4:0.05 and 5:20:4:0.08.

Table 3.8: Optimum dilution ratio and the added amounts of glycerol

Pig manure substrate (g)	Water (mL)	Inoculum (g)	Mass (g) of glycerol	Percentage of glycerol
5.0	20.0	4.0	0.03	0.5%
5.0	20.0	4.0	0.05	1.0%
5.0	20.0	4.0	0.08	1.5%

Fresh pig manure substrate had %VS of 74.05 ± 0.08 percent. The %VS of pig dung is about 80 percent of the TS (Fulford, 1988). The digestate had %VS of about 66-69%, meaning, therefore, that there was a reduction of the initial VS of the fresh pig manure substrate. This suggests, therefore, that a portion of the %VS (≈ 14%) was converted to biogas. The primary %TC for the fresh pig manure substrate was 41.14 ± 0.1 percent. The total carbon content of pig dung is about 53% (Fulford, 1988). The digestate had %TC of around 36-38%. This also means, therefore, that there was a reduction of the initial %TC of the fresh manure substrate; implying that a portion of the %TC (≈ 5%) was converted to biogas. However, some of the carbon is bound up in indigestible lignin, meaning that the carbon is partially removed from the digested material (Fulford, 1988; Kirchmann & Witter, 1992; Moller *et al.,* 2008). The pH values of the digestate fell near the desired range of 6.8 to 8.5 for optimum biogas production.

Table 3.9: Physico-chemical characteristics of the digestate bearing the glycerol supplement

Ratio	TS (%)	MC (%)	VS (%)	TC (%)	pH
5:20:4:0.03	3.98	96.00	69.13	38.40	6.55
	±0.11	±0.08	±0.13	±0.05	±0.06
5:20:4:0.05	4.72	95.67	66.10	36.72	6.97
	±0.08	±0.08	±0.09	±0.02	±0.10
5:20:4:0.08	3.97	96.01	67.88	37.71	6.63
	±0.09	±0.01	±0.14	±0.05	±0.07

Values are mean ± standard deviation, n = 3

Figure 3.18 presents the specific daily biogas productions post glycerol supplementation. The three sets of biogas digesters were supplemented with glycerol at varying amounts, Table 3.8. Steady biogas productions were observed throughout the monitoring period in all the three sets of anaerobic digesters. Biogas production trends were practically the same for ratios 5:20:4:0.05 and 5:20:4:0.08 where from the beginning onwards to the third and fourth days of observation for the two ratios, respectively, biogas productions increased steeply, Figure 3.18. The quick response in gas production was likely due to the fact that the added readily biodegradable soluble COD in the glycerol was converted to biogas within the three and four observation days for 5:20:4:0.05 and 5:20:4:0.08 ratios, respectively, as similarly observed by Wohlgemut *et al.* (2011). From Figure 3.18, on the third and the fourth day of observation onward, ratios 5:20:4:0.05 and 5:20:4:0.08 showed a decline in biogas production through to the twelfth and the fifteenth day of observation which can be credited to the fact that normal hydrolysis was taking place and perhaps consequently lead to a stationary phase of microbial growth (Ntengwe *et al.,* 2010). On the other hand, a sharp increase in biogas production in seen on the twelfth and the fifteenth day of observation for ratios 5:20:4:0.08 and 5:20:4:0.05, respectively, onward to the twenty-first day of observation where an optimum biogas production was observed followed by a drop in biogas production for the two dilution

ratios. This increase in gas production is doubtlessly due to the fact that on the microorganisms exhausting the readily biodegradable soluble COD in the glycerol they embarked on degrading and converting to biogas the actual substrate in this case pig manure that had already undergone hydrolysis (Wohlgemut *et al.,* 2011).

The other ratio of 5:20:4:0.03 had a passably good start of biogas production to the second day where a decrease was seen onward up until the fourth day of observation. The increase can be put down to the fact that the relatively small amount of added glycerol was hastily exhausted by anaerobes (Wohlgemut *et al.,* 2011), which was then followed by a decrease in biogas production most likely because of the start of the hydrolysis process. After the fourth day, a gradual increase is seen all the way to the tenth day of observation where a decrease in biogas production is observed through to the thirteenth day of observation. A sharp increase in biogas production is observed from the thirteenth day onwards where an optimum biogas production is attained on the twenty-second day of observation followed by a decline in biogas production. Comparing the control; 5:20:4 (Figure 3.18); with the rest of the ratios, it is obvious that the control had a fluctuating biogas production trend and managed to attain its optimum biogas production on the twenty-second day of observation. It could be that the addition of glycerol stabilizes biogas production and that the sharp fluctuations are lowered or done away with as is particularly evident from the graphs in Figure 3.18.

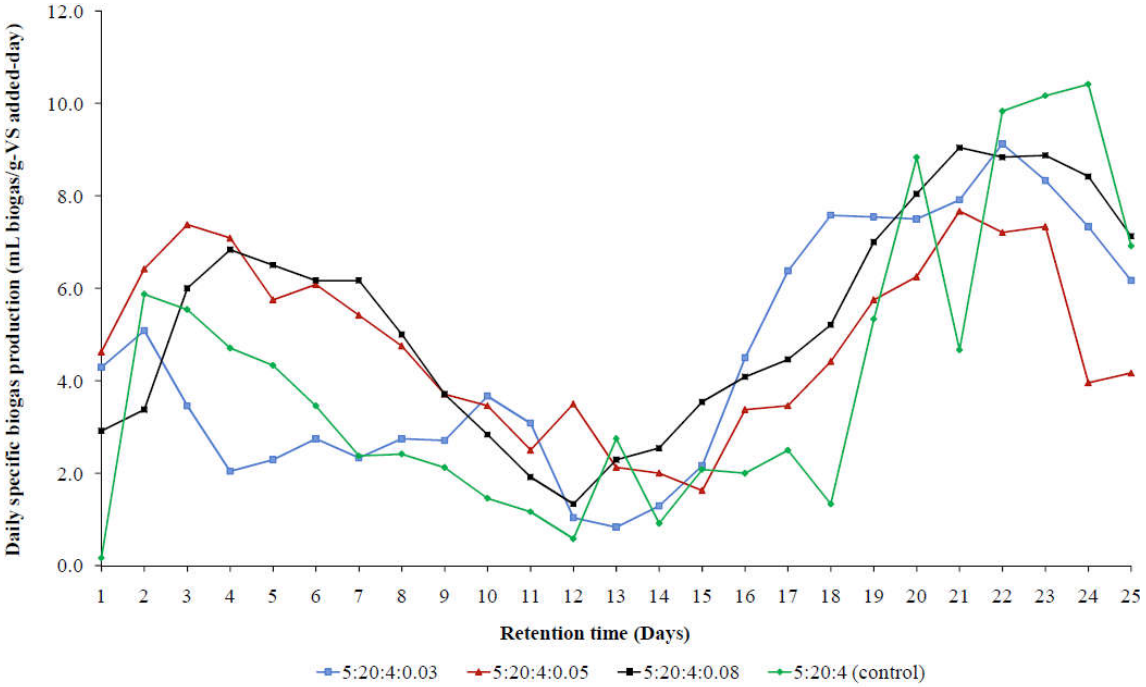


Figure 3.18: Specific daily biogas productions (mL biogas/g-VS added-day) from the optimum dilution ratio of 5:20 (g/mL) supplemented with varying amounts of glycerol at the optimum mesophilic temperature of 40 °C for 25 days' RT

Figure 3.19 shows the collected total biogas volumes from the three anaerobic digester sets supplemented with glycerol. The biogas digester set 5:20:4:0.08 supplemented with 1.5% glycerol generated the highest total biogas yield of 132.2 mL biogas/g-VS added. The least total biogas yield was collected from 5:20:4:0.03 supplemented with 0.5% glycerol with a total biogas yield of 112.2 mL biogas/g-VS added. The anaerobic digester sets in order of the total biogas collected: 5:20:4:0.08 > 5:20:4:0.05 > 5:20:4:0.03 > 5:20:4 (control). The response in biogas production in the anaerobic digester set 5:20:4:0.08 supplemented with 1.5% glycerol was most likely as a result of the breakdown of the readily biodegradable soluble COD in the glycerol that was relatively more in quantity compared to the other biogas digester sets 5:20:4:0.03 and 5:20:4:0.05 supplemented with 0.5% and 1% glycerol, respectively (Wohlgemut *et al.,* 2011). The results obtained, therefore, seem to indicate that biogas yields increased with

increasing glycerol amounts. There is a need, however, to maintain a stable digestion process, and therefore the amount of glycerol added has a limiting concentration level (Amon *et al.,* 2006; Holm-Nielsen *et al.,* 2007; Fountoulakis *et al.,* 2009).

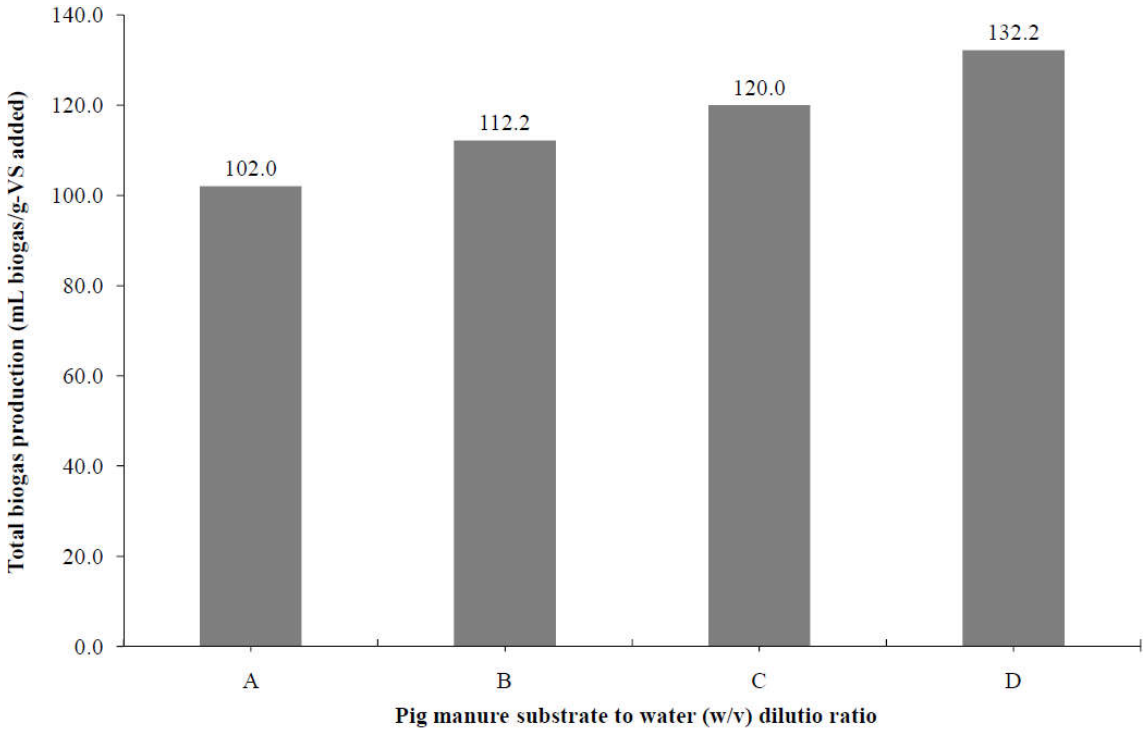


Figure 3.19: Total biogas productions (mL biogas/g-VS added) from the optimum dilution ratio of 5:20 (g/mL) supplemented with varying amounts of glycerol at the established optimum mesophilic temperature of 40 °C for 25 days' RT

The letters in Figure 21 represent pig manure substrate to water (m/v) to inoculum to glycerol ratios as follows: A = 5:20:4 (control; without glycerol supplement), B = 5:20:4:0.03, C = 5:20:4:0.05 and D = 5:20:4:0.08.

Figure 3.20 shows the cumulative volumes of biogas generated from the three sets of biogas digesters supplemented with glycerol. Trends of cumulative biogas production with time in the three digester sets were very similar and followed a sigmoidal curve (S-curve) as it generally occurs in batch growth curve in which biogas production rate directly corresponds to the specific growth rate of methanogens in anaerobic bacteria

(Beuvink & Kogut, 1993; Chinwendu *et al.,* 2013). In the first fifteen days of observation, biogas productions were gradual; this is probably because of the lag phase of microbial growth in addition to pH fluctuations. From the fifteenth day onward, biogases were rapidly produced and significantly increased yields were achieved, owing to the exponential growth of microorganisms caused by the optimum conditions of temperature, pH and dilution ratio. Towards the final observation day, decreases in biogas productions were noted, probably caused by the stationary phase of microbial growth; suggesting completion of the AD process or process breakdown possibly as a result of methane inhibitors in the substrate (Ogunwande *et al.,* 2013).

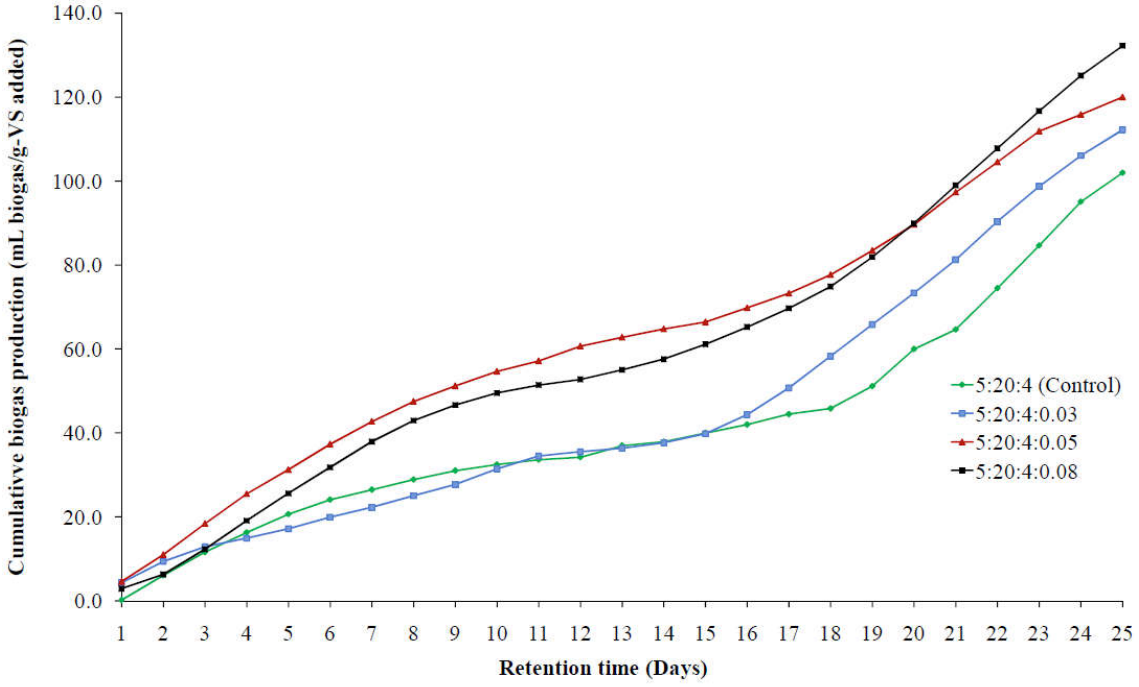


Figure 3.20: Cumulative biogas productions (mL biogas/g-VS added-day) from the optimum dilution ratio of 5:20 (g/mL) supplemented with varying amounts of glycerol at the optimum mesophilic temperature of 40 °C for 25 days' RT

Comparing the control's total biogas yield that had a yield of 102.0 mL biogas/g-VS added with the glycerol supplemented ratio, it can be seen from Figures 3.19 and 3.20 that there was a substantial increase in biogas production even for the 0.5% (5:20:4:0.03)

glycerol supplemented biogas digester which gave the least total biogas yield among the glycerol supplemented biogas digesters. From the control; 5:20:4; with a total biogas yield of 102.0 mL biogas/g-VS added (refer to Figures 3.19 and 3.20), based on the total biogas yields, the percentage increases in biogas productions were 10.00% for; 5:20:4:0.03; 0.5% (m/m) glycerol supplementing, 17.65% for; 5:20:4:0.05; 1% (m/m) glycerol supplementing and 29.61% for; 5:20:4:0.08; 1.5% (m/m) glycerol supplementing.

Based on the Buswell formula and the ideal gas law, the control's total biogas volume of 102.0 mL Biogas/g-VS added as well as a normalization factor of 1.2, the expected total biogas yields for 0.03, 0.05 and 0.08 g of added glycerol are 122.5, 136.2 and 167.6 mL Biogas/g-VS added, respectively. The expected respective percentage increases in biogas yields were 20.1, 33.5 and 53.6% for 0.03, 0.05 and 0.08 g of added glycerol. The actual total biogas yields; 112.2, 120.0 and 132 mL Biogas/g-VS added were lower (Figure 3.19) compared to the expected volumes, that is, 122.5, 136.2 and 167.6 mL Biogas/g-VS added, respectively. The actual biogas yield should always be lower than the theoretical value, because part of the substrate feed will always be used for cell growth, and some substrate will leave the biogas digester without being degraded (Poulsen, 2003). In addition, the exact biogas yield will also depend on the various environmental conditions, such as feedstock, temperature and microbial populations (Lusk, 1998).

It is clearly observable from Figures 3.19 and 3.200 that the more glycerol supplement added, the higher the biogas yield. In view of this, it is therefore of prime importance to note that glycerol addition has a limiting concentration effect. If too much glycerol is added, it reaches a point where the rate of glycerol conversion to VFAs is rapider than the rate of conversion of the organic acids to biogas. The organic acids eventually accumulate leading to a high pH values in the digester subsequently becoming toxic to methanogens. In due course, biogas production is upset. This makes it necessary to ensure that minimal concentrations are added. Fountoulakis *et al.* (2010) evaluated the

feasibility of adding crude glycerol to the anaerobic digesters treating sewage sludge in wastewater treatment plants. Results from this study showed that adding glycerol can increase biogas yields if it does not exceed 1% (v/v) concentration in the feed. They found that any further increase in glycerol caused a high imbalance in the AD process.

3.3 Conclusions and Recommendations

3.3.1 Conclusions

The primary objective of the study was to determine the effect of glycerol supplementation on biogas production while applying the established optimal mesophilic temperature, and the optimum cattle and pig manure substrates dilution ratios. Based on the results of this study, the following conclusions could be drawn:

3.3.1.1 Cattle Manure Substrate

The optimum mesophilic temperature for AD of cattle manure substrate was ascertained to be 40 °C with a maximum cumulative biogas yield of 180.6 mL biogas/g-VS added measured against other experimentation temperatures. The optimum cattle manure substrate to water dilution ratio (m/v) was determined to be 17.5 g of cattle manure substrate to 7.5 mL of water, that is, 17.5:7.5 (g/mL) with a maximum total biogas yield of 210 mL biogas/g-VS added. The percentage increases in biogas yields over the control for supplements of glycerol of 0.5%, 1% and 1.5% of the mass of cattle manure substrate charged into the batch biogas digesters were 6.363%, 12.54% and 21.89%, respectively.

3.3.1.2 Pig Manure Substrate

The optimal mesophilic temperature for pig manure substrate was established to be 40 °C with a maximum cumulative biogas yield of 30.6 mL biogas/g-VS added. The optimum pig manure substrate to water dilution ratio (m/v) was found out to be 5 g of pig manure substrate to 20 mL of water, that is, 5:20 (g/mL) with a maximum total biogas yield of 102.0 mL biogas/g-VS added. The percentage increases in biogas yields

over the control for supplements of glycerol of 0.5%, 1% and 1.5% of the mass of pig manure substrate charged into the batch biogas digesters were 10.00%, 17.65% and 29.61%, respectively.

Taken as a whole, it can therefore be concluded that, one, the upper limit of the mesophilic temperature range gives a higher biogas yield, two, too much or too little water will influence biogas yield as established by the current research and three, glycerol can be successfully applied as a supplement in anaerobic biogas digesters without any adverse impact on biogas production. In this respect, the conclusion can, therefore, be drawn that this study provides an exciting opportunity to advance our knowledge of AD of agricultural wastes with a view to making the process more economical for the farmer.

3.3.2 Recommendations

3.3.2.1 Recommendation from this Study

The application of the determined optimum mesophilic temperature, optimum substrates to water dilution ratios and glycerol supplementation percents can feasibly improve the AD of agricultural wastes subsequently making the process more economical for the farmer.

3.3.2.2 Recommendation for Further Work

- A number of possible future studies using the same experimental setup are apparent. Further investigation and experimentation under thermophilic condition are strongly recommended. It would be interesting to assess the effects of excessive glycerol supplementation.
- A comparative analysis of digester effluent; bio-fertilizer versus inorganic fertilizers is highly recommended.
- Although the experimental design (set-up) was carried out as a laboratory bench-scale test, it can be developed into a pilot-scale experiment with a view to

disseminating the technology to the community for utilization with the aim of making anaerobic biogas digesters more economical.

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