

**BIOGAS PRODUCTION FROM PRETREATED CHICKEN-GOAT AND
UNTREATED COW MANURES AT DIFFERENT INOCULUM AND
TOTAL SOLIDS LEVELS**

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for the Master of Science Degree in Agricultural Engineering of Egerton University**

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This Thesis is my original work and has not been presented in this or any other University for the award of a degree.

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DEDICATION

This thesis is dedicated to my parents Mr. Enos Waswa Mayende and Mrs. Penina Machuma Waswa, my brothers Emmanuel Musungu and Ian Kuka, and sisters Sharon Nanjala and Trizzer Nafuna. Special dedication also goes to Ms. Wendy Ofula.

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ABSTRACT

Kenya's efforts to offer food security (as envisaged in the Big Four Agenda) to its population, has significantly increased agro-waste generation, for instance chicken, goat and cow manure. A great deal of agro-based emissions come from livestock manure dropped on pasture, hence the need to mitigate the emissions in an eco-friendly way. Anaerobic digestion of manure (s) offers an effective route for energy recovery and emissions control prior to their use as bio-fertilizer, while achieving SDGs 7 and 13. Little information that exists on optimal biogas production from pretreated goat-chicken and cow manure at different substrate to inoculum ratios and total solids formed the basis of this research. Chicken and goat manure were pretreated through soaking (6, 12 and 18 hours), mechanical mincing (2 mm, 3 mm and 4 mm) and heating (at 60°C, 80°C and 100°C), co-digested with untreated cow manure at constant temperature of 35 °C and mix ratio of 1:1:1 volatile solids basis. Different substrate: inoculum levels of 2:1, 3:1, 4:1, 5:1 and 6:1, while total solid levels between (7.5% and 10.5%) at intervals of 0.5% were used to study their effects on biogas production. Optimization of biogas production was by Response Surface Methodology coupled with Central Composite Design that suggested the required mix of experiments, predictions and their associated statistics. Experiments were carried out in a 0.15 m³ laboratory scale batch digester. Mechanical pretreatment gave mean biogas production rate of 0.60 m³/m³d, followed by thermal (0.57 m³/m³d) and hydro (0.55 m³/m³d), against the control (0.54 m³/m³d). Within each treatment, the levels yielded different biogas production rates - the highest increase in production were noted for the; 6 hour soaking time (9%), 3 mm manure particle sizes (19%) and 80°C of heating (15%). Co-digestion improved mean biogas production rate over mono-digestion by 68% (chicken), 82% (goat) and 9% (cow manure). Increasing inoculums and total solids resulted in increased biogas production rates with peaks at a substrate to inoculum ratio of 4:1 (0.61 m³/m³d) and 9% total solids (0.63 m³/m³d). Optimization showed that substrate to inoculum ratios, effective minced particle size and total solids, and their interaction, significantly affected biogas production. Highest biogas production of 0.59m³/m³d was achieved at S/I of 4:1, total solids of 9% and particle size of 3 mm, with a close agreement between obtained and predicted production rates generated by the model. With these positive results, local farmers can improve uptake of chicken and goat manure as substrates for sustainable renewable energy production and reduce uncontrolled greenhouse gas emissions from open manure dumps.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

AD	Anaerobic Digestion
ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
BBD	Box–Behnken Design
EPA	Environmental Protection Agency
C _{CD}	Percentage of Carbon of cow manure in substrate, wet basis (%)
CCD	Central Composite Design
C _{CM}	Percentage of Carbon of chicken manure in substrate, wet basis (%)
C _{GM}	Percentage of Carbon of goat manure in substrate, wet basis (%)
CD	Cow manure
CH ₄	Methane
CM	Chicken Manure
C/N	Carbon to Nitrogen ratio
CO ₂	Carbon dioxide
FFD	Fractional Factorial Design
GHGs	Greenhouse Gases
GM	Goat manure
GRA	Grey Relational Analysis
H ⁺	Hydrogen ions
H ₂ O	Water vapour
H ₂ S	Hydrogen Sulphide
H ₂ SO ₄	Hydrogen Sulphate
HCl	Hydrogen Chloride
MC	Moisture Content (%)
MC _{CD}	Moisture content of cow manure in substrate, wet basis (%)
MC _{CM}	Moisture content of chicken manure in substrate, wet basis (%)
MC _{CD}	Solid mass of cow manure in substrate, wet basis (%)
MC _{GM}	Moisture content of goat manure in substrate, wet basis (%)
MC _{CM}	Solid mass of chicken manure in substrate, wet basis (%)
M _{GM}	Solid mass of goat manure in substrate, wet basis (%)
MOO	Multi Objective Optimization
N ₂ O	Nitrogen (IV) Oxide

NACOSTI	National Commission for Science, Technology and Innovation
NaOH	Sodium Hydroxide
N _{CD}	Percentage of Nitrogen of cow manure in substrate, wet basis (%)
N _{CM}	Percentage of Nitrogen of chicken manure in substrate, wet basis (%)
N _{GM}	Percentage of Nitrogen of goat manure in substrate, wet basis (%)
NH ₃	Ammonia
NH ₄ ⁺	Ammonium
KALRO	Kenya Agricultural and Livestock Research Organization
KCSAP	Kenya Climate Smart Agriculture Project
KNBS	Kenya National Bureau of Statistics
KPLC	Kenya Power and Lighting Company
LPG	Liquefied Petroleum Gas
LSD	Least Significant Difference
RSM	Response Surface Methodology
OLR	Organic Loading Rates
PBD	Plackett-Burman Design
PCA	Principal Component Analysis
PID	Partial Integrals Differential
PLC	Program Logic Controllers
SDGs	Sustainable Development Goals
SSA	Specific Surface Area
SRT	Solids Retention Time (Days)
TS	Total Solids (%)
VFAs	Volatile Fatty Acids
VS	Volatile Solids (%)

CHAPTER ONE

INTRODUCTION

1.1 Background

Countries are aspiring to significantly increase the share of renewable fuels in the energy mix because of increased negative environmental effects of fossil fuels, therefore biomass production and consumption chains are being promoted in generation of eco-friendly and sustainable fuels (Oranusi & Dahunsi, 2013). Biogas production from wastes, residues, and energy crops can play a vital role in many countries to limit emission of greenhouse gases (GHGs). The issue of fossil fuel supply is emotive as only a number of countries produce the product, whose reserves (mostly located in politically unstable regions) are depletable. Though, biomass is abundant to many nations, it's scattered and may need concentration. In Kenya, clean bio-energy offers an important way by which the country can achieve sustainable development goal seven (SDG 7) by ensuring access to affordable, reliable and sustainable energy for all.

Today, the Government of Kenya, is implementing its Big Four Agenda (Pillar 2: provision of food and nutrition security) for its population and as a result, poultry and livestock industries are growing rapidly along with their products' consumption. Consequently, animal wastes are increasing from these enterprises and they need proper management as they may lead to odours, attract pests, insects and rodents, release pathogens, and contaminate groundwater; among others (Sakar et al., 2009). Uncontrolled greenhouse gases (CH₄, CO₂ and NH₃) emitted from waste storage units pollute the air (Yetilmezsoy & Sakar, 2008) and contribute to global warming. The government is also promoting climate smart agricultural practices among farmers, aimed at enhancing sustainable agriculture, farming systems resilience to climate change and reduction of agro-based emissions. Achievement of sustainable development goal thirteen (SDG 13) entails taking action to combat climate change and its impacts, hence anaerobic digestion (AD) offers a viable means to solve the GHG emissions problem while generating clean energy for the population (Atilade et al., 2014). The country's economy is agriculture-based and hence biogas has a role to play in the environmental management of wastes to substitute the use of wood for fuel.

Pretreatment entails selection of right material composition and preparing it for effective biodegradation. Contents and concentration of substrates must be compatible with the designed digestion equipment (Zupancic & Grilc, 2012) and should be accompanied by an appropriate pretreatment method that is cost-effective, has low pollution emissions and low energy demand (Rusanowska et al., 2018).

Biomass for digestion may be subjected to physical, biological, chemical or combined-method pretreatment to help break the substrates' barriers to digestion. Biodegradation of non-pretreated lignocellulose substrates may however not exceed 20% (of fed quantity) (Kratky & Jirout, 2011). Farmers though, locally soak manure in water for several hours to enhance their digestion. Pretreatment of chicken and goat manure prior to their co-digestion with cow manure, has the potential to help open up their structures for bacterial attack to enhance the hydrolysis process.

When starting up a batch digester, a certain amount of inoculum should be added to the digester to provide the first microorganisms that improve the initial (rate) limiting hydrolysis stage for the process (Kameswari et al., 2012). Substrate to inoculum (S/I) ratio is the quantitative relation of the amount of volatile solids in the substrate per those contained in the inoculum at the start of each batch digestion process (Feng et al., 2013; Yoon et al., 2014). That is; each manure has its suitable substrate to inoculum ratio (Lesteur et al., 2010), because of the material-specific quantity of volatile fatty acids (VFAs) and its capacity to buffer against the VFAs that accumulate throughout its biodegradation process.

Information on the optimal total solids in the chicken-goat-cow manure substrate is also critical and useful in determining an efficient biogas production system from these materials. A variation in total solids content influences changes in microbial activity and therefore affects the amount of gas produced (Igoni et al., 2008; Pavan et al., 2000; Yi et al., 2014). Total solids (TS) content is the dry matter of a feed stock, manure or substrate and includes both volatile and dissolved solids.

Co-digestion, as a system component for the chicken, goat and cow manure biodegradation; can offer a suitable Carbon to Nitrogen (C/N) ratio within the range of 20:1 to 30:1 (Yangin-Gomec & Ozturk, 2013) for stability, improve biogas quality, reduce the cost biogas production and may encompass a more efficient use of bio-digesters (Macias-Corral et al., 2008). It is the anaerobic bio-degradation of a homogenous multi-substrate mixture and a major situation is where the main substrate is mixed at a larger ratio compared to the other minor substrate(s) (Braun et al., 2003). If one substrate has Carbon or Nitrogen deficiency, its co-substrate should hence provide the complementary growth factor to give a balanced C/N ratio (Mata-Álvarez et al., 2011).

1.2 Statement of the Problem

Uptake of chicken and goat manure as anaerobic digestion substrates is low due to their low C/N ratios of about 5 to 10 (Yangin-Gomec & Ozturk, 2013) and an average of 18 (Zhang

et al., 2013) respectively, low specific surface area (SSA) and high dry matter that inhibits the anaerobic process (Chen et al., 2008). Households pretreat dry chicken and goat manure by soaking in water for hours before digestion; however, incorrect duration of soaking prior to digestion may hamper biogas output. Improper total solids and substrate to inoculum ratios of chicken and goat manure has also resulted in the failure of digesters, fluctuating level of microbial activity and unavailability of nutrients for bacteria involved in biogas production (Masinde et al., 2020). Cow manure has a better C/N ratio of about 22.71 (Ardaji et al., 2016), but biodegrades slower than chicken and goat manure (Tufaner & Avsar, 2016). Non-existent information on optimal mix of levels of factors affecting biogas production has hindered the realization of biogas potentials from manure. Previous studies again offer scarce information about the effect of pre-treatment, total solids and substrate to inoculum ratios of the mixture of chicken and goat manure on biogas production when co-digested with cow manure.

1.3 Objectives

1.3.1 Broad Objective

The broad objective is to evaluate the effect of pretreatment, inoculum and total solids levels of chicken and goat manure on biogas production when co-digested with cow manure.

1.3.2 Specific Objectives

The specific objectives were to;

- i. Determine the effect of hydro, thermal and mechanical pretreatment of chicken and goat manure on biogas production when co-digested with cow manure
- ii. Determine the effect of different inoculum and total solids levels of pretreated chicken-goat and cow manure, when co-digested, on biogas production.
- iii. Optimize biogas production based on selected chicken-goat manure pretreatment method, inoculum and total solid levels when co-digested with cow manure

1.4 Research Questions

- i. How does hydro, thermal and mechanical pretreatment of chicken and goat manure affect biogas production when co-digested with cow manure?
- ii. How does varying substrate to inoculum ratios and total solids of do pretreated chicken-goat and cow manure, when co-digested, affect biogas production?
- iii. How can optimization be used to maximize biogas production from selected chicken-goat manure pretreatment method, inoculum and total solid levels when co-digested with cow manure?

1.5 Justification

Biogas is an eco-friendly renewable source of energy when efficiently harnessed compared to fossil fuels. Availability of chicken, goat and cow manure as residuals of agricultural activities presents a feasible fuel option that can be developed through anaerobic digestion.

Chicken and goat manure have lower carbon to nitrogen (C/N) ratios and higher methane potentials (Atilade et al., 2014) while cow manure has a lower methane potential, but a suitable C/N ratio. Goat manure balls also have hard outer covers that do hinder penetration by anaerobic bacteria. There is need, therefore, to pretreat them so as to increase their specific surface area for hydrolysis (Rusanowska et al., 2018) prior to their co-digestion to increase their anaerobic biodegradability and biogas production (González-Fernández et al., 2012). Mixing the three can provide the right C/N ratio range of between 20:1 to 30:1 (Dioha et al., 2014) for anaerobic digestion and knowledge about their best pretreatment method can result in optimal biogas production.

Co-digesting the three at varying total solids and inoculum levels is to help in establishing the optimal operating specifics, inform on the materials design parameters (density, water solid ratio etcetera), ensure system stability, and improve digester durability. Re-feeding spent slurry (contains washed out microbes) into the digester can also be way of improving biogas production (Sreekrishnan et al., 2004).

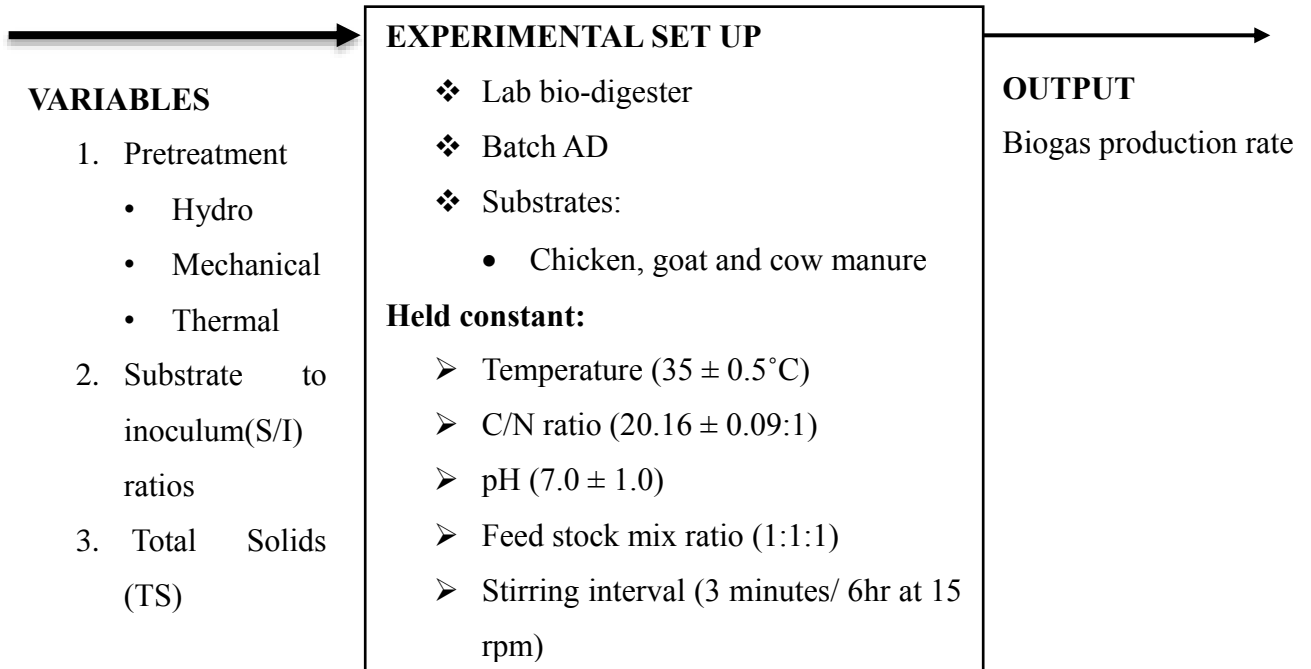
This research is also aimed at studying interaction effect of total solids and inoculum levels on biogas production of the pretreated materials. Information about optimal level combinations of the suitable pretreatment method, total solids and inoculums for substrates can hence assist AD plant designers and operators in improving biogas production from these systems. In the long run, this can help realize SDG 7 and 13 by ensuring access to affordable and sustainable energy for combating climate change.

1.6 Scope and Limitations

Pretreated chicken-goat and untreated cow manure were co-digested, with anaerobically digested cow manure from the Egerton University biogas plant being used as inoculum. Chicken manure was sourced from broiler production systems for its high dry matter content that allowed easier handling while cow and goat manure were sourced from their respective semi-intensive rearing systems; all from the university's Tatton Agricultural Park (TAP). Pretreatment (hydro, mechanical and thermal), inoculum and total solids levels were the three factors considered for this study. Other factors affecting biogas yield like temperature,

C/N ratio, pH, organic loading rates, stirring intervals and feedstock mix ratios were predetermined and monitored throughout the experiments. All experiments were carried out in a 0.15 m³ fixed dome laboratory scale batch bio-digester.

Sourcing of chicken manure occasioned by the shortage of the material at the university poultry enterprise greatly affected experimental timeliness. The university closure due the COVID 19 pandemic outbreak prevented access to the laboratory and unskilled labour.



1.7 Definition of Terms

- a. Co-digestion: Anaerobic digestion of multiple biodegradable organic substrates in anaerobic digestion system.
- b. Biogas quality A measure of the proportion of methane in biogas produced in percentage.
- c. Buffering Capacity Ability of the digester to resist change in pH.
- d. Dry digestion: Anaerobic biodegradation of organic substrates with total solids greater than 20%.
- e. Feedstock Any renewable, bio-material that can be utilised directly as fuel or converted into another form of fuel or energy product.
- f. Hydro pretreatment: Soaking of feedstock in a definite amount of water and leaving it uninterrupted for a given period of time prior to feeding it into the digester.
- g. Substrate: Biodegradable material that has been prepared ready for feeding into the digester for digestion and subsequently, production of biogas.
- h. Ultrasonication Feed stock pretreatment method that utilizes ultrasound waves to periodically compress material particles and rarefaction when propagating through the medium, causing the micro bubbles formed during this process to violently collapse due to powerfully induced hydro-mechanical shear forces.
- i. Wet digestion Anaerobic digestion of a substrate that has been prepared by adding of water, normally to contain a total solids content of less than 20%.

CHAPTER TWO

LITERATURE REVIEW

2.1 Chicken, Goat and Cattle Manure Production and Characteristics in Kenya

2.1.1 Production of Animal manure in Kenya

Livestock farming, in the country, is practiced in medium to high rainfall, arid and semi-arid areas hence uncontrolled methane gas emission from their wastes can be a national issue. The rise in demand for food has led to intensification of livestock production systems, whose wastes should be monitored and managed to prevent environmental pollution (Nabarro & Wannous, 2014). Livestock's more than 18% contribution to the global greenhouse gases is more than what the combination of all fossil fuel based modes of transportation (cars, planes, trains, among others) contribute (Steinfeld et al., 2006). In 2013, Kenya emitted 60.2 Metric tonnes of CO₂, representing 0.13% of the global GHG emissions; agriculture was the country's main source of greenhouse gas emissions with 62.8% of the country's total emissions (WRI CAIT 2.0, 2014) where 55% of the agro-based emissions came from livestock and 36.9% was uncontrolled GHG emissions from manure dropped on pasture. Methane's warming effect is 23 times stronger than carbon dioxide (Nabarro & Wannous, 2014) and requires to be controlled before reaching the ozone layer.

Kenya has approximately 43.8 million chicken, 26.7 million goats and 18 million cattle (KNBS, 2019) with each animal/bird producing 0.08 kg, 1 kg and 4.5 kg of manure, respectively, on average per day (Jorgensen, 2009). There are differences in physical and chemical structures of manure from poultry, arising from layer (egg) and broiler (meat) farming production systems. Cattle and goat rearing, on the other hand, is practiced using intensive, semi-intensive and extensive beef and dairy production systems.

2.1.2 Manure Characteristics

Suitability of different animal manure for anaerobic biodegradation also depends on their physical and chemical characteristics. This can largely attributed be to the animal's digestive system length and type, feed composition and feeding habits. Table 2.1 shows the various physical and chemical characteristics of chicken, goat and cow manure.

Table 2.1: Manure characteristics

Manure	TS (%)		VS (%)		C/N ratio		Density (Kg/m ³)	Source
	Range	Average	Range	Average	Range	Average		
Chicken	81.63- 90.15	85.89	47.50- 84.46	65.98	3.80- 14.44	6.06	827.59	Budiyono et al. (2014), Kafle and Chen (2016), Maramba et al. (1978) and Parnes (2013)
Cow	9.40- 22.75	16.08	10.25- 26.64	18.45	10.50- 26.64	18.57	990.73	Kafle and Chen (2016), Li et al. (2015), Maramba et al. (1978), Parnes (2013) and Pham et al. (2013)
Goat	17.40 – 37.03	27.22	16.30- 76.49	46.40	16.00- 20.00	18.00	878.01	Andrade et al. (2016), Lawal et al. (2016) and Parnes (2013)

Chicken manure is a highly potent organic substrate for biogas production. However, its higher nitrogen content results in ammonia inhibition that makes it problematic for anaerobic digestion (Bujoczek et al., 2000; Gangagni et al., 2008) while its high total solids content leads to system failure due to blockages (Fen et al., 2017). According to Hamilton (2014), the use of antibiotics for therapeutic and sub-therapeutic purposes on confined animals resulted in their subsequent droppings and residues having inhibitory effects on the anaerobic digestion. Zhang et al. (2014) observed that free ammonia inhibited methanogenic activity; for instance, acetoclastic methanogenesis was significantly inhibited, whereas hydro-genotrophic methanogenesis was resistant to ammonium stress. Chicken manure is hard/ dry and requires pulverization/ mixing with water before digester loading (Kossmann et al., 1999). Niu et al. (2013) states that chicken manure is often diluted with water to decrease its total solids and in turn, mitigate inhibition of ammonia. However, this leads to increased water use, a decline in biogas output per unit digester volume and increased slurry conveyance costs. Co-digestion of chicken manure with other livestock wastes like straw, cow and sheep manure can help stabilize ammonia imbalance (Abouelenien et al., 2009). Wang et al. (2012) co-digested chicken manure with cattle dung and realized maximum methane potential at chicken manure: cow manure mix ratio of 3:2 and C/N of 27.2:1. Ammonia can be stripped off chicken manure, by evaporating it into gas phase, to enable the use of chicken manure as a single substrate (Nie et al., 2015); however, this comes with an added cost of production.

Cow manure gives about 63% of methane (Yohannes, 2010) and can also provide required methanogenic bacteria. It however has a lower specific gas production, compared to most substrates, because of this pre-fermentation in the rumen. Single substrate digestion of cow manure, however yields lower methane compared to other animal manures because of its excess lignocellulosic complexes that lower its biodegradability to an average of about 47% (Rico et al., 2007; Monteiro et al., 2011). Also, lignin content and efficiency of hydrolysis are inversely related (Triolo et al., 2013). Abubakar and Ismail (2012) stated that cow manure is an effective feedstock for anaerobic digestion hence favourable for co-digestion. It ensures digester stability and offers nutrients such as metals, vitamins and other compounds that are necessary for microbial growth. It neutralizes pH and also has high water content which helps dilute the concentrated organic wastes (Gashaw et al., 2014).

Goat manure (GM) is a good raw material for anaerobic digestion because it is insensitive to acidification (Kanwar & Kalia, 1993). However, its hard outer coating inhibits its attack by microorganisms, delays or completely makes hydrolysis impossible, and hence,

discourages its uptake as a substrate by local biogas plant operators. Disintegration of goat manure balls is therefore necessary prior to their feeding into digesters so as to hasten the initial hydrolysis process and expose its nutrients to microbial attack for improved biogas production. GM has an average C/N ratio of 18 (Andrade et al., 2016; Lawal et al., 2016), that potentially increases its nitrogen content and proves unsuitable for AD; hence co-digesting it with other animal wastes with higher C/N ratios like cow manure can potentially achieve digester stability.

2.1.3 Biogas Potential from Chicken, Goat and Cattle Manure

Different animal wastes for use in biogas generation have varied specific biogas yields and methane content in the biogas produced. According to Maithel (2009) chicken and cow manure digested at total solids of 10% at a temperature of 35°C have methane content potentials of 69% and 58% after retention periods of 15 and 30 days respectively. Goat manure has a methane content of 65% (Jorgensen, 2009). Table 2.2 shows biogas/ methane production potentials of the three feed stocks.

Table 2.2: Biogas Potentials of each manure type

Manure	Specific biogas yield (m ³ / kg of VS)		Methane content (%)	Source
	Range	Average		
Cow	0.17-0.27	0.22	58	Kafle and Chen (2016), Maithel (2009); Pham et al. (2013) and Sakar et al. (2009)
Chicken	0.19-0.38	0.28	69	Budiyono et al. (2014), Kafle and Chen (2016) and Maithel (2009)
Goat	0.09-0.31	0.20	65	Andrade et al. (2016), Jorgensen (2009) Lawal et al. (2016) and Orrico et al. (2007)

Anaerobic digestion is good option for energy recovery from goat, chicken and cow manure, and their subsequent treatment before application to the soil.

2.2 Digester Types and Substrate Feeding Regimes

2.2.1 Digester Types

Digesters provide anaerobic conditions for biogas generation from biomass. The digester design and size vary depending on specific geographical conditions, substrate type, quantity available and availability of construction materials (Rajendran et al., 2012). The main digester designs used in developing countries are; fixed dome, floating dome and plug digesters.

Fixed dome digesters are non-portable two tank systems, usually built underground to protect them from temperature fluctuations and to save space (Vogeli et al., 2014). Digester feeding is through an inlet pipe that reaches the bottom level of the digester chamber, gas produced is accumulated at the gas collection chamber just above it before piping to a separate chamber, while slurry is collected through the expansion chamber (Rajendran et al., 2012). Gas pressure is created by level differences between the slurry in the digester and that in the expansion chamber; this helps push the digestate out. This study will employ the use of a fixed dome laboratory scale batch digester.

Floating drum digesters may have a well-shaped underground digester unit with a movable inverted drum acting as a gas holder or storage tank (Regattieri et al., 2018). They help produce gas at constant pressure and variable volume whereby the movable drum moves up and down depending on the amount of gas generated in the digester (Green & Sibisi, 2002; Rajendran et al., 2012). The drum's weight helps to pressurize gas flow through pipelines for conveyance, distribution and use. Its position above the digester also helps indicate the amount of biogas held.

Plug flow digesters are constant volume portable digesters but produce biogas at variable pressure (Green & Sibisi, 2002). They consist of a long, narrow, heated and insulated cylindrical tank whereby substrates are fed from one end while the gas and digestate are collected from the other end (Chen & Neibling, 2014); they may be partially or fully built below the ground and covered by a flexible or rigid roof. They are inclined to produce a two-phase system by facilitating the separation of acidogenesis from methanogenesis longitudinally (Rajendran et al., 2012).

2.2.2 Substrate Feeding Regimes

Batch fed digesters involve placing a portion of fresh substrate into the digester and leaving it for a given period before removing all the digestate once gas production ceases (Khalid et al., 2011). They are mostly utilized on large farms where feedstock supply is

intermittent throughout the year. Digesters for batch feeding are the simplest to construct and are often employed during dry digestion. Due to their simplicity in design and lower investment and operational costs, these systems can easily be adopted in developing countries like Kenya.

Continuous feeding regimes involve substrate being fed at regular intervals as an equivalent volume of digestate exits, usually where organic matter is available daily. Material movement through the system is either mechanically or pressure aided, where pressure is generated by feeding new substrate into the digester to push the digestate out (Al Seadi et al., 2008). Continuous digesters achieve predictable gas production without interruption from new influent loading and digestate unloading. This research employed the use of a batch feeding regime.

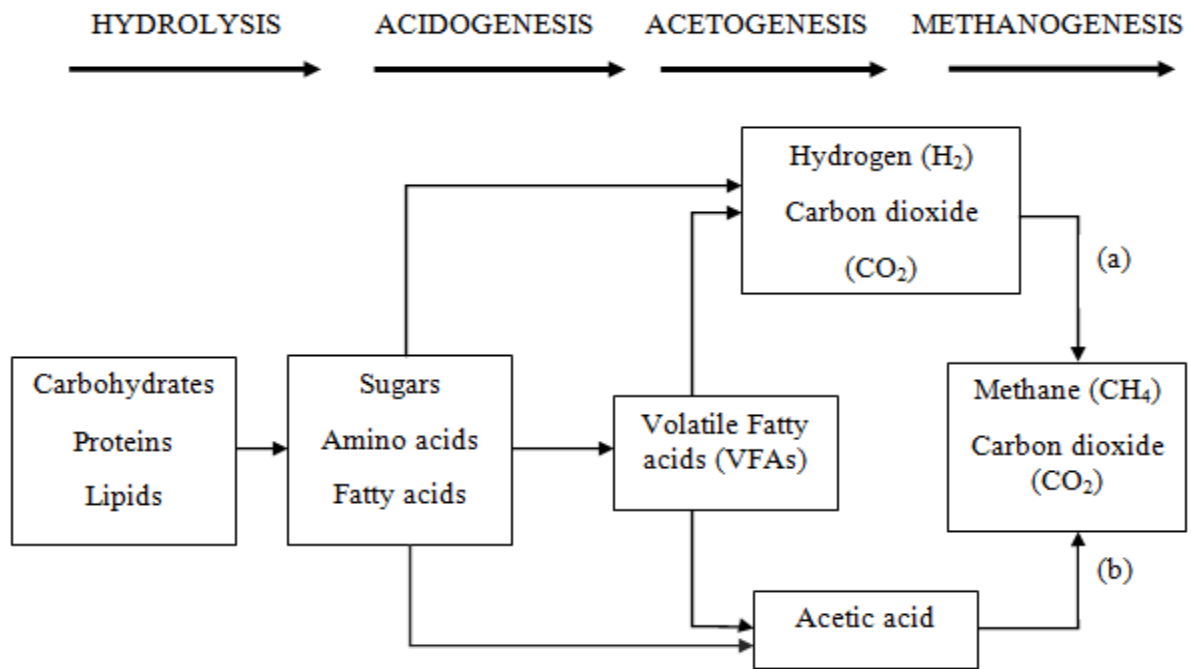
2.3 Anaerobic Digestion

Anaerobic digestion process is the decaying of biodegradable materials with the help of a diverse population of bacteria in an environment with low or no oxygen supply to release biogas plus liquid and solid effluents (Chen & Neibling, 2014). Table 2.1 shows biogas typical composition from anaerobic digestion while Fig 2.1 shows the anaerobic digestion pathway.

Table 2.3: Typical Composition of Biogas

Compound	Symbol	Mass Percentage (%)	Average (%)
Methane	CH ₄	50–70	60
Carbon Dioxide	CO ₂	30–40	35
Hydrogen	H ₂	5–10	7.5
Nitrogen	N ₂	1–2	1.5
Water Vapour	H ₂ O	0.3	0.3
Hydrogen Sulphide	H ₂ S	Traces	<1

Source: Adapted from Karki (2009)



Key: (a) - Hydrogenotrophic methanogenesis (b) - Aceticlastic methanogenesis

Fig 2.1: Anaerobic Digestion pathway (Adapted from; Wilson, 2014)

Anaerobic digestion to produce biogas and digestate (semi-solid matter) takes place by natural bacterial action in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis, as described below;

Hydrolysis, the initial stage, involves hydrolytic bacteria enzymes in reconstructing polymeric materials (proteins, carbohydrates, fats and cellulose) to smaller water-soluble monomeric molecules (monosaccharides, amino acids, fatty acids and water). Majority of the molecules are still large and are further broken down into smaller molecules like acetic acid for methane production. However, some products at this stage like hydrogen and acetate may be used directly by the methanogens for gas production (Biarnes, 2013).

Acidogenesis is done by acid-forming bacteria that take in the separate molecules from hydrolysis to further the material transformation. Acidogenic bacteria consume the rest of oxygen to provide a suitable anaerobic environment for methanogenic bacteria. It is the fastest stage and results in the formation of volatile fatty acids like acetic acid, butyric acid and propionic acid (Chen & Neibling, 2014).

Acetogenesis is the stage where acid-forming bacteria make the first materials for methanogenesis from the volatile organic acids: acetic acid, carbon dioxide and hydrogen. The hydrogen gas formed in this stage is viewed as a waste product as it inhibits the acetogenic

bacterial activity; but importantly, can be utilized by Hydrogenotrophic methanogens to create methane (Al Seadi et al., 2008).

Methanogenesis stage results in the formation of methane (CH₄), carbon monoxide (CO) and water by methanogens reducing carbon dioxide (CO₂) and breaking down acetic acid into methane gas and other trace products. This stage results in 90% of CH₄ formation with 70% provided by acetic acid. It is an important and slowest biochemical reaction step of the anaerobic digestion process (Al Seadi et al., 2008).

2.4 Manure Pretreatment

Different substrates have different types and degrees of limitations to optimal performance of anaerobic digestion that can be solved by different pre-treatment mechanisms (Carlsson et al., 2012). Conversion of biodegradable material to biogas is always limited by the rate and extent of hydrolysis. Animal manure has high lignocellulose content hence pretreatment is important in increasing carbohydrate protein and fatty acids accessibility along with hydrolysis efficiency (Rusanowska et al., 2018). Pretreatment reduces manure size to increase surface area and reduce cellulose crystallinity for improved hydrolysis yield by 5-25%, to potentially enhance biogas yield and reduce retention time by about 23-59% (Kratky & Jirout 2011). Assefa et al. (2014) found that there was no significant difference in pH and organic matter content between poultry- substrates subjected to different temperature and sodium hydroxide (NaOH) pretreatments prior to digestion. The study sought to find out if selected manure pretreatment methods affect biogas production.

Several pretreatment methods have been developed; mechanical, thermal, chemical and biological. Most of these methods are high-energy dependent, for instance mechanical and thermal pretreatments. Some methods like enzymatic treatments may be unprofitable (Teghammar et al., 2012) and the methods are discussed in subsequent sections.

2.4.1 Hydro Pretreatment

Hydro pretreatment increases the specific surface area to volume ratio of manure prior to digestion by soaking in water for a given period of time for loosening their tough outer coatings. Bolaji et al. (2017) found that soaking maize in water for a period up to 36 hours increased the specific surface area and sphericity of the grains. The practice of soaking hard covered feedstocks like sheep, goat and chicken manure by farmers in Kenya (Smith et al., 2013) has been common in most rural areas although proper process details and procedures of

approach to this pretreatment are not documented (little/ non-existent). This research thus sought to provide information on hydro pretreatment effects of manure on biogas production.

2.4.2 Mechanical Pretreatment

Mechanical pretreatment involves grinding, mincing, and milling or extrusion of manure into easily fermentable components through reduction in resistance to flow and making mixing within the digester easier. Knives and mills are used to break open the cellular structure to increase the specific surface area for bacterial attack, especially for lignocellulosic substrates (Montgomery & Bochmann, 2014). Shearing and compressive forces acting on biomass reduces its crystallinity, particle size and, increases specific surface area and bulk density (Kratky & Jirout, 2011).

Rusanowska et al. (2018) found that mechanical pretreatment of lignocellulosic biomass respectively increased biogas production and degradation by about 22% and 6%. On particle sizes, Schell and Harwood (1994) recommended 1mm to 2mm as the effective size for hydrolysis of lignocellulosic materials. Izumi et al. (2010) found that biogas production from food waste at sizes less than 0.7mm in mesophilic conditions increased by 28%. According to Taherzadeh and Karimi (2008), extruders and colloid mills are more suited to reducing sizes of materials with over 15-20% moisture content (wet basis) while hammer and knife mills are suited for dry biomass at 10-15% (wet basis) moisture content. Effectiveness and uniformity of grinding of a given feed stock depends on its moisture content.

This study used metallic meat mincers for the mechanical pretreatment of the manure due to their availability, low cost of acquisition and high moisture content of goat manure that was one of the feed stocks.

2.4.3 Thermal pretreatment

Thermal pretreatment involves subjecting a given material to heat at given temperatures over a period of time to help achieve thermo-chemical disintegration of cell membranes and tough bonds holding material components together in order to increase solubilization of organic compounds and their biodegradation (Ariunbaatar et al., 2014). Dry manure would though require hydration prior to pretreatment. Water and heat helps break down the hydrogen bonds of the cellulosic and lignocellulosic complexes, hence increasing the manure specific surface area (Garrote et al., 2007). It also achieves pathogen removal, increases flow of the digestate and the subsequent handling (Carlsson et al., 2012; Val Del Rio et al., 2011).

Biogas production increases with temperature of pretreatment up to a certain optimum above which production decreases (Bochmann et al., 2010; Zhang et al., 2011) because of certain xylose formation and lignin breakdown that become toxic to anaerobic bacteria (Montgomery and Bochmann 2014). Ariunbaatar et al. (2014) noted that low temperature-thermal pretreatment (<110°C) and subsequent anaerobic digestion of feed stocks achieved a more cost-effective performance compared to other methods. Assefa et al. (2014) found that thermally pre-treating poultry litter-cow manure substrate at 80°C respectively increased gas production and volatile solids removal by 46.3% and 26.1%. Climent et al. (2007) recorded a 50% increase in biogas production from organic solid waste subjected to 70°C thermal pretreatment prior to digestion at thermophilic conditions. On a laboratory scale, thermal pretreatment is done using microwave heaters, pressure cookers and autoclaves (Montgomery & Bochmann, 2014). This study used pressure cookers for the thermal pretreatment, as they are cheap to acquire and are available.

2.4.4 Chemical and Biological Pretreatment Methods

a) Chemical pretreatment

Chemical pretreatment utilizes strong alkalis, acids and oxidants to destroy organic bonds in lignocellulosic and cellulosic manure;

Alkali pretreatment commonly uses sodium hydroxide (NaOH), although other alkalis can be used. Assefa et al. (2014) pretreated cow and chicken manure with 0.45 g, 1.35 g, and 2.25 g of NaOH and recorded respective 0.03, 21 and 56% increases in cumulative biogas yield. However, according to Montgomery and Bochmann, (2014), alkali pretreatment of substrates results in increase in system pH and in a continuous anaerobic process, salt build up and ammonium-ammonia balance that may inhibit methane formation.

Acid pretreatment often uses acids like Hydrochloric (HCl) and Sulphuric (H₂SO₄) acids, typically in combination with heat, to break down hemicellulose and disrupt ether bonds between lignin and hemicellulose. According to Monlau et al. (2012) pretreatment of sunflower stalks with Hydrochloric acid (HCl) led to a 20% increase in methane yield. The researcher fails to provide information about the acid's concentration.

Oxidative pretreatment involves the use of hydrogen peroxide or ozone that causes swelling of lignocelluloses to increase substrate SSA and cause partial lignin solubilization for improved hydrolysis. However according to Montgomery and Bochmann (2014), the high costs associated with this pretreatment method is one of the factors preventing its large scale adoption.

b) Biological pretreatment

Biological pretreatment method uses micro-organisms to aerobically or anaerobically break down manure structures to increase their surface area for hydrolysis. Anaerobic microbiological pretreatment involves separation of the first and second stages of anaerobic digestion (hydrolysis and acidogenesis) from methanogenesis, also called two stage digestion, pre-acidification or dark fermentation (Montgomery & Bochmann, 2014). Aerobic microbial pretreatment occurs naturally by use of natural mixed cultures that generate enzymes for degrading cellulose, lignin and hemicellulose hence increase in surface area of substrate for the bacteria to act on.

2.5 Factors Affecting Biogas Production

Biogas production is affected by various factors whose variation within a range, proper monitoring and control enables biogas production systems to operate efficiently and achieve maximum biogas yield (Gashaw, 2014; Sidik et al., 2013). The factors (including total solids, volatile solids, pH, co-digestion, temperature; among others) are discussed in subsections below;

2.5.1 Total Solids

The role of volatile solids (VS) and total solids (TS) on activities of anaerobic bacteria is always important in order to increase the efficiency of the anaerobic digestion process (Orhorhoro et al., 2017). Too much TS leads to clogging of the system while too much dilution decreases biogas digestion. Igoni et al. (2008) thus acknowledged that quantity of biogas produced is a power function of the total solids content and hence production increases with an increase in % TS until an optimum point. Assefa et al. (2014) found that thermal and chemical pretreatment of poultry litter- substrates, prior to digestion resulted in significant reduction in TS and VS contents after AD, and this trend increased with increases in temperature and NaOH additions respectively.

In their separate studies; Paramaguru et al. (2017) reported that anaerobic digestion of food waste at TS of 10% gave the highest biogas yield compared to 5%, 15% and 20%, while, Abbassi-Guendouz et al. (2012) found that increasing TS from 10% to 25% resulted in a decrease in the total methane production from cardboard. On the hand, Orhorhoro et al. (2017) noted that biogas production from cow manure occurred at TS of 8%, whereas effects of TS on biogas yield by AD of cow manure and fruit waste, water hyacinth, pig dung, were optimal at

around 10.16% TS. This study investigated the effects of total solids (within this optimal range of 8-10%) of the chicken-goat-cow manure mixture on biogas production.

2.5.2 Volatile solids

Volatile solids (VS) are the proportion of solids with strength in terms of the available portion of the feed stocks that is readily available as nutrients for biogas producing bacteria. Substrates from agricultural wastes like chicken and goat manure are highly lignocellulosic with high nitrogen content; hence their high VS may not necessarily translate to high biogas yield due to the presence of non-available volatile solids in form of lignin (Igoni et al., 2008; Ituen et al., 2009). Co-digestion of chicken and goat manure with cow manure will provide a balance between the lignin content and the carbon to nitrogen ratio (Nuhu et al., 2013).

2.5.3 pH Range

pH is a measure of acidity or alkalinity of the substrate and is affected by the volatile fatty acids and Carbon dioxide produced during the anaerobic process. pH range is desired at 6.8-7.2 for proper digester functioning (Chen et al., 2008) as it affects growth of microbes during digestion. Ogiehor and Ovueni (2014) confirmed in their work that influent substrate of pH 7 consistently produced higher gas yields.

2.5.4 Inoculums and Substrate to Inoculum (S/I) ratio

Inoculums are small amounts of substance that contain bacteria from pure culture and offer the first microbial communities for new culture establishment, digestion and ensure continuity of bacteria generation and growth. All digested waste contains inoculum, although, fresh undigested cow manure contains a unique set of inoculum that are generated from the animal's rumen. Dennis (2015) found that substrates with cow manure inoculum exhibited higher cumulative and specific biogas production per VS added compared to those with animal manure and water alone. The researcher further noted that the best range of inoculum addition was 12.5% to 50%. In this study, spent slurry from cow manure digestion was used.

Substrate to inoculum ratio can be defined as a quantitative relation of the amount of volatile solids originating from the substrate to the amount of volatile solids in the inoculum (Yoon et al., 2014). The volume of inoculum influences the amount of methane produced, and this production may cease if microorganisms are not enough for the biodegradation (Zhou et al., 2011). According to Liu et al. (2009), the substrate to inoculum ratio is a significant parameter that guides on the appropriate volume of inoculum that will offer the required

population of bacteria for digestion to take place. If substrate to inoculum ratio is too high it may make system toxic, otherwise, it may prevent induction of enzymes necessary for biodegradation if it was too low (Prashanth et al., 2006). Most studies focused on the effect of S/I ratios alone on biogas yield; however, this research sought to find the optimum substrate to inoculum ratio and corresponding total solids for anaerobic co-digestion of chicken, goat and cow manure.

2.5.5 Temperature

Anaerobic fermentation occurs at different temperature ranges; psychrophilic, mesophilic and thermophilic, however most activity occurs within mesophilic and thermophilic temperature ranges (Yadvika et al., 2004). Table 2.4 presents operation temperature ranges for different anaerobic bacteria.

Table 2.4: Anaerobic Temperature Ranges

Anaerobe type	Temperature range	Source
Psychrophilic	<30	Adepoju et al. (2016), Connaughton, et al. (2006), Nozhevnikova et al. (2003), Russell and Fukunaga (1990)
Mesophilic	30 – 40	Adepoju et al. (2016), Bolzonella et al. (2005), Connaughton et al. (2006), Obiukwu and Grema (2014), Sibisi and Green (2005), Yadvika et al., (2004)
Thermophilic	50 – 60	Adepoju et al.(2016), Brock (2012), Russell and Fukunaga (1990)

Methanogenic bacteria, are however temperature sensitive and operate optimally at between 33-38°C (Sibisi & Green, 2005). Mesophilic conditions are more widely used because they provide higher stability and lower operational costs compared to thermophilic conditions (Tufaner & Avsar, 2016). Digester temperature hence affects the duration of fermentation. This study was under controlled mesophilic conditions of 35°C.

2.5.6 Moisture

Moisture is essential for substrate hydrolysis and other subsequent anaerobic digestion events. Excessive moisture content can dissolve readily degradable substrates and affect the process while too little water can make it difficult for microbes to attack the material and hence delay the process. According to Khalid et al. (2011), high methane production occurs when substrate has a moisture content of 60 % to 80%.

2.5.7 Retention Time

Retention time is duration that volatile solids take to remain in an anaerobic digester for the digestion process (Ezekoye et al., 2011). Hydraulic retention time (HRT) is the average time that a given volume of input slurry stays in the digester before it gets out (Chen & Neibling, 2014), while substrates retention time (SRT) is the ratio of solids retained in the digester to solids released in the effluent and controls the rate of microbial growth (Gray, 2004). Smaller digesters result in shorter HRTs but may not offer enough duration to reach optimal levels of biogas production, destruction of pathogens, total solids and green house gas emission control (Chen & Neibling, 2014). SRT is always a characteristic of batch feeding regimes and depends on digester temperatures, waste composition and digester type. According to Gray (2004), shorter retention time leads to risks of active bacterial wash outs while longer retention times require larger digester volumes hence increased costs. Lee and Rittmann (2011) reported that the digestion process of waste activated sludge was stable for SRT greater than 5 days whereas soluble organic compounds in the effluent were observed at retention times lower than 2 days owing inadequate hydrolysis and methanogenic washout. According to Carrere et al. (2016), pre-treatment greatly reduces the retention times needed for anaerobic digestion.

2.5.8 Carbon to Nitrogen Ratio

Carbon to Nitrogen (C/N) ratio is the proportion of the mass of carbon to that of nitrogen in a substance, manure or feed stock. Dioha et al. (2014) studied the effects of carbon to nitrogen ratio on biogas production and found that microorganisms required a ratio range of 20:1 to 30:1. Co-digestion of C/N deficient manure with those that are C/N rich balances the ratio and nutrients of the resultant substrate hence preventing inhibition of digestion. Nitrogen plays an important role in amino acid synthesis and the formation of ammonia to neutralize volatile acids generated by acid forming bacteria hence offering suitable pH levels for digestion (Tufaner & Avsar, 2016). However excess amounts of nitrogen lead to ammonia (NH₃) inhibition of the process as it is highly toxic to the bacteria. Ammonia is always at equilibrium with ammonium (NH₄⁺) ion (Equation 2.1) and this state is maintained by the acidity, pH and temperature of the system.



Where;



NH_3 = Ammonia

H^+ = Hydrogen ion

Compared to ammonia, the ammonium ion is less toxic hence it is always essential to maintain this state. Cow manure has a C/N ratio of about 22.71 (Ardaji et al., 2016), hence its co-digestion with C/N deficient goat and chicken manure ensures an effective and system stable anaerobic process.

2.6 Co-digestion

Co-digestion mitigates inhibitory effects of unfavourable substrates, balances nutrients and increases organic loading with resultant higher methane yields while diversifying and synergizing the bacterial populations that carry out methanogenesis (Shah et al., 2015). Sompong et al. (2012) compared biogas production from empty fruit bunches and co-digestion with palm oil mill effluent and found that the two enhanced biodegradability with 25–32%, increased methane production and their pretreatment resulted in 98% improvement in methane production.

Co-digestion potential is confirmed by various researchers as; cow manure with energy rich substrates like pig and chicken manure increases biogas yield (Zielinski et al., 2019), chicken and cow manure enhanced biogas yield by 50% under mesophilic conditions as compared to their mono- digestion (Sebola et al., 2015) and a maximum biogas yield attained at a mix ratio of 1:1. Biogas production from chicken and cow manure (s) under co-digestion increased by 69.6% (Afazeli et al., 2014), whereas, pretreated maize silage with chicken manure in a continuous digestion system at mesophilic conditions of 37°C recorded a 24% increase in biogas yield (Bojti et al., 2017).

2.7 Optimization of Biogas Production

Optimization is a decision-making process of attaining the most favourable design or result relative to a set of predetermined variables. The most commonly used parameter optimization techniques include; Response Surface Method (RSM), Artificial Neural Network (ANN), Multi-Objective Optimization (MOO), Taguchi method, Grey Relational Analysis (GRA), among others. The techniques are discussed in subsequent sections;

2.7.1 Response Surface Methodology

Response surface methodology (RSM) is a group of statistical and mathematical techniques that can be utilized for modeling and analyzing tasks or processes where a certain response of interest (y) is affected by several variables (x_1, x_2, \dots et cetera) with the aim of optimizing the response (Olawoye, 2016). Some of the popular strategies designed for RSM application include; Central composite Design (CCD), Box-Behnken Design (BBD), Doehlert matrix, Plackett–Burman Design (PBD), and full or fractional factorial design (FFD).

Safari et al. (2018) optimized biogas yield from canola residues co-digested with cattle manure using response surface methodology (RSM) and Box - Behnken design of the experiment to study the effects of inoculum, total solids (TS), temperature and stirring time. They achieved optimum biogas production of $0.4036 \text{ m}^3/\text{kg}$ of VS at $52.49 \text{ }^\circ\text{C}$ (thermophilic conditions), 7.02% TS, 3.12 min/ day of stirring time, and 22.17% inoculum.

Jacob and Banerjee (2016) modeled and conducted optimization of process parameters affecting biogas production from the co-digestion of potato waste and aquatic waste using central composite design (CCD) under response surface methodology (RSM) and artificial neural network (ANN) coupled genetic algorithm (GA) model. Process parameters were 5% total solids at mesophilic temperatures. Methane yield of $0.4474 \text{ m}^3/\text{kg}$ VS was realized based on ANN-GA model, and this value was 6% higher compared to the CCD-RSM based approach

For this study, CCD was used owing to its advantages over other methods. It allows the researcher to know the factors' effect on response if they would have been beyond or below the chosen factor levels. Central Composite Design caters for 2^n coded factorial runs denoted with ± 1 , $2n$ axial points $(\pm a, 0, 0 \dots 0)$, $(0, \pm a, 0 \dots 0) \dots (0, 0, \pm a \dots 0)$ and n_c centre points that would take care of experimental error (Behera et al., 2018). The design takes in a minimum of 2 factors with 3 levels each and has an extra point; axial (star) point; added to the 3 level factors. The star point is normally denoted as (α) increases the levels from 3 to 5, therefore allowing for experimental design flexibility. The number of experimental runs can be computed as (Equation 2.2);

$$N = 2^n + 2 \times n + n_c \quad (2.2)$$

Where;

N = number of runs

n = number of factors

n_c = number of centre points desired by the researcher

2.7.2 Artificial Neural Networks

Artificial neural networks (ANNs) are biologically inspired computational networks based on the study of the brain and the nervous system and are used to solve many real complex problems (Park & Lek, 2016). These computations are based on multilayer perceptrons that involve a supervised procedure that consists of three layers: input, hidden, and output layers.

Kana et al. (2012) used Artificial Neural Network (ANN) coupling Genetic Algorithm (GA) to model the non-linear behaviour of the anaerobic process and optimize biogas production from mixed substrates of saw dust; cow dung, banana stem, rice bran and paper waste. When the optimal profile was evaluated, it yielded 0.0103 m³ of biogas (an 8.64% increase) in production over that predicted by the optimized substrate profile. Production from the non-optimized profile started on the 8th day, compared to that of the 3rd day from the optimized one.

Ghatak and Ghatak (2018) used ANN to model, predict and optimize specific biogas production using cattle dung as co-substrates, separately with bamboo dust, sugar-cane bagasse and saw dust in mesophilic as well as in thermophilic conditions. The effect of substrate composition and temperature were considered and optimal biogas production of 0.45 m³/kg VS was realized from the cattle dung – bagasse mixture at 53 °C after 45 days.

Limitations of ANN are; the lack of fixed guidelines for optimal ANN architecture, its “black-box model” behaviour and insufficient concepts of ecology and relations (Basheer & Hajmeer, 2000; Thuiller, 2003).

2.7.3 Multi - Objective Optimization

Multi-objective optimization (MOO) involves more than one objective function that need to be optimized simultaneously and requires finding the right mix of all the variables affecting a given process that result in desired multiple outputs. According to Lee and Kang (2018) when solving an MOO problem, the decision maker may be forced to compromise an output that provides the greatest satisfaction, for the sake of other conflicting objectives or outputs; hence their preference is critical in finding the solution.

Mahanty et al. (2014) used combined desirability function based multi-objective optimization technique where co-digestion scenarios, incorporating main and interaction effects, from different industrial sludges were obtained to predict the maximum possible methane yield of 1161.53 m³. Validation experiments resulted in biogas yields of 1130.33 m³ (2.71%) and 1045.65 m³ (9.97%) that were close to the optimized scenario. Senthilkumar et al. (2016) used multi-objective techniques; grey relational analysis (GRA) and multi-variate

principal component analysis (PCA) to optimize biogas production and volatile solid removal efficiency from food/ poultry waste and reported optimal conditions as 7.5 % TS, pH of 7, operational temperature of 50 °C and co-digestion at a 7:3 mix ratio.

Limitations of multi-objective optimization technique include high computational costs, extremely difficult or impossible encoding of the objective functions for certain applications if there are too many objectives. It is also difficult to determine the appropriate weight coefficients for use when information about the problem is insufficient, particularly for real world applications (Chiandussi, 2012).

2.7.4 Taguchi Optimization Technique

Taguchi optimization technique is used for minimizing the number of experimental runs to be performed within the acceptable limit of factors and levels (Meena et al., 2018). Special orthogonal arrays are used to study all the design factors and control product variability more so in cases where high quality is dependent on low variability.

Senthilkumar et al. (2016) used Taguchi - Grey relational analysis (GRA) technique for multi-response optimization of process parameters in biogas production from food waste and each parameter's percentage contribution was determined by analysis of variance. It involved selection of an L₁₆ orthogonal array with five parameters being varied over four levels in Taguchi experimental design. Optimum process conditions were; TS of 7.5%, pH of 7, temperature of 50 °C, a C/N ratio of 20.19 and ultra-sonication pretreatment, with pretreatment being the prominent parameter that contributed to the output responses. Sa et al. (2018) optimized biogas production from poultry litter using Taguchi grey relational analysis (GRA) and achieved 13.5% TS, pH of 7.4, temperature of 26 °C and C/N of 15.3 as optimal levels of operational parameters.

Taguchi is however unable to explain all the variability where available data has no outcomes (Sukthomya & Tannock, 2005). The need for more complicated designs also hinders its utilization in evaluating factor interaction (Raajpoot et al., 2008).

This study utilized CCD with RSM to design and analyze the experimental runs and data. It sought to determine combined effects and interactions of substrate to inoculum ratios, total solids and effective minced particle sizes of chicken and goat manure (s) on biogas production. Response Surface methodology helped explore and establish the relationship between optimal variable conditions and biogas production (Ravindran et al., 2016).

2.8 Summary of Literature Review

This research intended to determine the optimal biogas production from varied pretreatments of chicken and goat manure; combined substrate to inoculum ratios and total solids of the pretreated manure and untreated cow manure when co-digested. Positive results when obtained can improve the uptake of chicken and goat manure as substrates by local farmers for sustainable renewable energy production, reduce uncontrolled greenhouse gas emissions from open manure dumps. The materials' parameters will have an impact in pre-knowing the parameters of importance when designing a digester for a particular volume or size. However, very little information exists with regard to the intention of this study.

The three objectives were chosen to inform data collection and analysis that could culminate into findings that are to help solve problems facing biogas plant operators. Additionally, to solve energy scarcity and mitigate negative environmental impacts due to over-reliance on non-renewable energy sources. The response surface methodology and central composite design for optimization were preferred over other techniques as they allowed evaluation of effects of multiple independent variables and their interaction on biogas production. They also save time and costs by minimizing the number of experimental runs

CHAPTER THREE

MATERIALS AND METHODS

This chapter presents the various activities that were undertaken to aid data collection, facilitate analysis and making of deductions. The study was carried out using a 0.15 m³ fixed dome laboratory bio-reactor at the Agricultural Engineering Department of Egerton University, under mesophilic conditions with manure characterization done in the Food Science Microbiology laboratory. Chicken, goat and cow manure were obtained from Tatton Agricultural Park (TAP) of Egerton University. Effluent from anaerobic digestion of cow manure at the Egerton University biogas plant was used as inoculum.

Randomized Block Design (RBD) was used for objectives one and two. Treatments (for objective one: hydro, mechanical and thermal pretreatment levels; and for objective two: inoculum and total solid levels) were randomized within blocks (days of biogas production). The only source of extraneous variability, therefore, emerged from reading the values of daily biogas production for each pretreatment, inoculum and total solids levels. Experimental data obtained was subjected to Analysis of Variance (ANOVA) using SAS 8.2 software to establish significant differences between the treatments. Fisher's Least Significant Difference (LSD) at $\alpha = 0.05$ was used to separate the means. Objective three was subjected to Response Surface Methodology (RSM) and Central Composite Design (CCD) by Design Expert 10.0.0.3 software for maximizing biogas output.

3.1 Biomass Reactor, Substrate Preparation and Temperature Control

3.1.1 Biomass Reactor Experimental Setup

Figure 3.1 shows the fixed dome laboratory bio-reactor used in the research;

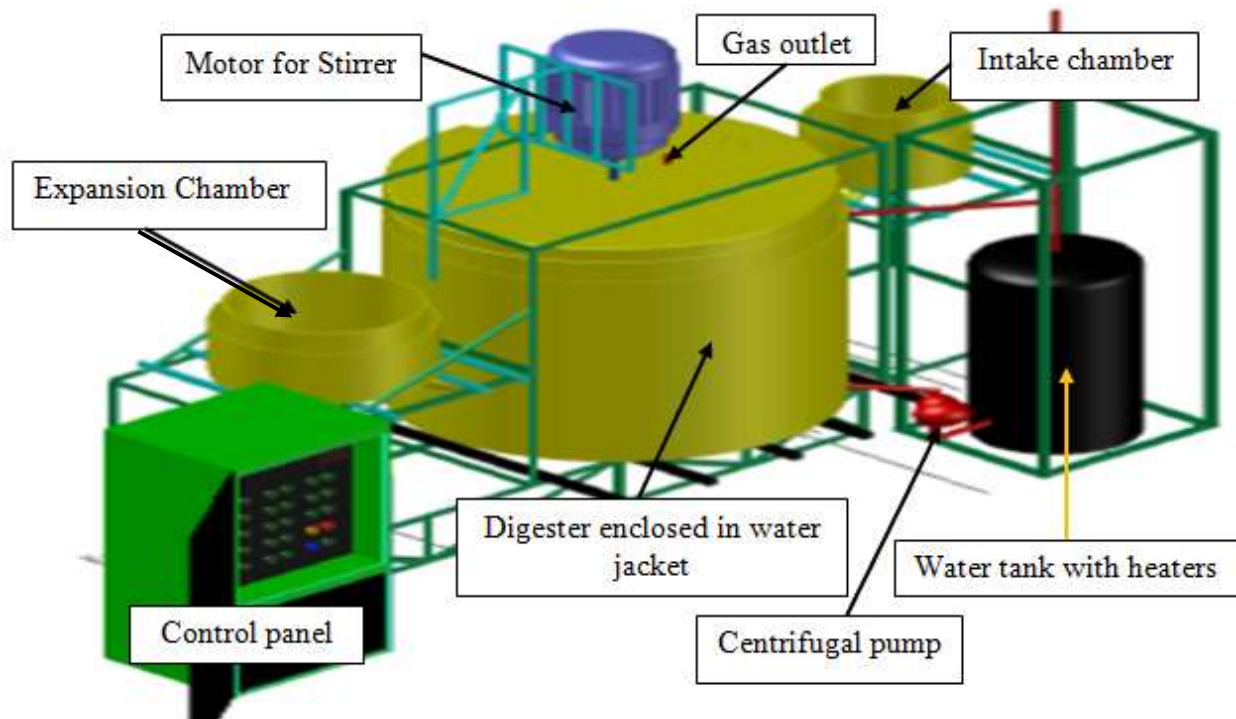


Fig 3.1: Fixed dome Laboratory bio-reactor used for the Research

Further images are as on Plates B1, B2, B3 and B4, while a detailed schematic drawing is presented on Plate B5 (all in Appendix B). The 0.15 m^3 digester was enclosed in a 0.1 m^3 cylindrical water jacket. The digester is metallic and welded to prevent gas and substrate leakage that may result from cracks or joints brought about by any slight expansion. Also, to help distribute organisms and temperature uniformly throughout the mixture is a perpendicularly above motor driven stirrer which is at the centre of the digester and agitates the substrate. Stirring was done at 15 revolutions per minute (rpm) for 3 minutes after every 6 hours. The agitation also, enhances substrate particle distribution and detachment during the digestion process, and gas removal. Adjacent to it is a 19 mm diameter gas removal pipe fitted with a gas outlet valve, from where a 13 mm diameter plastic pipe is fitted for gas collection and measurements by the water displacement method.

The water jacket provides thermal insulation for effective biological activity. The set-up has a control panel as an interface between the operator and other remote system components. Ambient, digester and water jacket temperature readings are taken on display and necessary thermal and motor revolution settings done. Adjacent is a 0.1 m^3 water heating tank with two 15 A, 250 V immersible heaters for switch on/ off upon the control panel sensors' instructions. A 3 speed Dayliff DQ 32/80 centrifugal pump, aids in forcing cold water into the water tank from the jacket, while hot water from the tank is circulated by convection to the jacket. A 10 litre inlet chamber (surrounded by an open 16 litre concentric insulation chamber)

fitted with a 50 mm diameter and 600 mm long inlet pipe was provided for digester loading while the 90 litre expansion chamber (surrounded by an open 100 litre concentric insulation chamber) with a 50 mm diameter and 500 mm long outlet pipe connected to the digester, aided effluent removal.

3.1.2 Substrate Preparation

Chicken, goat and cow manure were manually sorted to remove stones, soil, plastics and other foreign materials using a spade at the TAP poultry structures, goat yard and zero grazing unit. Thereafter, they were separately placed into 20 litre buckets and lid covered prior to transfer to the Agricultural Engineering laboratory. Physiochemical characteristics (total solids, moisture content, volatile solids, bulk density and carbon to nitrogen ratio) of each manure were determined (at the Food Science and Agricultural Engineering, Egerton University and KALRO Kabete laboratories), prior and after pretreatment; except for hydro pretreatment where only C/N ratio was determined as diluting the material to 8% total solids would alter the other original characteristics. Physiochemical characterization for hydro, thermal and mechanical pretreatment were respectively done at 12 hours, 80 °C and 3 mm. Raw data is presented in Appendix A. All measurements were recorded per parameter as (Equation 3.1a);

$$\bar{x} + \sigma \tag{3.1a}$$

Where;

\bar{x} = the sample mean of the material variable

σ = sample standard deviation, which could be found by (Equation 3.1b);

$$\sigma = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$$

(3.1b)

Where;

N = number of values of the measured variable, i...N

x_i = individual values of the measured variable

Total solids, moisture content and volatile solids were determined using Method 1684 of US EPA standards (Telliard, 2001), as below;

a. Total Solids

Total solids of manure prior to and after mixing and pretreatment were determined to guide on the manure: water mix ratio for the preparation of the influent (Yongabi et al., 2009). Three (3) samples of each of the manures were placed in oven metal cans of known weight “W_{can}”, spread evenly, covered with the lids, weighed and labeled as “W_{sample}”. These were then oven-dried at 105 °C for 24 hours, after which, the samples in cans were cooled for 30 minutes, weighed and the final weight recorded as “W_{total}” as (Equation 3.2);

$$\%TS = \frac{W_{total} - W_{can}}{W_{sample} - W_{can}} \times 100 \quad (3.2)$$

Where;

TS = total solids content of the manure

W_{can}= Weight of can (g)

W_{sample}=Weight of wet sample and can (g)

W_{total}=Weight of dried residue and can (g)

The results are presented in Table 4.1, with their ANOVA in Tables D1 and D2 of Appendix D, and discussed in section 4.1.1.

b. Moisture Content

In determining the moisture content, empty crucibles were heated in an oven for 1 hour at 105 °C, removed and cooled in a desiccant for 30 minutes. Each manure sample weight was recorded as “W_{wet}”, placed onto the crucibles and oven-dried for 24 hours at 105 °C. The crucibles were removed from the oven, cooled in desiccators to room temperature and weights recorded as “W_{dry}”. The difference in weight prior to and after drying represented the moisture content. MC was expressed on wet basis as (Equation 3.3);

$$MC = \left(\frac{W_{wet} - W_{dry}}{W_{wet}} \right) \times 100\% \quad (3.3)$$

Where;

MC = Moisture content of manure (%)

W_{wet} = Weight of wet manure (g)

W_{dry} = Weight of oven-dry manure (g)

The results are presented in Table 4.2 and discussed in section 4.1.2. The ANOVA of the data is presented in Tables D3 and D4 of Appendix D.

c. Volatile Solids

Volatile Solids (VS) were determined by incineration of the samples in a muffle furnace at 550 ± 5 °C for 2 hours. Volatile solids of individual manure inform on their volumetric and weight proportions in the final substrates (Appendix C1). Volatile solids were computed as (Equation 3.4);

$$\% \text{ VS} = \frac{W_{\text{total}} - W_{\text{volatile}}}{W_{\text{total}} - W_{\text{dish}}} \times 100 \quad (3.4)$$

Where;

VS = volatile solids content of the manure

W_{dish} = Weight of dish (g)

W_{volatile} = Weight of residue and dish after ignition (g)

W_{total} = Weight of dried residue and dish (g)

The results are presented Table 4.3 and discussed in section 4.1.3. The results were subjected to analysis of variance that culminated into Tables D5 and D6 of Appendix D.

Goat, chicken and cow manure were mixed at a ratio of 1:1:1 (Langat et al., 2018; Rahman et al., 2017; Sebola et al., 2015) based on their volatile solids content (Achinass et al., 2018), hence proportions of each manure type in the final influent would not be equal because of their varying VS. The respective mass proportions in the final substrate are as presented in Appendix C2. The respective masses of the three solid manure types and water (for dilution) were thereafter determined based on their initial and final total solids.

d. Bulk Density

Density is an important physical property that determines handling, storage and transportation characteristics of manure for anaerobic digestion and was determined according to ASAE D384 standards (Daniel et al., 2002). The process of determining manure bulk density was accomplished as follows; using a vernier caliper, the inside diameters and heights of metallic cans were measured at six positions at 90° each and their respective volumes calculated. Three (3) samples of each manure type prior to and after the three pretreatment methods were placed in metallic cans of known weight " W_{can} ", spread evenly to the brim, weighed using a pan balance and labeled as " W_{sample} ". Density was computed using (Equation 3.5);

$$\rho = \frac{W_{\text{sample}} - W_{\text{can}}}{V_{\text{can}}} \quad (3.5)$$

Where;

ρ = Manure bulk density (g/cm³)

W_{can} = Weight of can (g)

W_{sample} = Weight of pretreated or non-pretreated sample and can (g)

V_{can} = Internal volume of can (cm³)

The results obtained were presented as in Table 4.4, with their ANOVA in Tables D7 and D8 of Appendix D, and discussed in section 4.1.4.

e. Carbon to Nitrogen ratio

Total Carbon (C) in the manure samples, prior to and after pretreatment was determined using the Colorimetric method (Nelson & Sommers, 1996), where one gram of each dry manure sample was ground to pass through a sieve of 0.5 mm mesh and placed in a 250 mL Erlenmeyer flask. 10 ml of 0.667 sodium dichromate was mixed with 5 M sulfuric acid added to the suspension and stirred in a circular motion (at 180 rpm) using a horizontal shaker for 10 minutes. After shaking, the suspension remained undisturbed for one hour and 50 mL distilled water was added. The supernatant was collected and the absorption of the solution at 660 nm was measured using a B220 photoelectric colorimeter to display the concentration (%) of Carbon in the manure samples.

Total nitrogen (N) in the samples, on the other hand, was determined by the Kjeldahl method (Jones Jr, 1991), where digestion of the manure was done to convert nitrogen into HNO₃. Thereafter, the ammonia released was distilled into an absorbing surface or medium and volumetric analysis of the ammonia formed during the digestion process done to determine the amount of Nitrogen in the sample (%).

The percentages of Carbon and Nitrogen in each of the manure samples were then represented in ratios. Overall C/N ratio of the chicken-cow-goat manure substrate was calculated based on Cornell University (1996), (Equation 3.6) and presented as in Appendix A4;

$$R = \frac{M_{\text{MCD}} [C_{\text{CD}} (100 - MC_{\text{CD}})] + M_{\text{MCM}} [C_{\text{CM}} (100 - MC_{\text{CM}})] + M_{\text{MGM}} [C_{\text{GM}} (100 - MC_{\text{GM}})]}{M_{\text{MCD}} [N_{\text{CD}} (100 - MC_{\text{CD}})] + M_{\text{MCM}} [N_{\text{CM}} (100 - MC_{\text{CM}})] + M_{\text{MGM}} [C_{\text{GM}} (100 - MC_{\text{GM}})]} \quad (3.6)$$

Where;

R = Overall substrate C/N ratio

M_{MCD} , M_{MCM} , M_{MGM} = Solid mass of cow, chicken and goat manure (kg) respectively in substrate, wet basis.

3.1.3 Temperature Control

MC_{CD} , MC_{CM} , MC_{GM} ; C_{CD} , C_{CM} , C_{GM} ; N_{CD} , N_{CM} and N_{GM} = Moisture content, percentage of Carbon and Nitrogen of cow, chicken and goat manure (%) respectively in substrate, wet basis.

The results are as presented in Table 4.5, and discussed in section 4.1.5. Analysis of variance of the data is in Tables D9 and D10 of Appendix D.

The digestion process was kept by a control panel set at an average mesophilic temperature of 35 °C. The thermostat was regulated by switching the immersion heaters on and off as the temperature oscillated above or below 35 ± 0.5 °C digester temperature. Thermocouples in the digester and water jacket relayed real time temperature information to the control panel throughout this process, until the set substrate temperature was achieved. Water circulated in the jacket surrounding the digester by use of a centrifugal pump. Heat transfer to and from the substrate, through the digester wall was by conduction and radiation.

Temperature was maintained with the help of partial integral differentials (PIDs) and program logic controls (PLCs). The PID algorithm controlled the output to the control point so that a digester temperature of 35 °C (set as a static variable in PLC) was achieved.

3.2 Effect of Manure Pretreatment on Biogas Production

The control experiments involved separate digestion of non-pretreated chicken, goat and cow manure, and a chicken-goat-cow manure mixture at TS of 8%, commonly used in Kenya as reported by Nyaanga (2011). Prior to pretreatment, initial particle sizes of chicken, goat and cow manure were 5.37 ± 1.06 mm, 10.60 ± 2.74 mm and 4.38 ± 0.68 mm respectively. The results are as presented in Table 4.6 and discussed in section 4.2.1. The ANOVA is presented in Table D11 of Appendix D.

Chicken (2.4 kg) and goat manure (12.9 kg) were pretreated separately and mixed with fresh untreated cow manure (23.8 kg) (mix ratio = 1:1:1, VS basis). The pretreatment of goat and chicken manure were because their initial physical and chemical states make them problematic for digestion. The mixture was diluted using 80.9 litres of water to generate 120 litres of substrate with 8% total solids then agitated for 5 minutes by a piece of wood for increased surface area of the particles prior to feeding into the digester.

Gas collection and measurement was by the water displacement method (Masinde et al., 2020) where a gas pipe was connected to an inverted 500 ml graduated volumetric cylinder full of water, placed in a bucket of water. A gate valve then opened for gas to displace water in the cylinder at intervals until all the gas for set duration of production was exhausted. The rate of biogas production was estimated from volume measured over set duration and translated into cubic metres per unit volume of digester volume per day ($\text{m}^3/\text{m}^3\text{d}$).

Gas samples were also taken daily through the experimental period using 500 ml gas bags for biogas quality determination. This was to know the day when biogas formed by meeting the 50% threshold of methane content. Biogas quality was indicated by the methane content using a digital gas analyzer screen and its values recorded as percentages (%), as on Plate B10. The three treatments (also in Appendix A1) that were administered are discussed in the following subsections;

3.2.1 Hydro Pretreatment

Chicken (2.4 kg) and goat manure (12.9 kg) were diluted with 24 litres and 37 litres of tap water, respectively, to attain total solids of 8% and were soaked for 6, 12 and 18 hours at room temperature (Plate B7). The procedure for Total Solids standardization is given in Appendix C Soaking times were based on local farmers' common practice of overnight soaking of materials in water overnight (12 hours) prior to digester feeding (T. Okeno, personal communication, September 28, 2019); the intervals were spaced around this known time. They were then mixed with 43.7 litres of fresh untreated cow manure substrate diluted to 8% total solids.

The results are presented in Table 4.7, and their ANOVA in Table D12 (Appendix D), and discussed in section 4.2.2.

3.2.2 Mechanical Pretreatment

Chicken and goat manure mixture was crushed using a metallic meat mincer (Plate B8) with aperture sizes of 2 mm, 3 mm and 4 mm. These were mixed with untreated cow manure and placed in 20 litre buckets for substrate preparation. Schell and Harwood (1994) recommended the effective particle size of about 1 mm to 2 mm. This study sought to observe the behaviour of larger particle sizes (2 mm to 4 mm) on anaerobic digestion, since achievement of smaller sizes is deemed to be more expensive.

The results and their analysis of variance are presented in Table 4.8 and D13 (Appendix D) and discussed in section 4.2.3.

3.2.3 Thermal Pretreatment

Chicken and goat manure were separately cooked in a 6 litre pressure cooker (Plate B9) in batches of maximum 6 kg, depending on the calculated masses (Appendix C2) at 60 °C, 80 °C and 100 °C for 20 minutes, based on Assefa et al. (2014) recommendations. These temperatures were regulated by the Thermo-regulation knob. The material was mixed with untreated cow manure and diluted with water to attain 8% total solids prior to feeding into the digester.

The results and their ANOVA are presented in Table 4.9 and D14 of Appendix D and discussed in section 4.2.4.

3.3 Effect of Inoculum and Total Solids levels of Pretreated Manure on Biogas Production

3.3.1 Effect on Inoculum levels on Biogas Production

Preparation of influent was done by mixing pretreated chicken, goat and fresh cow manure with spent cow dung slurry at different ratios. Inoculum was passed through a 2 mm sieve to eliminate any biodegradable material remnants prior to preparation of S/I ratios.

The effect of inoculum levels (ratios) on biogas production was investigated at 2:1, 3:1, 4:1, 5:1 and 6:1, for 10 days each. These values were distributed around an optimal ratio of 4:1 that is reported in literature when fresh cow manure (Haider et al., 2015) was used in the co-digestion of food waste and rice husks. The ratio was also used when anaerobically digested cow manure was used to inoculate wheat straw digestion (Hashimoto, 1989) and pig slurry effluent to enhance the biodegradation of pig slaughterhouse wastes (Yoon et al., 2014). The base S/I ratio is within the optimal range reported by Dennis (2015) for cow manure and rumen inoculum. Table 3.1 presents volumes of substrate and inoculum used to prepare digester influent at different ratios;

Table 3.1: Influent preparation at different Inoculum levels

Substrate to Inoculum Ratio	Substrate		Inoculum added	
	%	Volume (litres)	%	Volume (litres)
2:1	66.67	80.00	33.33	40.00
3:1	75.00	90.00	25.00	30.00
4:1	80.00	96.00	20.00	24.00
5:1	83.33	100.00	16.67	20.00
6:1	85.71	102.85	14.29	17.15

The results and ANOVA are as presented in Table 4.12 and D16 (Appendix D), with their discussions in section 4.3.1.

3.3.2 Effect of Total Solids on Biogas Production

Substrate for digester feeding was prepared by separately diluting different weights of pretreated chicken, goat and fresh cow manure with computed quantities of tap water to attain different influent total solids. This was because pretreated chicken, goat and untreated cow manure had average total solids of 88.97%, 33.53% and 14.72% respectively, hence required separate dilution to predetermined influent total solids prior to their mixing.

The effect of total solids on biogas production was investigated at 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, 10.0% and 10.5%; each for 10 days. The choice of total solids for the study were guided by Abbassi-Guendouz et al. (2012), Budiyo and Sumardiono (2014), Orhororo et al. (2017) and Paramaguru et al. (2017) who reported a TS range of 8% to 10% as optimal for biogas production from most feedstocks/ manures. Preparation of influent at every TS level was done using (Equations 3.8 a and b);

$$\text{Manure} = \left(\frac{F_{ts}}{I_{ts}} \times \text{TI} \right) \quad (3.8a)$$

$$\text{Water} = \left(1 - \frac{F_{ts}}{I_{ts}} \right) \times \text{TI} \quad (3.8b)$$

Where;

F_{ts} = Final total solids of the substrate (%)

I_{ts} = Initial total solids of fresh manure (%)

TI = Total substrate, comprising of water and manure

Table 3.2 represents individual manure to water ratios used in their dilution to attain the different total solids in the final influent as per equations 3.7a and 3.7b. Their exact weights are presented in appendix A3. Table 4.12 and D17 (Appendix D) present the obtained and analyzed data while their discussions are done in section 4.3.2.

Table 3.2: Manure to water ratios used in attaining respective Total solids

Total solids (%)	Manure to water ratio			
	Chicken	Goat	Cow	Total substrate
7.5	1:10.7	1:3.5	1.0:1	1:2.4
8.0	1:10.0	1:3.2	1.2:1	1:2.2
8.5	1:9.3	1:2.9	1.4:1	1:2.0
9.0	1:8.7	1:2.7	1.6:1	1:1.8
9.5	1:8.3	1:2.5	1.8:1	1:1.7
10.0	1:7.8	1:2.4	2.1:1	1:1.5
10.5	1:7.4	1:2.2	2.5:1	1:1.4

3.4 Optimization of Biogas Production

The optimization process requires designing experiments statistically based on individual factors, carrying them out, estimating the factor coefficients in a mathematical model and predicting the response while checking the adequacy of the model (Körbahti & Rauf, 2008; Mondal et al., 2013). Individual and interactive factor effects of substrate to inoculum ratio, total solids (%), and effective minced particle size (mm) on biogas production rate ($\text{m}^3/\text{m}^3\text{d}$) were studied.

3.4.1 Multi-Variate Design of Experiments

The Design Expert version 10.0.0.3 software (Expert, 2016) was used to generate the experimental design, statistical analysis and regression model. The Response Surface Methodology (RSM) was based on linear and quadratic models and was used with central composite design (CCD) to study simultaneous effects and determine optimum conditions of independent variables; substrate: inoculum ratio (S/I: X_1), total solids (TS: X_2) and effective minced particle size (PS: X_3) of chicken - goat and untreated cow manures in the substrate on biogas production (response, Y).

Each of the variables was divided into three levels where the highest level was coded as +1, the centre point as 0, and the lowest level as -1 and the real values of the variable ($-\alpha$

and $+\alpha$) in relation to the coded variables were computed using (Equation 3.9a) while the response, Y due to X_1 , X_2 and X_3 was given by a general second order polynomial (Equation 3.9b);

$$\text{Coded Variable} = \frac{X - X_0}{\lambda} \quad (3.9a)$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \quad (3.9b)$$

Where;

X_0 = value of variables at the central level: for $X_1 = 4:1$, $X_2 = 9\%$ and $X_3 = 3$ mm

X = input variable: for; $X_1 = 2:1, 4:1, 6:1$; $X_2 = 8\%, 9\%, 10\%$ and $X_3 = 2$ mm, 3 mm, 4mm

λ = step change of the variable: for $X_1 = 2:1$, $X_2 = 1\%$ and $X_3 = 1$ mm

Y = biogas yield ($\text{m}^3/\text{m}^3\text{d}$)

X_1, X_2, X_3 = independent variables

β_0 = offset co-efficient

$\beta_1, \beta_2, \beta_3$ = linear coefficients

$\beta_{11}, \beta_{22}, \beta_{33}$ = quadratic coefficients

$\beta_{12}, \beta_{13}, \beta_{23}$ = interactive coefficients

ε = residuals associated

Input of the values for each actual factor level into Equation 3.9a resulted in real and coded values for each variable as presented in Table 3.3. The values guided RSM to design the experimental matrices.

Table 3.3: Experimental Variables and their Coded values

Factor	Symbol	Units	Coded and Real Values					
			Coded:	$-\alpha$ (-1.68)	-1.00	0.00	+1.00	$+\alpha$ (+1.68)
S/I	x_1	Ratio		0.64	2.00	4.00	6.00	7.36
TS	x_2	%	Real:	7.32	8.00	9.00	10.00	10.68
Size	x_3	Mm		1.32	2.00	3.00	4.00	4.68

The range distance between axial points and the centre points was $\alpha = (2)^{0.75} = 1.68$. $\alpha = 2^{k/4}$, k is the number of variables (Aslan, 2007) and k = 3 for this study. The software used this distance from fixed '2n' axial points together with the centre point runs to generate model quadratic terms (Behera et al., 2018); and avoid error. A total of 20 experimental runs (6 centre and 14 axial points) was designed. Axial points checked for analyses for variance of the model prediction and needed to be equidistant in all directions from the design centre while centre points helped in estimation of experimental error (Yi et al., 2010). The results of the experiments are presented in Table 4.13 and F1 and F2 (Appendix F), and discussed in sections 4.4.1 and 4.4.2.

3.4.2 Validation of the Prediction Model

The prediction model suggested by the software for biogas production rate was quadratic and consisted linear, quadratic and cross product terms (Brown & Brown, 2012 as given by (Equation 3.10), which is a solution to (Equation 3.9b);

$$Y = -275.20 + 12.25X_1 + 69.42X_2 + 28.16X_3 + 1.53X_1X_2 - 0.97X_1X_3 + 1.22X_2X_3 - 2.88X_1^2 - 4.57X_2^2 - 6.22X_3^2 \quad (3.10)$$

Where;

Y = biogas production rate (m³/m³d)

X₁ = substrate to inoculum ratio (2:1 - 6:1)

X₂ = total solids (8 - 10%)

X₃ = effective minced particle size (2 - 4 mm)

Equation (3.10) was validated statistically by performing analysis of variance (ANOVA) to evaluate the model's significance as recommended by Khoobakht et al. (2016) including dividing the complete variation in the data into two, by isolating variation due to experimental errors from that associated with the model to test for their significance in line with Yousuf et al. (2018). Fisher's (F) and probability (P) tests were used for this analysis. The adequacy of this model equation was tested by the coefficient of determination R² and its significance determined by an F-test.

The validation experiments (to confirm and verify the accuracy) of the prediction model emanating from Equation 3.9b upon inserting the coefficients was done at optimal factor levels (S/I = 4:1, TS = 9% and Size = 3 mm) in 3 replications at a temperature of 35 ± 0.5 °C. Experimental (observed and measured) and predicted biogas production rates were compared as in Table F3 of Appendix F and their discussions done in section 4.4.3.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Manure Characteristics

Anaerobically digested cow manure used as inoculum, for the determination of the effect of substrate to inoculum ratios on biogas production, had an average total solids, moisture content, volatile solids, bulk density and C/N ratio of $8.74 \pm 0.69\%$, $91.26 \pm 0.69\%$, $38.20 \pm 1.20\%$, $1007.58 \pm 2.56 \text{ kg/ m}^3$ and 22:1, respectively.

Hydro, thermal and mechanical pretreatment of the manure (s) was done for 12 hours, 80°C and 3mm respectively (average variable values) for manure characterization. With regards hydro pretreatment, only C/N ratio was determined because, prior to soaking for 12 hours, the manure (s) was diluted to 8% total solids, therefore altering their other original physical characteristics.

The characteristics of the different manures prior to and after pretreatment are presented and discussed in the following sub-sequent sections;

4.1.1 Total Solids

The average total solids for chicken, goat and cow manure are given in Table 4.1 based on raw data (Table A2) and ANOVA (Tables D1 and D2).

Table 4.1: Manure Total Solids before and after Pretreatment

Manure	Total Solids (%)				LSD
	Before pretreatment	After pretreatment			
		Hydro	Mechanical	Thermal	
Cow	14.72 ± 0.08	-	-	-	-
Chicken	$87.96^c \pm 0.50$	$8.03^d \pm 0.06$	$88.97^a \pm 0.20$	$88.40^b \pm 0.48$	0.34
Goat	$31.02^k \pm 1.24$	$8.02^l \pm 0.13$	$33.53^j \pm 0.98$	$31.53^k \pm 0.90$	1.00

The values are the mean \pm standard deviation of the triplicate measurements

Means followed by the same letter(s), (a, b, c, d), in the same row (for chicken manure); and (j, k, l) for goat manure are not significantly different at $\alpha = 0.05$

From results obtained, the total solids of goat (31.02%), cow (14.72%) and chicken (87.96%) manures (as in Table 4.1), before pretreatment were close to their respective reported values of 33.65%, 16.28 % and 85.38 % (Barooh et al., 2015; Jha et al., 2012; Zhang et al.,

2013). The difference between total solids determined in this research and that reported in literature for same manure types could be due to the differences in animal production and manure handling systems and practices (before and after generation) including animal breeds, husbandry, feeds, manure collection, handling methods and other products in the manure stream like feathers, blood and urine (Wang et al., 2019) that could have had a significant effect on the manure's physical characteristics.

Pretreatment significantly affects manure total solids ($\alpha = 0.05$) (Yousuf et al., 2018) as deduced from Table 4.1, D1 and D2 (Appendix D). Manure mincing to 3 mm particle sizes increased total solids for chicken and goat manure by 1.01% and 2.51%; while thermal pretreatment at 80°C respectively improved manure total solids by 0.44% and 0.51%. This is attributed to increase in effective specific surface areas and reduction of pore spaces in the manure due to mechanical pretreatment, thus enhanced total solids per unit volume of the biomass material (Hess et al., 2020). Evaporation of moisture from the manure during heating could have also increased the amount of effective total solids.

Initial total solids provides information on the right amount of water required for diluting the manure (as in section 3.3 in Materials and Methods and Appendices C1 and C2) to make the substrate prior to digester feeding (Yongabi et al., 2009), This is because loading the digester with excess total solids may clog the system while insufficiency may lead nutrient deficiency for the microorganisms.

4.1.2 Moisture Content

The average moisture for chicken, goat and cow manure are given in Table 4.1 based on raw data (Table A3) and ANOVA (Tables D3 and D4).

Table 4.2: Moisture Content of Manure before and after Pretreatment

Manure	Moisture content (%)				LSD
	Before pretreatment	After Pretreatment			
		Hydro	Mechanical	Thermal	
Cow	85.28 ± 0.04	-	-	-	-
Chicken	12.04 ^b ± 0.50	91.97 ^a ± 0.06	11.03 ^d ± 0.40	11.60 ^c ± 0.48	0.34
Goat	68.98 ^k ± 1.24	91.98 ^j ± 0.13	66.47 ^l ± 0.98	68.47 ^k ± 0.89	1.44

The values are the mean ± standard deviation of the triplicate measurements

Means followed by the same letter(s), (a, b, c, d), in the same row, for chicken manure; (j, k, l) for goat manure are not significantly different at $\alpha = 0.05$

The moisture content (MC) before pretreatment of chicken manure from TAP of $12.04 \pm 0.50\%$ was basically the same as that reported by Roeper et al. (2005) of 12.37%. This could be attributed to the fact that chicken are globally reared and fed on the same. The MC of untreated TAP goat manure of 68.98% was nearly twice as much as that reported by Zhang et al. (2013) of 36.55%. This could be due to the fact the TAP goat manure was collected from an open pen/shed soon after the rains while those in China were exposed to the sun before collection.

It is evident that mechanical and thermal pretreatment significantly ($\alpha = 0.05$) reduce manure moisture content. Percentage moisture content due to 3 mm manure mincing (Table 4.2) reduced by 2.51% and 1.01% for chicken and goat manure respectively and by 0.44% and 0.51% when thermally pretreated at 80 °C. Mincing led to particle size reduction that opened up biomass materials and could have led to the escape of moisture from the manure as they were being subjected to elevated temperatures that could have resulted in increased evaporation hence decreasing in their moisture content. However, the change in mc was significantly different among all the 2 pretreatments (mechanical, thermal and hydro) at $\alpha = 0.05$ (Table 4.2 and Appendix D3 and D4).

Moisture content and total solids of the manure helps in predetermining the volume of substrate and choice of device/ system to be used for their pretreatment (Aa, 1996); and amount of water required for substrate preparation. An adequate water amount helps in dissolving readily available bio-degradable organic matter in line with Fujishima et al. (2000) who states that the amount of moisture in a substrate influences carbohydrate degradation and activates protein degradation, hence has a positive effect on biogas production. That is, substrates with a moisture content of about 70% would result in maximal gas yield (Mir et al., 2016). As in Table 4.1, manure requires more water for substrate preparation after thermal and mechanical pretreatment (refer to Appendix C).

4.1.3 Volatile Solids

The volatile solids of the three manures before and after pretreatment are presented in Table 4.3 and Table as in Table A4, D5 and D6 of Appendix

Table 4.3: Manure Volatile Solids before and after Pretreatment

Manure	Volatile Solids (%)				LSD
	Before	After pretreatment			
	Pretreatment	Hydro	Mechanical	Thermal	
Cow	20.12 ± 1.15	-	-	-	-
Chicken	33.39 ^a ± 1.50	33.36 ^a ± 1.80	34.10 ^a ± 1.19	33.55 ^a ± 1.21	9.73
Goat	17.63 ^j ± 1.15	17.72 ^k ± 0.24	18.03 ^j ± 0.14	16.25 ^k ± 0.23	1.18

The values are the mean ± standard deviation of the triplicate measurements

Means followed by the same letter(s), (a), in the same row for chicken manure; (j, k) for goat manure are not significantly different at $\alpha = 0.05$.

The volatile solids of non-pretreated cow (20.12%), chicken (33.29%) and goat (17.63%) manure (Table 4.3) were within the respective reported ranges of 10.79-28.8%, 17-37.21% and 16.3-76.49% (Andrade et al., 2016; Lawal et al., 2016; Li et al., 2013; Lorimor et al., 2004). Chicken manure had higher volatile solids content compared to the other livestock wastes due to its higher proportion of biodegradable organic matter (Bujoczek et al., 2000), prior to and after pretreatment.

Hydro, thermal and mechanical pretreatment methods had no significant differences on chicken manure volatile solids since none of the methods had chemical impacts on chicken and goat manures ($\alpha = 0.05$) (Table 4.3 and also D5 and D6 of Appendix D). Pretreatment, however, had a significant effect on goat manure VS as a lower value was recorded due to dissolution of small volatile molecules into macromolecular organic compounds like CO₂, VFAs; among others (Liu et al., 2012). These changes could further translate in variations in methane yield, as acknowledged by Molaey et al. (2018).

The VS of the individual manure dictated their volumetric proportions in the final substrate mixture; hence the use of a constant ratio of 1:1:1 (goat, chicken and cow manure) as also has been reported by Achinas et al. (2018), Langat et al. (2018) and Sebola et al. (2015). Their proportions in the final influent were therefore not equal because of the varying VS as in appendix C2.

4.1.4 Bulk Density

The bulk densities of the three manures obtained prior to and after pretreatment are presented in Table 4.4.

Table 4.4: Manure Bulk Density

Manure	Bulk Density (kg/ m ³)				LSD
	Before pretreatment	After pretreatment			
		Hydro	Mechanical	Thermal	
Cow	961.35 ± 17.23	-	-	-	-
Chicken	710.85 ^b ± 38.70	999.03 ^a ± 2.29	717.75 ^b ± 7.30	674.05 ^b ± 26.54	48.14
Goat	813.46 ^k ± 16.20	999.40 ^j ± 1.43	817.03 ^k ± 11.20	784.71 ^k ± 34.19	45.29

The values are the mean ± standard deviation of the triplicate measurements

Means followed by the same letter(s), (a, b), in the same row for chicken manure and (j, k) for goat manure are not significantly different at $\alpha = 0.05$, LSD = 48.14 and 45.29 respectively

As seen in Table 4.4; bulk densities of the manures before and after pretreatment were lower than values reported values of 990.73 kg/ m³, 827.59 kg/ m³ and 878.01 kg/ m³ for untreated cow, chicken and goat manures respectively (Parnes, 2013). These could be attributed to variations in manure handling during mass and volumetric measurements; shaking containers, for instance, could have allowed loose particles that make up the material to settle, increasing the overall bulk density as explained by Amidon et al. (2009) and Wang et al. (2019). These practices vary from region to region.

As shown after data analysis in Table D7 and D8 (Appendix D), hydro pretreatment had a significant influence while other methods had insignificant effect on manure bulk density at $\alpha = 0.05$. Bulk densities of soaked chicken (999.03 kg/m³) and goat manure (999.40 kg/m³) were close to a value of 1093 kg/m³ reported by Mahmoodi- Eshkaftaki et al. (2017). Manure subjected to hydro pretreatment had higher bulk density because their dilution with water to attain 8% total solids prior to soaking at 6 hours increased their mass per unit volume. Expansion of the material per unit volume due to their intra and extracellular temperature increase (with their effective particle mass remaining constant) led to a decrease in their bulk density after thermal pretreatment compared to those of untreated manure (Chung et al., 2011).

For mechanical pretreatment, increase in specific surface area of the particles reduced total pores between particles, therefore increasing the number, mass and eventually manure particles per unit volume (bulk density) (Bashour & Sayegh, 2007).

4.1.5 Carbon to Nitrogen (C/N) ratio

Results of C/N ratios of the three manures obtained prior to and after pretreatment, are presented in Table 4.5, as generated from raw (Table A6) and analyzed data (Table D9 and D10) in the Appendices.

Table 4.5: Carbon to Nitrogen ratios of the three Manure types

Manure	C/N ratio				Mean	LSD
	Before pretreatment	After pretreatment				
		Hydro	Mechanical	Thermal		
Cow	27.91 ± 0.06	-	-	-	-	-
Chicken	13.67 ^a ± 0.04	13.62 ^a ± 0.05	13.63 ^b ± 0.03	13.40 ^c ± 0.02	13.58	0.03
Goat	18.86 ^j ± 0.07	18.89 ^j ± 0.15	18.65 ^j ± 0.09	18.82 ^j ± 0.30	18.81	0.27

The values are the mean ± standard deviation of the triplicate measurements

Means followed by the same letter(s), (a, b, c), in the same row for chicken manure and (j) for goat manure are not significantly different at $\alpha = 0.05$

Chicken manure C/N ratios were significantly affected by mechanical (13.63:1) and thermal pretreatment (13.40:1) but had no significant effect on goat manure C/N ratios (mechanical = 18.65:1; thermal = 18.82:1) ($\alpha = 0.05$) (Table 4.5, D9 and D10 in Appendix D). The manure C/N ratios before and after pretreatment varied from the reported values of non-pretreated chicken (11:1) (Amanullah, 2007), cow (22.71:1) (Ardaji et al., 2016) and goat manure (18:1) (Andrade et al., 2016; Lawal et al., 2016). This difference could be attributed to animal protein intake. Chemical composition of animal manure depends on the properties of animal feeds consumed, where for instance, increased protein in the diet results in increased Nitrogen excretion as wastes may contain unused feed (ASAE, 2005).

Carbon is an energy source for anaerobes while nitrogen helps synthesize amino acids, proteins and nucleic acids (Khanal et al., 2019) to enhance growth of microbes (Mir et al.,

2016). Part of the nitrogen forms ammonia that neutralizes volatile fatty acids and the pH range. Knowledge of individual manure C/N ratios helped in the determination of an overall substrate ratio as in appendix A3. The C/N ratios were computed and found to be 20.21:1 before pretreatment and; 20.26:1, 20.15:1 and 20:01 after hydro, mechanical and thermal pretreatment respectively. The substrate therefore had C/N ratios was within the range 20:1 to 30.1 (Dioha et al., 2013; de Gannes et al., 2018) suitable for anaerobic digestion. According to Khalid et al. (2011), higher C/N ratios above 30:1 result in rapid nitrogen depletion, while ammonia accumulation is experienced in digesters with very low ratios (< 20:1); causing low gas production in both scenarios as reported by Mir et al. (2016).

4.2 Effect of Manure Pretreatment on Biogas Production

4.2.1 Biogas production from Untreated Manure

The control experiments involved the mono-digestion and co-digestion of untreated cow, chicken and goat manure with data being presented in Tables 4.6a, 4.6b and Figure 4.1.

Table 4.6a: Methane Content of Biogas from Various Manures

Substrate Retention Time (Days)	Biogas quality (% Methane)			
	Goat Manure (GM)	Chicken Manure (CM)	Cow manure (CD)	GM+CM+ CD
1	38.00	41.00	40.00	48.00
2	43.00	47.00	45.00	62.00
3	48.00	56.00	47.00	65.00
4	53.00	58.00	55.00	69.00
5	57.00	66.00	56.00	74.00
6	60.00	67.00	56.00	82.00
7	64.00	67.00	57.00	83.00
8	65.00	68.00	57.00	83.00
9	67.00	68.00	57.00	84.00
10	68.00	69.00	58.00	84.00
11	68.00	69.00	58.00	84.00
12	68.00	69.00	58.00	86.00
13	69.00	70.00	59.00	86.00
14	68.00	70.00	59.00	87.00
Mean	64.27	66.42	57.27	79.15

The flammable/ignitable biogas from pure cow and goat manure began on the 4th day and 3rd day for chicken manure; while their mixture began on the 2nd day (Table 4.6a) which agrees with Edache et al. (2017) and Ugwuoke et al. (2016) who reported that biogas started burning on the 4th, 3rd and 2nd days, respectively. On these days, gas produced from the different manures had methane content of more than 50% which also agrees with that reported by Karki (2009) since goat, chicken and their mixed manures had 53%, 55%, 56% and 62%, respectively. Average values of methane content of the first days of gas flammability to the 14th day concur with reported values of 65% (goat), 58% (cow) and 69% (chicken) (Jorgensen, 2009; Maithel, 2009).

Table 4.6b: Daily Production from Goat, Chicken, Cow and Mixed Manures

Day	Gas yield for different Control Experiments			
	Per unit digester volume (m ³ /m ³ d)			
	Goat Manure (GM)	Chicken Manure (CM)	Cow manure (CD)	GM+CM + CD
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.43
3	0.00	0.16	0.00	0.49
4	0.15	0.17	0.30	0.56
5	0.15	0.18	0.35	0.63
6	0.15	0.18	0.37	0.76
7	0.16	0.28	0.48	0.69
8	0.29	0.35	0.56	0.55
9	0.32	0.54	0.64	0.50
10	0.46	0.38	0.51	0.43
11	0.36	0.36	0.47	0.40
12	0.34	0.34	0.45	0.36
13	0.33	0.29	0.36	0.32
14	0.31	0.26	0.34	0.26
Cumulative	3.02	3.48	4.83	6.38
Average	0.27 ^c	0.29 ^c	0.44 ^b	0.49 ^a

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$, LSD = 1.00

The biogas production rate from goat, chicken and cow and a mixture of the manures before pretreatment is given in Table 4.6b. The highest daily biogas rate of $0.49\text{m}^3/\text{m}^3\text{d}$, was obtained from mixture of the three manures (co-digestion), followed by pure cow of $0.44\text{m}^3/\text{m}^3\text{d}$ whose value agrees with Nyaanga (2011). The rate from pure chicken of $0.29\text{m}^3/\text{m}^3\text{d}$ and goat manure's $0.27\text{m}^3/\text{m}^3\text{d}$ were lower which can be attributed to the low C/N ratios in these manures as has been argued by Yangin-Gomec and Ozturk (2013) and Zhang et al. (2013) and low specific surface area that inhibited the digestion process as indicated by Zhang and Banks (2013). Despite chicken manure's higher average specific gas yield and methane potential of $0.28\text{m}^3/\text{kg}$ of VS (Kafle & Chen, 2016) and 69% (Maithel, 2009); its high Nitrogen content hinders methanogenic bacteria survival. Therefore mixing it with other Carbon – rich biomass like cow manure prior to their digestion would offer a balanced C/N ratio for their effective biodegradation. Chicken and goat manure can also be combined with inoculum to help realize their methane potential and enhance biogas production (Hanafiah et al., 2017).

Co-digestion of the manures significantly increased biogas production, at $\alpha = 0.05$, $\text{LSD} = 1.00$ (Table D11 in Appendix D), compared to mono-digestion, and represented respective increases of $0.20\text{ m}^3/\text{m}^3\text{d}$ (69%), $0.22\text{ m}^3/\text{m}^3\text{d}$ (81%) and $0.20\text{ m}^3/\text{m}^3\text{d}$ (9%) in biogas production over the values from the individual digestion of chicken, goat and cow manure. This may be due to the positive synergetic effect offered by co-digestion (Jianzheng et al., 2011; Li et al., 2009) that provided more nutrient balance, improving the buffering capacity and minimizing effect of toxicity of the compounds. The large gas increase reported here is also attributed to stirring of the substrate within the digester for 3 minutes after each 6 hours at 15

rpm; throughout the process.

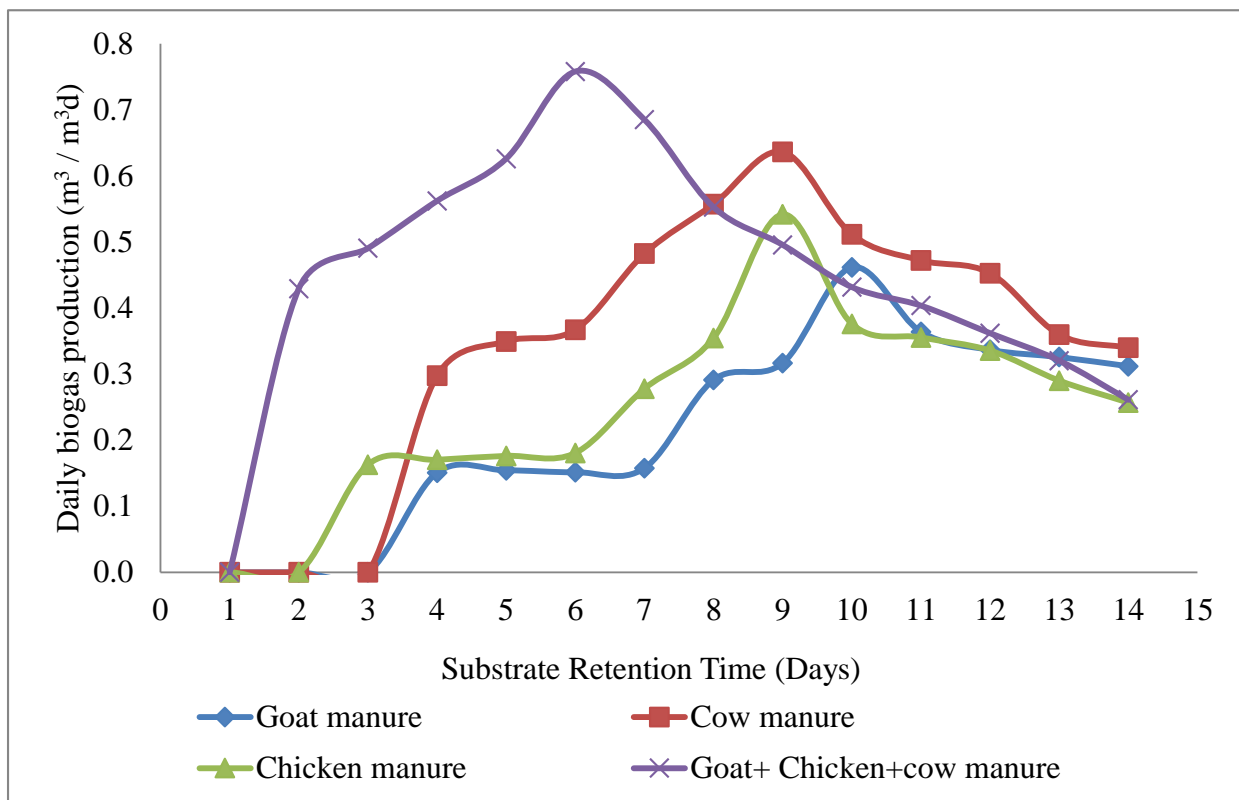


Fig 4.1: Biogas yield from Mono and Co-digestion of the three Manure types

Degradation of mixed and individual substrates happened between the 1st, 2nd and 3rd days for mixtures, chicken and cow/goat manures respectively (Fig 4.1). This was followed by system balance for a rise in biogas production up to the respective peaks. Biogas production from pure cow (0.30m³/m³d) and goat manure (0.15m³/m³d) began on the 4th day, 3rd day for chicken manure (0.16m³/m³d) as pure substrates; and their mixtures (0.43m³/m³d) on the 2nd day. These start days concur with studies by Edache et al. (2017) who respectively reported the 3rd, 4th and 1st day after digester feeding. Ugwuoke et al. (2016) however reported that production started for goat manure on the 4th day.

Quicker biogas produced from co-digestion may be due to bio-degradable organic matter in the substrate being readily available and the initial high population of methanogenic bacteria (Aragaw et al., 2013), provided by cow manure. It can also be attributed to a shorter lag phase caused by a positive synergetic effect of co-digesting the three. Suitable C/N ratio in the digester was obtained by a balanced mixture of nitrogen rich goat and chicken manure, and carbon-rich cow manure (Chukwuma et al., 2013). The biogas production indicated provision and development of a conducive environment for microbial activity as AD took place.

Peak biogas production rate for co-digestion (0.76m³/m³d) was on the 6th day, cow (0.64m³/m³d) and chicken manure (0.54m³/m³d) were on the 9th day, while that of goat manure

(0.46m³/m³d) occurred on the 10th day These findings differ with Ojolo et al. (2007) and Ugwuoke et al. (2016) who reported the 14th day for peak biogas production for cow and chicken manure and 21st day for goat manure at a digestion temperature of 30 ± 0.5 °C. This contrast may be due a higher (35°C) operating temperatures and periodic stirring (for 3 minutes after every 6 hours at 15 rpm) done when carrying out the experiments for this study.

At the initial stages, biogas production rate is low which is attributable to acclimatization of the bacterial consortium (in the inoculum) to the multi-component substrates used. Differing start and peak biogas production days for individual substrates' may be due to varying physiological and nutritional requirements of anaerobic bacteria - that are specific to each. There were steady rises in daily gas yield up to peaks, followed by falls for all experiments and may be linked to a decrease in nutrient availability.

4.2.2 Hydro Pretreatment

Hydro pretreatment was done by the separate soaking of chicken and goat manure at 0, 6, 12 and 18 hours prior to co-digesting them with untreated cow manure at mix ratios 1:1:1 volatile solids basis. Results of daily biogas production rate are presented in Table 4.7 and the ANOVA in Table D12 of Appendix D.

Table 4.7: Daily Biogas Production after Hydro Pretreatment

Day	Gas yield from different soaking times				
	Production per unit digester volume (m ³ /m ³ d)				
	Soaking times (hours)				
	0 (Control)	6	12	18	Average
1	0.00	0.00	0.00	0.00	0.00
2	0.41	0.46	0.43	0.39	0.42
3	0.47	0.54	0.52	0.44	0.49
4	0.54	0.59	0.56	0.53	0.55
5	0.61	0.65	0.63	0.62	0.63
6	0.73	0.76	0.72	0.72	0.73
7	0.65	0.67	0.65	0.62	0.65
8	0.53	0.62	0.60	0.52	0.57
9	0.48	0.56	0.53	0.44	0.50
10	0.41	0.49	0.43	0.39	0.43
Cumulative	4.83	5.35	5.07	4.67	4.98
Mean	0.54 ^c	0.59 ^a	0.56 ^b	0.52 ^d	0.55

Control= No soaking

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$, LSD = 0.02

Co-digesting chicken, goat and cow manure (as in Table 4.7) recorded highest mean gas production rate of 0.59m³/m³d, after 6 hours of soaking prior to digester feeding. This was followed by 12 hours, 0 hours (control) and 18 hours of soaking with mean gas production rates of 0.56, 0.54, and 0.52m³/m³d. At Fisher's LSD = 0.02, (Table D12 of Appendix D). It is with 95% confidence that biogas yield rate after different soaking times were significantly different.

Soaking chicken and goat manure prior to co-digestion resulted in biogas production rate increases of 0.05 (9.23%) and 0.02 m³/m³d (3.70%) after 6 hours and 12 hours respectively while after 18 hours, there was 0.02 m³/m³d (3.70%) reduction in production rate. There is an increase in gas yield with an increase in soaking duration up to 6 hours, after which a decline; it even falls below that of the control after 18 hours. This could be attributed to the possible death of anaerobic microbes in the feed stocks due to its longer time of exposure to aerobic conditions outside the digester. Methanogens are strictly anaerobes according to Brioukhanov et al. (2002) and can be oxygen tolerant for several hours before they die off (Kiener &

Leisinger, 1983). For instance, some bacteria (*Methanobacterium thermoautotrophicum* and *Methanosarcina barkeri*) exhibit some aero-tolerance for up to 30 hours while others (*Methanococcus voltae* and *Methanococcus vannielii*) may die off within 10 hours of oxygen exposure as stated by Kiener and Leisinger (1983). Their viability and effectiveness of catalysis reduces with increased time of aerobic exposure, as it happens with soaking chicken and goat manure.

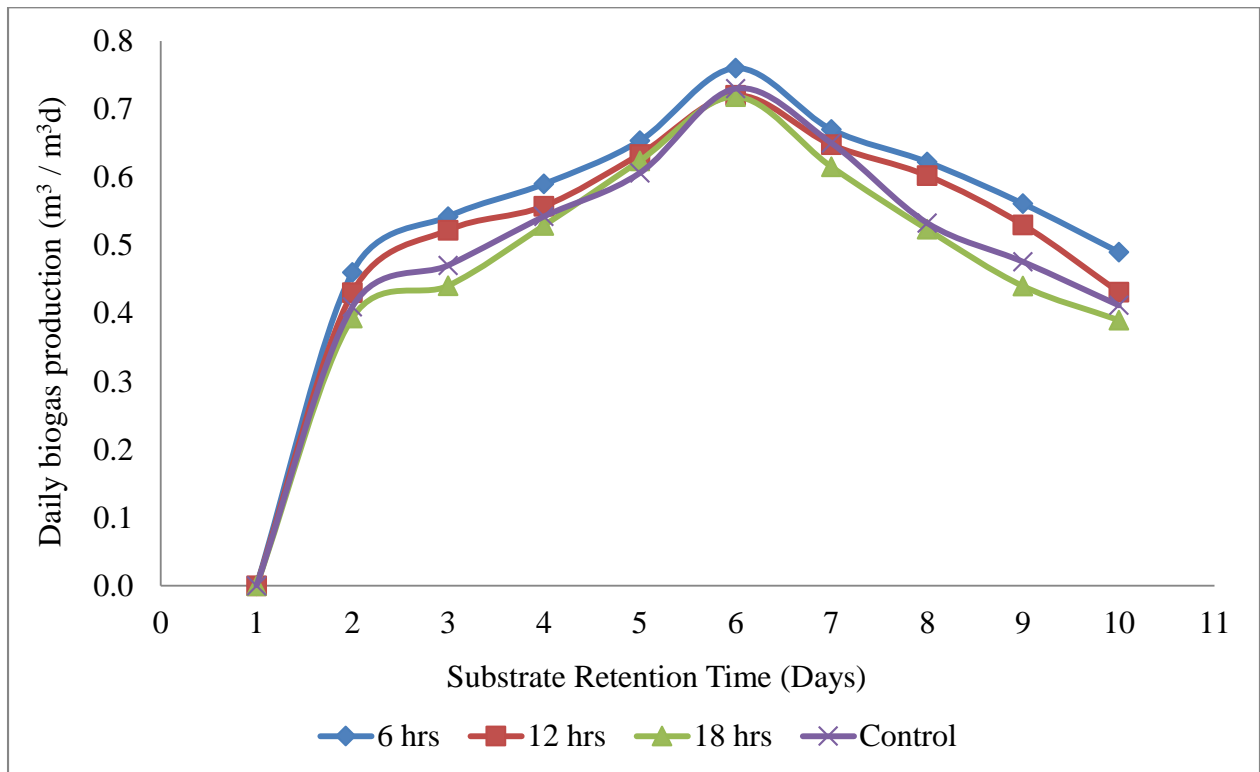


Fig 4.2: Biogas yield at different Soaking times

Biogas production for each soaking time, as in Figure 4.2, shows a low starting rate within the first few days of observation and may be referred to us the system’s lag phase. Biogas production start rates of $0.41\text{m}^3/\text{m}^3\text{d}$, $0.46\text{m}^3/\text{m}^3\text{d}$, $0.43\text{m}^3/\text{m}^3\text{d}$ and $0.39\text{m}^3/\text{m}^3\text{d}$ were obtained after 0, 6, 12 and 18 hours respectively. The death (last) phase involved retention of living cells and little growth of the population (Nopharatana et al., 2007). This behaviour can be attributed to establishment and customization of methanogens to internal digester conditions. The exponential and stationary growth phases of microbes then follow.

Co-digesting soaked chicken and goat manure with fresh cow manure significantly increased gas production (from a mean of $0.54\text{m}^3/\text{m}^3\text{d}$ at the control to peak at a mean $0.59\text{m}^3/\text{m}^3\text{d}$ after 6 hours of soaking) as seen in Table 4.7 and D12 in Appendix D ($\alpha = 0.05$, $\text{LSD} = 0.02$). Of the four times, 6-hour soaking had the highest peak daily production rate of $0.76\text{m}^3/\text{m}^3\text{d}$. Soaking times significantly influenced production differently and therefore, if

other factors of production were kept constant, soaking chicken and goat feed stocks for 6 hours before digestion, would result in the highest average production rate (for this study, $0.59\text{m}^3/\text{m}^3\text{d}$).

4.2.3 Mechanical Pretreatment

Biogas production data (subjected to ANOVA) and trends after size reduction of the chicken and goat manure by mincing at different sieve sizes are presented in Table 4.8 and D13 of Appendix D, and Figure 4.3.

Table 4.8: Daily Biogas Production after Mechanical Pretreatment

Day	Gas yield from different mincing sizes				
	Production per unit digester volume ($\text{m}^3 / \text{m}^3\text{d}$)				
	Effective particle size (mm)				
	Control	2	3	4	Average
1	0.00	0.00	0.00	0.00	0.00
2	0.41	0.38	0.51	0.48	0.44
3	0.47	0.58	0.62	0.57	0.56
4	0.54	0.66	0.68	0.67	0.64
5	0.61	0.73	0.74	0.73	0.70
6	0.73	0.75	0.78	0.77	0.76
7	0.65	0.72	0.74	0.73	0.71
8	0.53	0.62	0.65	0.63	0.61
9	0.48	0.53	0.56	0.54	0.53
10	0.41	0.48	0.52	0.48	0.47
Cumulative	4.83	5.45	5.81	5.59	5.42
Mean	0.54 ^c	0.60 ^b	0.64 ^a	0.62 ^{ba}	0.60

Control = No feed stock mincing; Means followed by the same letter(s), (a, b, c), are not significantly different at $\alpha = 0.05$, $LSD = 0.02$

Daily biogas yield from co-digesting chicken, goat and cow manure after being subjected to mechanical pretreatment is shown Table 4.8. Mincing chicken and goat manure to a particle size of 3 mm resulted in highest observed mean daily rate of $0.64 \text{ m}^3/\text{m}^3 \text{ d}$, followed by 4 mm ($0.62 \text{ m}^3/\text{m}^3\text{d}$) and 2 mm ($0.60 \text{ m}^3/\text{m}^3\text{d}$). Biogas yield from 2 mm, 3 mm and 4 mm average particle sizes resulted in $0.06 \text{ m}^3/\text{m}^3\text{d}$ (11.11%), $0.10 \text{ m}^3/\text{m}^3\text{d}$ (18.52%) and $0.08 \text{ m}^3/\text{m}^3 \text{ d}$ (14.81%) increase in production rates over the values from the of co-digestion non-pretreated

manure (control). Smaller particles increase the surface area for substrate adsorption by microbes and increased gas production up to a certain size limit below which, it may result in digester clogging and inhibit the digestion process (Yadvika et al., 2004).

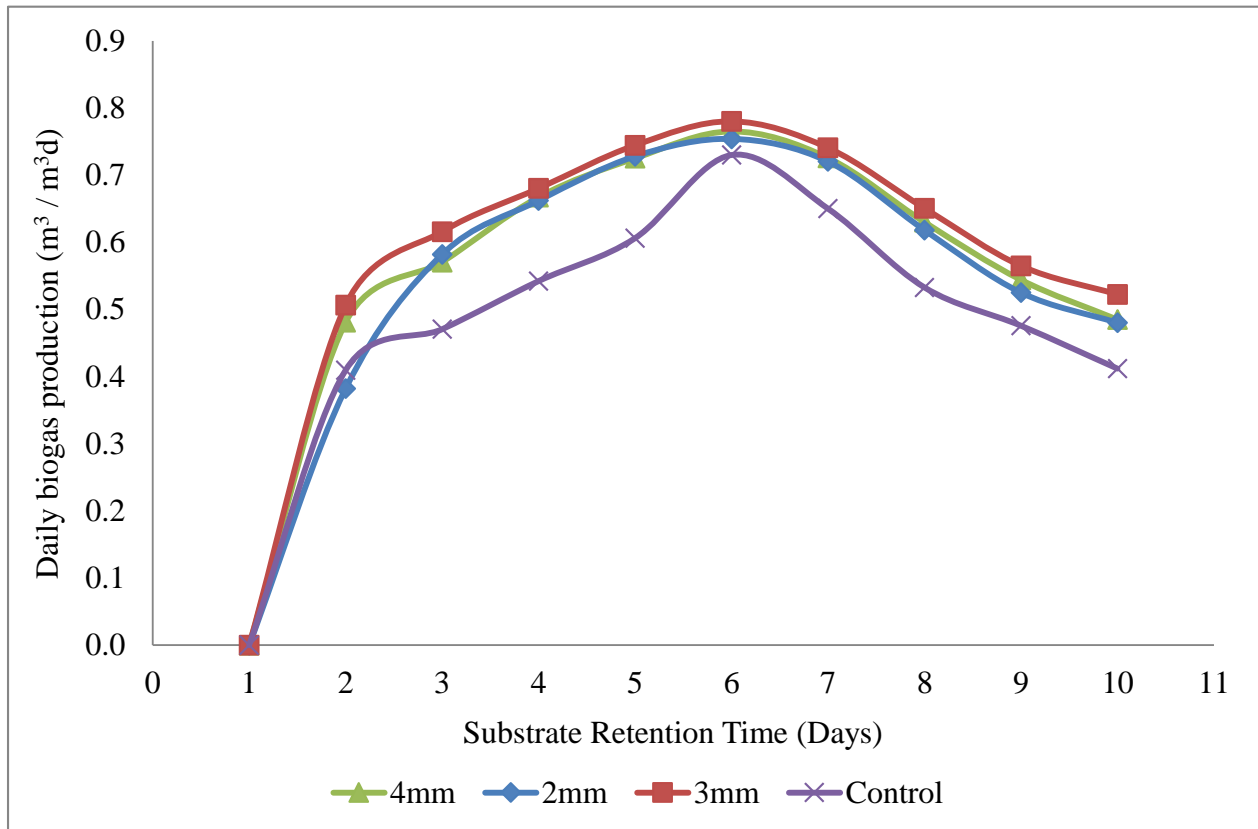


Fig 4.3: Biogas yield trend after Mechanical pretreatment

Plots of biogas yield in Fig. 4.3 indicate highest start and peak production rates with the 3 mm particle size (0.51 and 0.78 m³/m³d), followed by 4 mm (0.48 and 0.77 m³/m³d), and 2 mm (0.31 and 0.75 m³/m³d) respectively. The results are better and outside Schell and Harwood (1994) recommendations of a particle size range of 1 mm to 2 mm as the best for biogas production. The researchers used individual biomass materials for their general recommendation, while this study investigated gas production with the three manure types under co-digestion and constant digester stirring. Biogas production improved with increase in particle size up to the 3 mm size, after which there is a fall. This is in agreement with Yadvika et al. (2004) who reported that smaller particles increase the surface area for substrate adsorption by microbes and consequently, increased biogas production.

Mincing goat and chicken manure before co-digesting with fresh cow manure significantly increased gas production (at $\alpha = 0.05$, LSD = 0.02). Biogas production from different effective particle sizes significantly varied from each other, therefore, 3 mm effective

manure particle size would result in the highest production if economics had no role in choosing the best mechanical pretreatment level.

4.2.4 Thermal Pretreatment

The effect of cooking chicken and goat manure at 60, 80 and 100 °C, prior to their co-digestion with untreated cow manure is presented in Table 4.9, Figure 4.4 and the ANOVA in Table D14 of Appendix D.

Table 4.9: Daily Biogas Production after Thermal Pretreatment

Day	Gas yield from different pretreatment temperatures				
	Per unit digester volume (m ³ /m ³ d)				
	Temperature (°C)				
	Control	60	80	100	Average
1	0.00	0.00	0.00	0.00	0.00
2	0.41	0.42	0.46	0.43	0.43
3	0.47	0.52	0.55	0.54	0.52
4	0.54	0.57	0.64	0.58	0.58
5	0.61	0.65	0.68	0.66	0.65
6	0.73	0.74	0.77	0.75	0.75
7	0.65	0.62	0.69	0.66	0.66
8	0.53	0.55	0.65	0.60	0.58
9	0.48	0.52	0.59	0.56	0.54
10	0.41	0.45	0.51	0.49	0.47
Cumulative	4.83	5.04	5.53	5.28	5.17
Mean	0.54 ^d	0.56 ^c	0.62 ^a	0.59 ^b	0.57

Control = No thermal pretreatment

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$, LSD = 0.02

Thermally pretreating chicken and goat manure (Table 4.9) at 80°C and co-digesting them with untreated cow manure resulted in the highest mean daily production rate of 0.62 m³/m³d, followed by 100 °C and 60 °C (0.59 and 0.56 m³/m³d); implying 0.08 m³/m³d (14.81%), 0.05 m³/m³d (9.26 %) and 0.02 m³/m³d (3.70%) increases in production rate over the control, respectively. Subjecting the manure to increased pretreatment temperatures steadily improves biogas production (Assefa et al., 2014) as seen at 60 °C to 80 °C. However this increase in production reaches the peak at 80 °C (an optimum) from which production

decreases (Bochmann et al., 2010; Zhang et al., 2011) as subsequent drop is witnessed at 100°C. This can be due to the formation of xyloses at higher pretreatment temperatures that started becoming toxic to microorganisms (Montgomery & Bochmann, 2014).

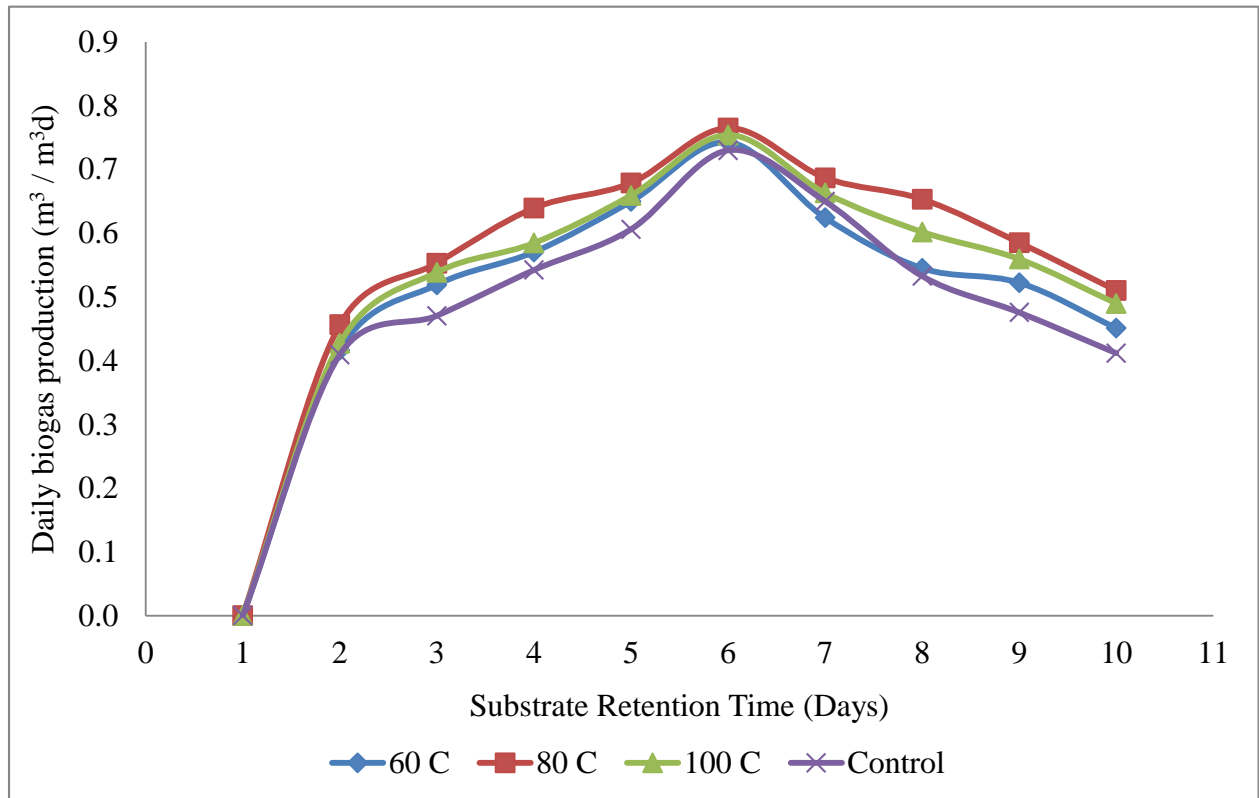


Fig 4.4: Biogas yield after different levels of Thermal pretreatment

The plot of trends indicate higher respective start and peak biogas yield rates (Fig. 4.4) after the three levels of thermal pretreatment (60°C = 0.42 and 0.74 m³/m³d, 80°C = 0.46 and 0.77 m³/m³d, 100°C = 0.43 and 0.75 m³/m³d) than those control (0.41 and 0.73 m³/m³d). This might have been caused by the thermo-chemical disintegration of cell membranes and tough bonds holding manure components together. This may have assisted in availing the organic compounds to bacterial attack and biodegradation (Ariunbaatar et al., 2014).

The slight stagnation in production happened between the 2nd and 3rd days on the control and '80 °C' gas yield plots and may be due to imbalance in favourable conditions for acid and methane formation. Such imbalances are common according to Trisaktia et al. (2015) as long as hydrolytic, acidogenic, acetogenic and methanogenic bacteria are working under the same digester conditions. Hydrolysis and acidogenesis release volatile fatty acids that reduce the digester's pH by overproducing hydrogen which consequently affect aceticlastic methanogens adversely (Iyagba et al., 2009). This can be due to the different bacteria involved in AD having varying physiological and nutritional requirements (Speece, 1985). Steady production was,

however, restored afterwards. Hydrogenotrophic methanogens rapidly consumed the excessive hydrogen with continued biogas production to consequently help maintain pH levels favourable for acetoclastic methanogens (Stams & Plugge, 2009).

The exposure of goat and chicken manure to elevated heat for 20 minutes before co-digesting with fresh cow manure significantly improved gas production ($\alpha = 0.05$, $LSD = 0.02$) as in Table D14 of Appendix D. Different heating temperatures had significant variation in gas production; therefore, heating chicken and goat manure at 80° C before digester feeding with cow manure would offer the best thermal pretreatment level, if costs of energy for heating were not of significance.

4.2.5 Comparative effect of Hydro, Thermal and Mechanical Pretreatment on Biogas Yield Rate

Different pretreatment methods have different degrees of manure disruption for enhanced hydrolysis and eventual biogas production. Table 4.10 and D15 in Appendix D compare effect of the three different pretreatment methods on biogas yield rate.

Table 4.10: Biogas Production rate after Pretreatment

Pretreatment method	Mean rate (m ³ / m ³ d)	Increase in production over control (%)
Control	0.54 ^d	-
Hydro	0.55 ^c	1.85
Thermal	0.57 ^b	5.56
Mechanical	0.60 ^a	11.11
Mean	0.57	5.56

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$ using $LSD = 0.01$

The three pretreatment methods had a significant effect on biogas yield rate and varied from each other at $\alpha = 0.05$, $LSD = 0.01$ (Table 4.10 and D14). Respectively, the highest yield was 0.60 m³ /m³d, followed by 0.57 and 0.55 m³ /m³d, which are 11.11%, 5.56% and 1.85% increases over and above the control. Higher increase in yield after mincing could be attributed to enhanced outer cell wall and cover disruption of goat and chicken manure that enhanced micro-bacterial attack for ease of hydrolysis (Montgomery & Bochmann, 2014; Mudhoo et al.,

2012). This was evident in the digestate that appeared homogenous; unlike from hydro and thermal pretreatment where undamaged goat manure balls were observed.

These findings also implied that pretreatment/co- digestion had a positive effect as it resulted in more specific gas yield above pure substrates. Since hydrolysis is a rate-determinant in anaerobic digestion (Yoon et al., 2014), pretreatment helps in particle disintegration prior to feeding into the digester, making the substrate more available to anaerobic bacteria and hence initiates earlier biogas production. This may also have been due to co-digestion and maintenance of correct operating temperatures at 35 °C that provided more favourable system conditions. This concurs with Sebola et al. (2014) who reported a temperature range of 35 °C - 37 °C as optimum for biogas production in anaerobic digestion under mesophilic conditions.

The percentage increases in gas production can guide in the choice of pretreatment method based on the prevailing comparative advantages at the area of production unless economics restrict the production. However economies of scale may offset pretreatment costs. The results imply that mechanical pretreatment method at 3 mm particle size can be recommended for large industrial and institutional plants and while hydro pretreatment can be adopted by local small- scale biogas plant operators to improve gas output from co-digestion of the three biomass materials; dependent on prevailing comparative advantages and economies of scale. Peak daily biogas production rate of 0.78 m³/m³d from 3mm mechanical mincing on the 6th day (as in Table 4.8) under controlled (laboratory) digestion conditions was used to design a similar digester. This presented in Appendix E and could offer the same production rate under uncontrolled (field conditions). The mechanical method was subsequently selected for experimental runs for objective two.

4.3.1 Effect of Inoculums and Total Solids levels of Pretreated Manure on Biogas Production

4.3.1 Effect on Inoculum levels on Biogas Production

Average biogas production rates from different inoculum levels of pretreated chicken-goat and untreated cow manure is presented in Tables 4.11 and D16 (Appendix D), with the different production trends being presented in Figures 4.5 and 4.6.

Table 4.11: Biogas Production Rates from different Substrate to Inoculum ratios

Day	Gas yield from different Substrate to Inoculum ratios					
	Production per unit digester volume (m ³ /m ³ d)					
	Substrate to Inoculum ratio					
	6:1	5:1	4:1	3:1	2:1	Mean
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.41	0.42	0.44	0.43	0.42	0.42
3	0.45	0.55	0.57	0.54	0.47	0.51
4	0.53	0.58	0.68	0.59	0.54	0.58
5	0.60	0.65	0.72	0.66	0.62	0.65
6	0.67	0.70	0.75	0.72	0.67	0.70
7	0.61	0.60	0.66	0.64	0.62	0.63
8	0.56	0.55	0.58	0.56	0.56	0.56
9	0.53	0.54	0.55	0.54	0.54	0.54
10	0.47	0.50	0.52	0.52	0.48	0.50
Cumulative	4.83	5.09	5.46	5.19	4.92	5.10
Mean	0.54 ^d	0.57 ^{cb}	0.61 ^a	0.58 ^b	0.55 ^{dc}	0.57

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$, $LSD = 0.02$

It is evident that increasing the amount of inoculum (Cow substrate inoculum – CSI) significantly increased biogas yield up to an optimal ratio of 4:1(20% inoculum addition) with a yield rate of 0.61 m³/m³d (Table 4.11). Further addition of inoculums led to a decrease in biogas production ($\alpha = 0.05$, $LSD = 0.02$) which can attributed to a better system balance offered the amount suitable amounts of substrate and inoculum in the digester at 20% inoculum addition (S/I = 4:1). This is also confirmed by Brown and Li (2013) who observed a decrease in biogas production with increasing S/I ratios of food yard waste and food waste. The other combinations may have received setbacks of unbalanced substrate to inoculum ratio (Asante-Sackey et al., 2018) due to excessive proportions of inoculum occupying more digester volume hence reducing its organic loading (Ma et al., 2019).

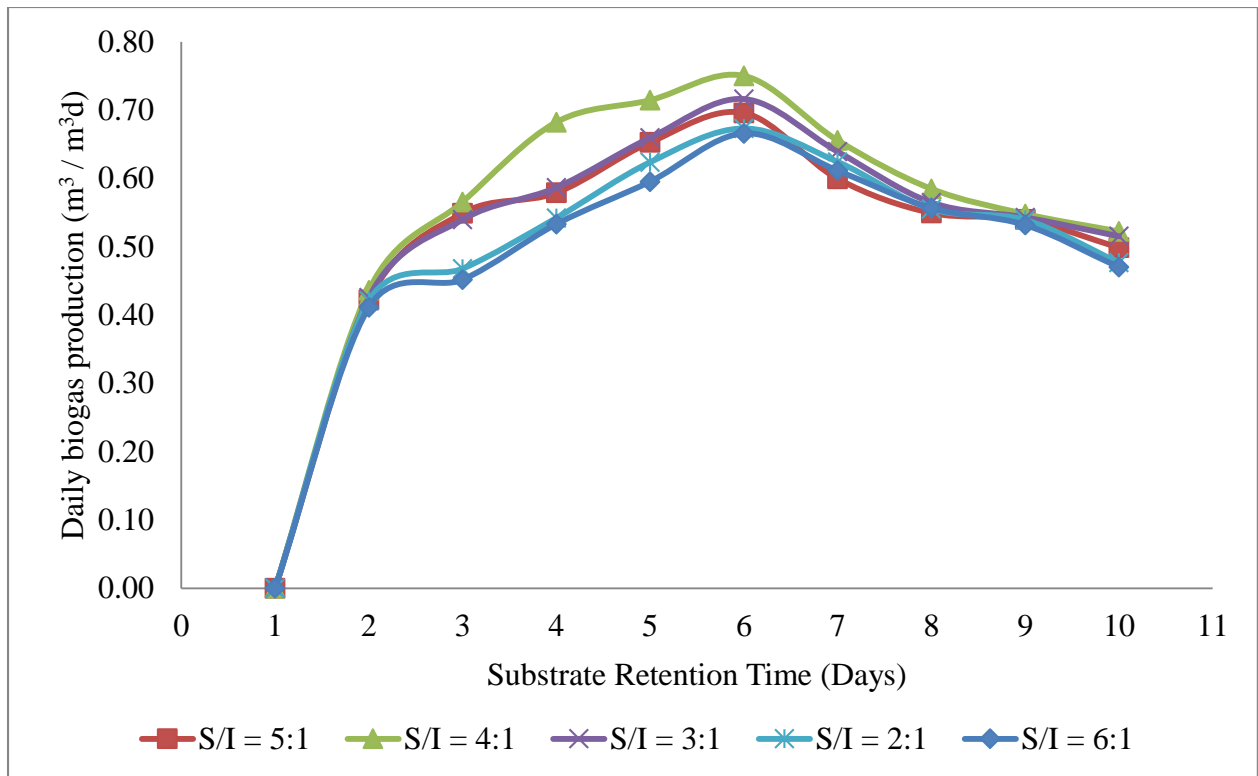


Fig 4.5: Biogas production at different Substrate to Inoculum ratios

Biogas from all the experimental runs began on the 2nd day throughout the entire period to the set 10 days of AD with the highest start rate of 0.44 m³/m³d at S/I = 4:1, followed by S/I = 3:1, 2:1, 5:1 and 6:1 (0.43, 0.42, 0.42 and 0.41 m³/m³d) (Fig 4.5). Peak production rates of 0.67, 0.70, 0.75, 0.72 and 0.67 m³/m³d were realized for S/I = 6:1, 5:1, 4:1, 3:1 and 2:1 respectively, on the 6th day. Higher gas yields at lower S/I ratios (more inoculum addition) may be attributed to the increased inoculums for more methanogenic bacteria that facilitated the effective conversion of volatile fatty acids into biogas (Lin et al., 2014; Yang et al., 2015). Biodegradation of inoculum volatile solids might be occurring simultaneously with that of the substrate in the reactor (Yoon et al., 2014).

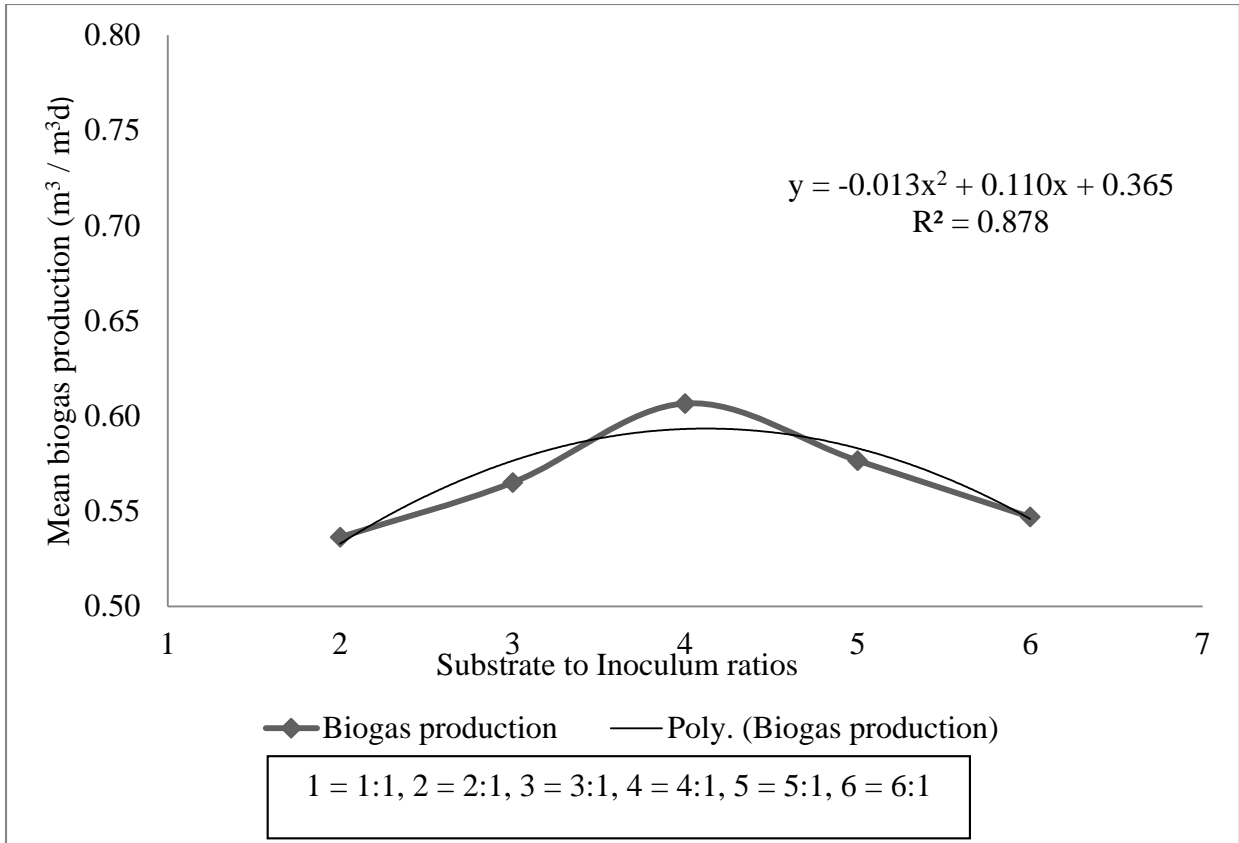


Fig 4.6: Average biogas production rate from different Substrate to Inoculum ratios

Anaerobically digesting the three at different inoculum levels resulted in highest mean biogas production rate of 0.61 m³/m³d (S/I = 4:1), followed by 0.58, 0.57, 0.55 and 0.54 m³/m³d (S/I = 3:1, 5:1, 2:1 and 6:1) (Fig 4.6). Average production rate from co-digesting the chicken, goat and cow manure at various substrate to inoculum ratios can be predicted (as guided by Fig 4.6) by using (Equation 4.1);

$$y = -0.013x^2 + 0.110x + 0.365 \quad (4.1)$$

Where;

y = Average biogas production rate (m³/m³d)

x = Substrate to Inoculum ratio

The model's R² value of 0.878, is within the range of R² = 0.75 – 1 that is recommended by Niladevi et al. (2009), hence it represents 87.8% of the variation in actual data. Biogas production exhibits an inverse relationship with increase inoculum (Liu et al., 2009; Zhou et al., 2017) as lower S/I ratios might cause system instability hence low gas output (Fathya et al., 2014). Excessive S/I ratios can lead to VFA accumulation, inhibition and incomplete biomass degradation, hence lower biogas yield (Budiyono et al., 2014; Sarker et al., 2019), and careful compromise on the ratios is key, given the type of substrate and inoculum.

Therefore, the relationship in equation 4.1 can be developed for different substrate conditions for future universality. This can be used by biogas plant operators to predict gas production from their plants at different ratios, depending on availability of substrate contents and cow manure inoculum.

4.3.2 Effect of Total Solids on Biogas Production

Biogas production data and trends from different total solids are presented in Tables 4.12 and D17 (Appendix D) and Figure 4.7. The experiments were set up as in section 3.3.2 of the Materials and Methods.

Table 4.12: Biogas Production at different Total Solids

Day	Gas yield from different Total Solids							
	Production per unit digester volume (m ³ /m ³ d)							
	Total solids (%)							
	7.50	8.00	8.50	9.00	9.50	10.00	10.50	Mean
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.41	0.42	0.44	0.43	0.43	0.43	0.41	0.42
3	0.46	0.55	0.57	0.57	0.56	0.45	0.43	0.51
4	0.53	0.68	0.68	0.69	0.68	0.58	0.52	0.62
5	0.60	0.70	0.71	0.72	0.71	0.66	0.59	0.67
6	0.67	0.73	0.75	0.76	0.76	0.68	0.66	0.72
7	0.62	0.66	0.68	0.70	0.70	0.64	0.62	0.66
8	0.58	0.62	0.63	0.65	0.63	0.60	0.58	0.62
9	0.50	0.56	0.56	0.61	0.55	0.56	0.50	0.55
10	0.43	0.47	0.53	0.54	0.53	0.51	0.43	0.49
Cumulative	4.81	5.39	5.55	5.67	5.55	5.11	4.75	5.26
Mean	0.53 ^d	0.60 ^b	0.62 ^{ba}	0.63 ^a	0.62 ^{ba}	0.57 ^c	0.53 ^d	0.58

Means followed by the same letter(s), (a, b, c, d), are not significantly different at $\alpha = 0.05$, $LSD = 0.02$

Mean biogas production rate significantly increased at $\alpha = 0.05$; $LSD = 0.02$ (Table D17 of Appendix D) with an increase in total solids up to 9% where maximum yield rate of 0.63 m³/m³d was realized. Continued increase in the total solids being fed to the digester exhibited an inverse relationship with biogas production as seen with 9.5%, 10.0% and 10.5% total solids (0.62, 0.57 and 0.53 m³/m³d). A slight increase in total solids marginally improves

gas yield up to some point (7.5% to 9.0%), after which further increase in total solids no longer results in increased biogas production (9.0% to 10.5%), as reported in previous works by Igoni et al. (2008) and Masinde et al. (2020). This is attributable to higher organic loading rates and volatile fatty acid in the digester that leads to mesophilic bacteria decline (Kiener & Leisinger 1983; Yi et al., 2014), and digester clogging (Igoni et al., 2008). Total solids of 7.5% to 9.0% might have provided more moisture content that enhanced mass transfer during the process (Lin et al., 2014; Yang et al., 2015) whereas the lower amount of water at higher total solids above 9.0% reduced microbial activity, hence lower biogas production Igoni et al. (2008).

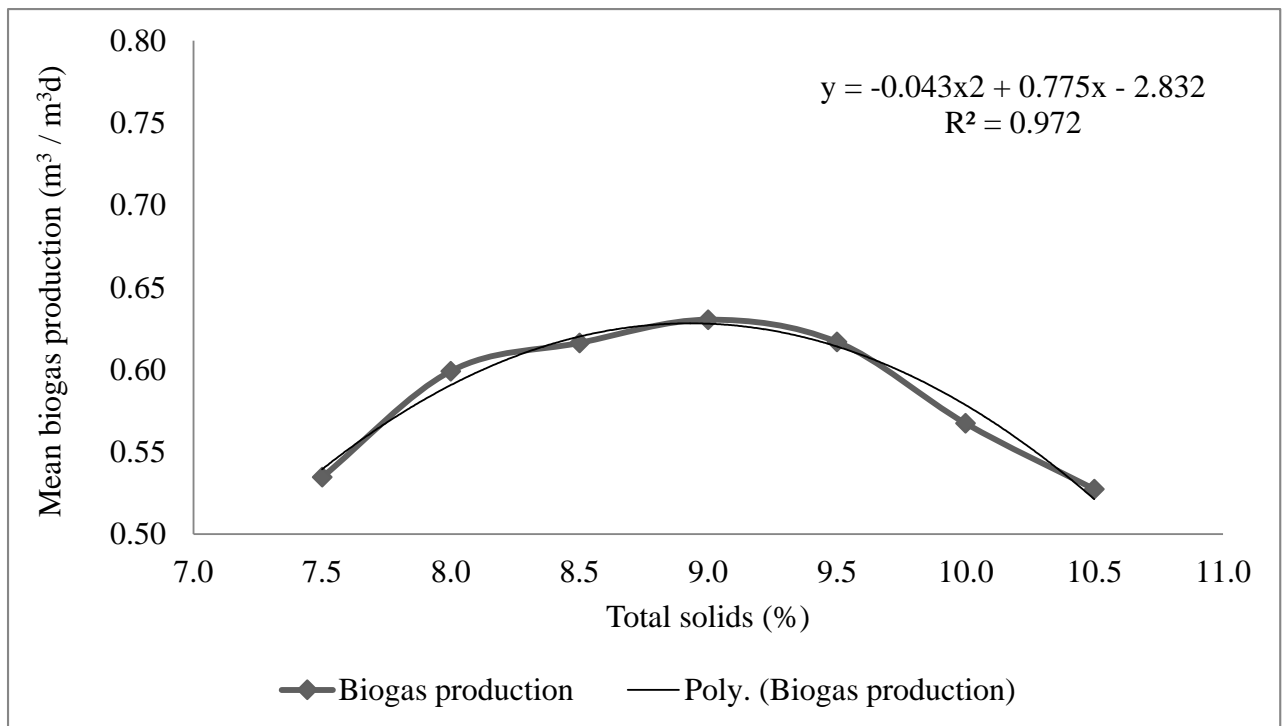


Fig 4.7: Average biogas production rate from different Total Solids

The trend in figure 4.7 indicates that average production rate from co-digesting the three at different total solids; it can be predicted using (Equation 4.2).

$$y = -0.043x^2 + 0.775x - 2.832 \quad (4.2)$$

Where;

y = Average biogas production rate (m³/m³d)

x = Total solids (%)

The model took care of 97.2% ($R^2 = 0.972$) of variations in actual data hence can be used by biogas plant operators to predict biogas plants' production when using chicken-goat-cow manure substrate, with the best total solids loading being 9.0%. Highest mean production rate of 0.63m³/m³d was realized at 9.0% total solids. This result is within the range of 8 – 10% for most feed stocks as reported by Budiyo and Sumardiono (2014), Abbassi-Guendouz et al. (2012); Orhororo et al. (2017) and Paramaguru et al. (2017).

Inadequate microorganisms (low inoculum volume) for substrate degradation may derail the methane production process (Prashanth et al. 2006; Zhou et al., 2011) whereas too high inoculum levels may lead to low cumulative biogas yield due to inadequate organic nutrients for the microorganisms leading to their decline. A careful integration between total solids and inoculums is therefore required to get high biogas production rates from the manures, as a result of enhanced microbial activity, facilitated mass transfer and nutrient stability offered to the methanogenic bacteria. Therefore an effective way of improving biogas yield from chicken, goat and cow manure would require optimizing the S/I ratio and TS of the influent.

4.4 Optimization of Biogas Production

The optimization process requires statistically designing experiments based on individual factors, carrying them out, estimating the factor coefficients in a mathematical model and predicting the response while checking the adequacy of the model (Körbahti & Rauf, 2008; Mondal et al., 2013).

4.4.1 Prediction of Biogas Production Rate

Table 4.13 shows the Central composite design matrix, observed and predicted values for biogas production rate. Analysis of Variance by Response Surface Methodology was done as in Table F1 of Appendix F. The predicted values that corresponded to each actual value for every factor mix, were generated using equation 4.3 below (similar to equation 3.10 in section 3.4.2).

$$Y = -275.20 + 12.25X_1 + 69.42X_2 + 28.16X_3 + 1.53X_1X_2 - 0.97X_1X_3 + 1.22X_2X_3 - 2.88X_1^2 - 4.57X_2^2 - 6.22X_3^2 \quad (4.3)$$

Where;

Y = biogas production rate ($\text{m}^3/\text{m}^3\text{d}$)

X₁ = substrate to inoculum ratio (2:1 - 6:1)

X₂ = total solids (8 - 10%)

X₃ = effective minced particle size (2 - 4 mm)

The positive coefficients indicated the synergistic effects whereas negative coefficients hinted antagonistic effects on biogas production rate, caused by individual factors (quadratic) or their double interactions (Behera et al., 2018; Sadhukhan et al., 2016). For instance, an interaction of substrate to inoculum ratio with effective minced particle size would cause a decrease in biogas production rate, while that involving total solids and effective particle size would positively affect yield rate. It is also evident that self-interaction within the three individual factors (quadratic) would negatively affect biogas production rate.

Table 4.13: Experimental and Predicted Biogas Production Rate

Run	Factors			Biogas production rate	
	X ₁ :S/I(ratio)	X ₂ : TS (%)	X ₃ :Size(mm)	Actual (m ³ /m ³ d)	Predicted (m ³ /m ³ d)
1	6.00	10.00	4.00	0.36	0.42
2	4.00	9.00	3.00	0.59	0.58
3	7.36	9.00	3.00	0.39	0.37
4	4.00	10.68	3.00	0.47	0.46
5	6.00	8.00	4.00	0.38	0.40
6	2.00	10.00	2.00	0.41	0.38
7	2.00	8.00	4.00	0.45	0.46
8	4.00	7.32	3.00	0.51	0.53
9	6.00	8.00	2.00	0.48	0.47
10	0.64	9.00	3.00	0.33	0.36
11	4.00	9.00	3.00	0.58	0.58
12	6.00	10.00	2.00	0.46	0.45
13	4.00	9.00	3.00	0.57	0.58
14	4.00	9.00	4.68	0.50	0.44
15	2.00	8.00	2.00	0.53	0.48
16	2.00	10.00	4.00	0.38	0.40
17	4.00	9.00	3.00	0.58	0.58
18	4.00	9.00	3.00	0.59	0.58
19	4.00	9.00	1.32	0.42	0.49
20	4.00	9.00	3.00	0.57	0.58
Mean				0.48	0.48

The predicted values were closer to the actual ones; with the highest biogas production rate being 0.59 m³/m³d at a substrate to inoculum ratio of 4:1, total solids of 9% and an effective minced particle size of 3mm (from Table 4.13). Differences between actual and predicted values were less than 20% (Subha et al., 2015) hence a good relationship between the two sets of data. Values generated by equation 4.3 are slightly above the reported 0.50 m³/m³d (Masinde et al., 2020) for cow manure in fixed dome laboratory digester and could be due to goat and chicken manure particle size reduction and co-digestion of the two with cow manure. Co-digestion balances the availability of nutrients, offers system buffering and reduces inhibition

by toxic compounds (Zamanzadeh et al., 2017), and increases substrate degradation and biogas production rates (Aichinger et al., 2015; Labatut et al., 2014).

Experimental errors like loss of gas and monitoring equipment could have caused the outliers exhibited in the table. The model had a high R^2 of 0.7681 (Table F1 in Appendix F), which indicates a good co-relation between observed and predicted data (Masinde et al., 2020).

4.4.2 Significance and Adequacy of the Model

The null hypothesis of the individual factors and their interactive effect on biogas production rate, and the probable models to predict biogas production rate was tested using the probability of F-statistics (Prob > F). ANOVA and adequacy tests for the response surface quadratic model suggested by CCD are as in Table F2 in Appendix F. The model gave a mean biogas production of $0.48\text{m}^3/\text{m}^3\text{d}$ an F-value of 7.99, an adjusted R^2 of 0.7681, an adequate precision of 7.538, a coefficient of variation of 8.59 and a standard deviation of 6.16.

Factors with a Prob > F value less than 0.05 were reported as being significant (Masinde et al., 2020; Saha et al., 2010). The model's F-value of 7.99 meant the model was significant and there was less than 0.01% probability the value could be due to noise. A greater calculated F-value for the model than the tabulated suggest that the model could adequately be used to predict experimental data (Ani et al., 2015). Quadratic effects of substrate to inoculum ratio (X_1^2), total solids (X_2^2) and effective minced particle size (X_3^2) were significant. Their linear effects (X_1, X_2, X_3), and their interactive effects (X_1X_2 , X_1X_3 and X_2X_3) on biogas production rate were insignificant.

An adjusted co-relation coefficient (R^2) value was estimated to be 0.7681, which was within the recommended range of 0.70 to 1 (Sadhukhan et al., 2016). This meant that 78.61% of the total variation on biogas production rate data could be described by the model. The model also had an adequate precision (signal-to-noise ratio) of 7.538 which was greater than the recommended base value of 4 (Mason et al., 2003; Sadhukhan et al., 2016), an indication that the model has an adequate signal. Since the above requirements are met, the quadratic model can therefore be used to explore the design space and obtain optimal conditions for biogas production rate from the chicken-goat-cow manure substrate.

A coefficient of variation value (CV) of 8.59% was below the recommended 10% and hence indicated sufficient reliability of the experiment (Rasouli et al., 2015). The Prob > F value was used to test the significance of each model by its terms to the preceding model. It can be seen (Table F2 in Appendix F) that adding the sequential sum of squares for the

quadratic terms (X_1^2 , X_2^2 and X_3^2) to the 2FI model (with individual: X_1 , X_2 , X_3 and two-factor interaction: X_1X_2 , X_1X_3 , X_2X_3) improved the prediction model since a small p-value (Prob>F) of 0.0001 was found. CCD suggested the quadratic model as it best fitted the data.

4.4.3 Verification of the Predicted Model

A comparison between verification and predicted biogas production rates at suggested optimal factor level combination is shown in Table F3 of Appendix F. It was observed that under optimal conditions suggested by the model, biogas production rate was 0.61 m³/m³d, 0.60 m³/m³d and 0.60 m³/m³d, representing 3.38%, 1.69% and 1.69% increases above the statistically predicted value of 0.59 m³/m³d. These differences were less than 20% (Subha et al., 2015) and therefore proved that verified values adequately matched the predicted result under optimal conditions.

Experimental design methods may offer direct benefits; however, the statistically estimated optimal conditions require validation (Mu et al., 2006). The results obtained therefore confirmed that the biogas production process from chicken-goat-cow manure could satisfactorily be experimentally designed and developed by RSM and CCD.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the findings from the three specific objectives, the following conclusions are drawn;

Hydro, mechanical and thermal pretreatment of goat and chicken manure averagely resulted in 0.01 m³/m³d (1.85%), 0.01 m³/m³d (11.11%) and 0.01 m³/m³d (5.56%) increase in mean biogas production rate respectively, when co-digested with cow manure. Maximum increase in production for each pretreatment was at 6 hour soaking time (9.30%), 3 mm effective manure particle sizes (18.52%) and 80°C of heating (14.81%) that represented the hydro, mechanical and thermal pretreatments of the manures. Co-digestion increased mean biogas production rate over mono-digestion by 0.20 m³/m³d: 68.97% (chicken), 0.22 m³/m³d: 81.84% (goat) and 0.05 m³/m³d: 8.80% (cow manure).

Biogas yield rate increased with the addition of inoculum up to a maximum of 0.61m³/m³d at S/I = 4:1 (20% inoculum addition), after which further increase in inoculum (lower S/I ratios) resulted in lower gas production. Biogas production increased steadily with increase in total solids from 7.5% to 9.0% (0.63 m³/m³d) and declines as total solids increase further above 9.0%. Therefore, inoculum addition of 20% and total solids of 9% would be ideal for the co-digestion of chicken, goat and cow manure.

Optimization of biogas production rate from chicken-goat-cow manure mixture showed that substrate to inoculum ratios, effective minced particle size and total solids had a significant effect on biogas production. The interaction among the factors enhanced biogas production rate as modeled by Response Surface Methodology. Highest biogas production of 0.59 m³/m³d was achieved at the following optimal factor levels; substrate ratio of 4:1, total solids of 9% and effective chicken-goat manure minced particle size of 3 mm. There was a close agreement between the expected and obtained production rates for each factor level combination.

With these positive results, local farmers can improve uptake of chicken and goat manure as substrates for sustainable renewable energy production and reduce uncontrolled greenhouse gas emissions from open manure dumps.

5.2 Recommendations

Further studies can be done to determine the following;

- i. Interactive effect of selectively combining the three manure pretreatment methods at their optimal levels of (6 hour soaking times, 3 mm effective sizes and heating at 80°C) on biogas production from the three materials.
- ii. Initial total solids of all promising manures and feed stocks in the study region versus mono and co-digestion of various manure combinations at different mix ratios to confirm the optimal range of substrate total solids and inoculum ratios
- iii. Undertake recommendations 1 and 2 in lab conditions as such versus ordinary field conditions for possible advice to biogas producers versus policy, management and economic quantification and qualifications.

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APPENDICES

Appendix A: Pretreatment Methods and Feed stock characteristics

Table A1: Pretreatment Methods

Pretreatment method	Experimental levels	Process Specifications	Gas production Rate (m ³ /m ³ d)
Hydro	H ₁	6 hr	
	H ₂	12hr	
	H ₃	18hr	
Thermal	T ₁	60°C	
	T ₂	80°C	
	T ₃	100°C	
Mechanical	M ₁	2mm	
	M ₂	3mm	
	M ₃	4mm	

To be completed

Table A2: Total solids

Feed stock	Total Solids (%) after Pretreatment				
	Trial	*None	Hydro	Mechanical	Thermal
Chicken manure	1	87.90	8.04	88.98	88.42
	2	88.32	8.08	88.94	88.38
	3	87.66	7.96	89.00	88.40
	Mean	87.96	8.03	88.97	88.40
Goat manure	1	31.93	7.94	33.52	31.49
	2	31.12	8.02	33.57	31.58
	3	30.00	8.10	33.49	31.51
	Mean	31.02	8.02	33.52	31.53
Cow manure	1	14.73			
	2	14.69	-	-	-
	3	14.75			
	Mean	14.72			

**None = No pretreatment*

Table A3: Moisture content

Feed stock	Pretreatment method				
	Moisture content (%)				
	Trial	*None	Hydro	Mechanical	Thermal
Chicken manure	1	12.34	92.04	11.00	11.60
	2	11.68	91.92	11.06	11.62
	3	12.10	91.96	11.02	11.58
	Mean	12.04	91.97	11.03	11.60
Goat manure	1	68.88	91.08	66.43	68.42
	2	68.07	92.96	66.48	68.51
	3	70.00	91.90	66.51	68.48
	Mean	68.98	91.98	66.47	68.47
Cow manure	1	85.25			
	2	85.31	-	-	-
	3	85.27			
	Mean	85.28			

**None = No pretreatment*

Table A4: Volatile Solids

Feed stock	Pretreatment method				
	Volatile Solids (%)				

	Trial	*None	Hydro	Mechanical	Thermal
Chicken manure	1	40.46	33.23	33.56	33.63
	2	23.32	33.57	33.47	34.72
	3	36.39	33.28	33.28	32.30
	Mean	33.39	33.26	34.10	33.55
Goat manure	1	18.50	17.55	19.18	19.65
	2	18.06	17.99	18.90	20.02
	3	16.32	17.62	19.10	20.09
	Mean	17.63	17.72	19.06	19.92
Cow manure	1	21.02			
	2	18.82			
	3	20.51	-	-	-
	Mean	20.12			

**None = No pretreatment*

Table A5: Bulk Density

Feed stock	Pretreatment method				
	Bulk Density (kg/ m3)				
	Trial	*None	Hydro	Mechanical	Thermal
Chicken manure	1	649.72	996.91	710.97	643.40

	2	750.32	998.72	716.79	689.54
	3	732.50	1001.45	725.48	689.20
	Mean	710.85	999.03	717.75	674.05
Goat manure	1	795.12	997.83	811.09	819.48
	2	819.48	999.75	810.05	783.52
	3	825.77	1000.63	829.95	751.13
	Mean	813.46	999.40	817.03	784.71
Cow manure	1	980.85			
	2	955.72			
	3	947.48	-	-	-
	Mean	961.35			

*None = No pretreatment

Table A6: Carbon to Nitrogen ratio (C/N)

Feed stock	Pretreatment										Mean
	Re p men t		*None		Hydro		Mechanical		Thermal		
			% in feed stock	C/N ratio	% in feed stock	C/N ratio	% in feed stock	C/N ratio	% in feed stock	C/N ratio	
Chicken manure	1	C	27.10	13.62	27.00	13.57	27.20	13.60	26.50	13.38	
		N	1.99		1.99		2.00		1.98		

	2	C	27.20	13.68	26.90	13.65	27.00	13.64	26.30	13.42	
		N	1.99		1.97		1.98		1.96		
	3	C	27.40	13.70	27.30	13.65	26.90	13.65	26.80	13.40	
		N	2.00		2.00		1.97		2.00		
Mean		C	27.23	13.67	27.07	13.62	27.03	13.63	26.53	13.40	13.58
		N	1.99		1.99		1.98				
Cow manure	1	C	39.60	27.89	39.90	27.90	39.80	28.03	38.80	27.71	
		N	1.42		1.43		1.42		1.40		
	2	C	39.50	28.01	39.70	27.96	39.90	28.10	39.20	27.80	
		N	1.41		1.42		1.42		1.41		
	3	C	39.80	27.83	39.00	27.86	39.70	28.16	38.30	27.75	
		N	1.43		1.40		1.41		1.38		
Mean		C	39.63	27.91	39.83	27.91	39.80	28.09	38.77	27.76	27.92
		N	1.42		1.42		1.42		1.40		
Goat manure	1.0	C	38.70	18.79	38.90	18.79	38.80	18.56	38.60	18.47	
		N	2.06		2.07		2.09		2.09		
	2.0	C	38.50	18.87	38.80	18.83	38.60	18.74	38.40	19.01	
		N	2.04		2.06		2.06		2.02		
	3.0	C	38.80	18.93	38.70	19.06	38.60	18.65	38.50	18.97	
		N	2.05		2.03		2.07		2.03		
Mean		C	38.67	18.86	38.80	18.89	38.67	18.65	38.50	18.82	18.81
		N	2.05		2.05		2.07		2.05		

*None = No pretreatment

Overall C/N ratio due to pretreatment was given by;

$$R = \frac{M_{CD}[C_{CD}(100 - MC_{CD})] + M_{CM}[C_{CM}(100 - MC_{CM})] + M_{GM}[C_{GM}(100 - MC_{GM})]}{M_{CD}[N_{CD}(100 - MC_{CD})] + M_{CM}[N_{CM}(100 - MC_{CM})] + M_{GM}[C_{GM}(100 - MC_{GM})]}$$

Substituting the average values of % Carbon and % Nitrogen into the above equations yielded the following;

$$R_{NP} = \frac{24.75[39.63(100 - 84.82)] + 2.5[27.23(100 - 11.61)] + 13.40[38.67(100 - 66.42)]}{24.75[1.42(100 - 84.82)] + 2.5[1.99(100 - 11.61)] + 13.40[2.05(100 - 66.42)]} = 20.21$$

$$R_{HP} = \frac{24.75[39.83(100 - 84.82)] + 2.5[27.07(100 - 11.61)] + 13.40[38.80(100 - 66.42)]}{24.75[1.42(100 - 84.82)] + 2.5[1.99(100 - 11.61)] + 13.40[2.05(100 - 66.42)]} = 20.26$$

$$R_{MP} = \frac{24.75[39.80(100 - 84.82)] + 2.5[27.03(100 - 11.61)] + 13.40[38.67(100 - 66.42)]}{24.75[1.42(100 - 84.82)] + 2.5[1.98(100 - 11.61)] + 13.40[2.07(100 - 66.42)]} = 20.15$$

$$R_{TP} = \frac{24.75[38.77(100 - 84.82)] + 2.5[26.53(100 - 11.61)] + 13.40[38.50(100 - 66.42)]}{24.75[1.40(100 - 84.82)] + 2.5[1.98(100 - 11.61)] + 13.40[2.05(100 - 66.42)]} = 20.01$$

Where;

R= Overall substrate C/N ratio resulting after no (NP), hydro (HP), mechanical (MP) and thermal pretreatment (TP)

Table A7: Feed stock and dilution water weight used to attain respective Total Solids

Total solids (%)	Chicken manure		Goat manure		Cow dung		Total Substrate (120 litres)	
	F	W	F	W	F	W	F	W
7.5	2.25	24.12	11.16	38.73	22.28	21.45	35.66	84.33
8.0	2.40	23.97	11.90	37.99	23.77	19.96	37.77	82.22
8.5	2.55	23.82	12.65	37.24	25.25	18.48	40.42	79.58
9.0	2.70	23.67	13.39	36.50	26.74	16.99	42.80	77.20
9.5	2.85	23.52	14.14	35.75	28.22	15.51	45.17	74.82
10.0	3.00	23.37	14.88	35.01	29.71	14.02	47.55	72.44
10.5	3.15	23.22	15.62	34.27	31.19	12.54	49.93	70.07

Key: F = Feed Stock, W = Water

Appendix B: Experimental Plates

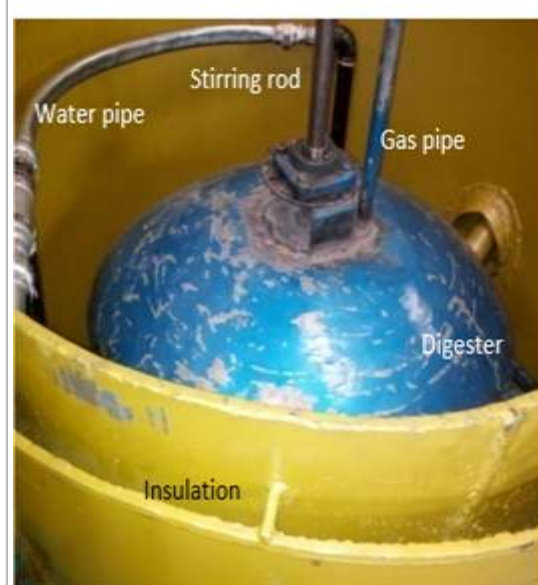


Plate B1: Assembly of Equipment for the Experiment

Plate B2: Bio-reactor



Plate B3: Expansion Chamber

Plate B4: Water tank with immersion heaters

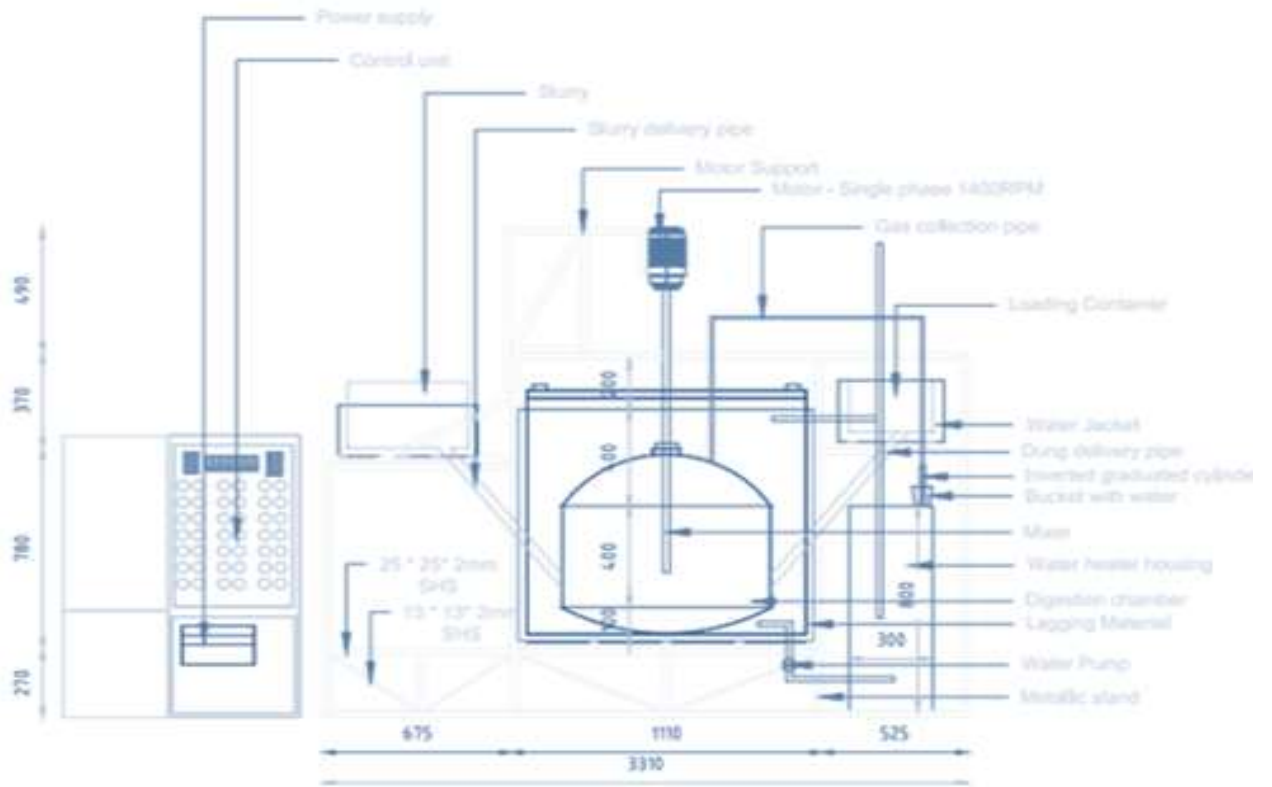


Plate B5: Cross section of the Laboratory bio-digester



Plate B6: Chicken, Cow and Goat manure prior to pretreatment



Plate B7: Hydro pretreatment



Plate B8: Mechanical pretreatment



Plate B9: Thermal pretreatment



Plate 10: Gas Analysis

Appendix C: Total Solids standardization

Regulation of TS content to x% and making 120 litres of substrate is by the following procedure;

$$TS_I = y\%$$

Hence for every 1kg of substrate,

$$M_{SI} = \frac{y}{100} kg$$

Where

TS_I = Initial substrate Total solids content

M_{SI} = Initial mass of solids in substrates

To prepare x % TS of influent, mass of influent required is given by;

$$M_I = \frac{\frac{y}{100} * 100}{x} = \frac{y}{x} kg$$

Where

M_I = Mass of influent required

But mass of substrate = 1kg,

Therefore mass of water to be added, M_w will be given by;

$$M_w = \frac{y}{x} kg - 1kg$$

Hence the water to substrate ratio will be $\left(\frac{y}{x} - 1\right) : 1$

For 120 kg of influent, respective masses of substrate and water will be given by equations

$$Substrate = \left(\frac{x}{y} * 120\right) kg$$

$$Water = \left(1 - \frac{x}{y}\right) * 120 kg$$

Mixing Feed Stocks in the ratio 1:1:1 using their Volatile Solids

Average VS was 0.3339 kg of VS/ kg for Chicken manure, 0.1762 kg of VS/ kg for goat manure and 0.2012kg of VS/ kg for cow manure.

Therefore 1 kg of VS is contained the following weights of feed stocks;

a. Chicken manure

0.3339 kg of VS = 1kg of feed stock

$$1\text{kg of VS} = \frac{1}{0.3339} = 2.9990\text{ kg of feed stock}$$

b. Goat manure

$$0.1762\text{ kg of VS} = 1\text{kg of feed stock}$$

$$1\text{kg of VS} = \frac{1}{0.1762} = 5.6754\text{ kg of feed stock}$$

c. Cow manure

$$0.2012\text{ kg of VS} = 1\text{kg of feed stock}$$

$$1\text{kg of VS} = \frac{1}{0.2012} = 4.9702\text{ kg of feed stock}$$

d. Inoculum

$$0.3820\text{ kg of VS} = 1\text{kg of inoculum}$$

$$1\text{kg of VS} = \frac{1}{0.3820} = 2.6178\text{ kg of feed stock}$$

Therefore, total mass of feed stocks for co-digestion prior dilution = sum of masses of chicken, goat and cow manure.

$$\text{Total mass} = 2.9990 + 5.6754 + 4.9702 = 13.6412\text{ kg of feed stock}$$

$$\text{CM: GM: CD} = 0.2198: 0.4158: 0.3644$$

Therefore chicken, goat and cow manure formed 21.96%, 41.58% and 36.44% respectively of the final substrate. Volumes of the three feed stocks in the substrate varied based on substrate to inoculum ratios.

For instance; where S/I ratio was 4:1 and total solids required were 8% to produce 120 L of influent for the digester, 96 L of the volume contained the substrate while 24 L was made up of the inoculum.

For this case, diluted volumes of chicken, goat and cow manure were as follows;

$$\text{Chicken manure} = \frac{21.98\%}{100\%} * 96L = 21.10L$$

$$\text{Goat manure} = \frac{41.58\%}{100\%} * 96L = 39.92L$$

$$\text{Cow manure} = \frac{36.44\%}{100\%} * 96L = 34.98L$$

To attain 8% TS:

Mass of each feed stock and water required for dilution was given by;

$$\text{Mass of feed stock} = \frac{TS_r}{TS_i} * V_{is}$$

Where, TS_r = standardized total solids (%)

TS_i = initial total solids (%)

V_{is} = volume of individual feed stock in substrate

i. Chicken manure

$$\text{Mass of feed stock} = \frac{8}{87.96\%} * 21.10\text{kg} = 1.92\text{kg}$$

$$\text{Mass of water for dilution} = 21.10\text{kg} - 1.92\text{kg} = 19.18\text{kg}$$

ii. Goat manure

$$\text{Mass of feed stock} = \frac{8\%}{31.02\%} * 39.92\text{kg} = 10.30\text{kg}$$

$$\text{Mass of water for dilution} = 39.92\text{kg} - 10.30\text{kg} = 29.62\text{kg}$$

iii. Cow manure

$$\text{Mass of chicken manure feed stock} = \frac{8\%}{14.72\%} * 34.98\text{kg} = 19.01\text{kg}$$

$$\text{Mass of water for dilution of CD} = 34.98\text{kg} - 19.01\text{kg} = 15.97\text{kg}$$

Masses of feed stocks and water used for preparation of substrates and inoculum for the different runs under selected substrate: inoculum ratios and total solids were as in the table below;

Appendix D: Data Analysis Sheet

The SAS System

The ANOVA Procedure

Table D1-a: Dependent Variable – Chicken Manure Total Solids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	14552.40327	2910.48065	101560	<.0001
Error	6	0.17193	0.02866		
Corrected Total	11	14552.57520			

	R-Square	Coeff Var	Root MSE	CM_Total_solids Mean
	0.999988	0.247702	0.169280	68.34000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.06140	0.03070	1.07	0.4001
Var	3	14552.34187	4850.78062	169279	<.0001

Table D1-b: t Tests (LSD) for Chicken Manure Total solids

t Tests (LSD) for CM_Total_solids

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha		0.05
Error Degrees of Freedom		6
Error Mean Square		0.028656
Critical Value of t		2.44691
Least Significant Difference		0.3382

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	88.9733	3	M
B	88.4000	3	T
C	87.9600	3	N
D	8.0267	3	H

H= Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D2-a: Dependent Variable – Goat Manure Total solids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1307.331275	261.466255	1054.33	<.0001
Error	6	1.487950	0.247992		
Corrected Total	11	1308.819225			

R-Square	Coeff Var	Root MSE	Goat_Manure_Total_solids Mean

0.998863	1.913681	0.497988	26.02250		
Source	DF	Type III SS	Mean Square	F-value	Pr > F
Rep	2	0.411050	0.205525	0.83	0.4810
Var	3	1306.920225	435.640075	1756.67	<.0001

Table D2-b: t Tests (LSD) for Goat Manure Total Solids

Alpha	0.05		
Error Degrees of Freedom	6		
Error Mean Square	0.247992		
Critical Value of t	2.44691		
Least Significant Difference	0.9949		
<i>t</i> Grouping	Mean	N	Var
A	33.5267	3	M
B	31.5267	3	T
C	31.0167	3	N
D	8.0200	3	H

Means with the same letter (s) (a, b, c, d) are not significantly different.

H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D3-a: Dependent Variable – Chicken Manure Moisture Content

Dependent Variable: Chicken_Manure_Moisture_Content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	14552.40327	2910.48065	101568	<.0001
Error	6	0.17193	0.02866		
Corrected Total	11	14552.57520			
R-Square	0.999988	Coeff Var	0.534679	Root MSE	0.169280
				Chicken_Manure_Moisture_Content Mean	31.66000
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.06140	0.03070	1.07	0.4001
Var	3	14552.34187	4850.78062	169279	<.0001

Table D3-b: t Tests (LSD) for Chicken Manure Moisture Content

t Tests (LSD) for Chicken_Manure_Moisture_Content

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.028656
Critical Value of t	2.44691
Least Significant Difference	0.3382

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	91.9733	3	H
B	12.0400	3	N
C	11.6000	3	T
D	11.0267	3	M

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D4-a: Dependent Variable – Goat Manure Moisture Content

Dependent Variable: Goat_Manure_Moisture_Content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1307.470775	261.494155	504.09	<.0001
Error	6	3.112450	0.518742		
Corrected Total	11	1310.583225			

R-Square	Coeff Var	Root MSE	Goat_Manure_Moisture_Content Mean
0.997625	0.973590	0.720237	73.97750

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.550550	0.275275	0.53	0.6135
Var	3	1306.920225	435.640075	839.80	<.0001

Table D4-b: t Tests (LSD) for Goat Manure Moisture Content

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

t Tests (LSD) for Goat_Manure_Moisture_Content

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.518742
Critical Value of t	2.44691
Least Significant Difference	1.439

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	91.9800	3	H
B	68.9833	3	N
B	68.4733	3	T
C	66.4733	3	M

Table D5-a: Dependent Variable – Chicken Manure Volatile Solids
 Dependent Variable: CM_Volatile_solids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	25.1464417	5.0292883	0.21	0.9450
Error	6	142.1620500	23.6936750		
Corrected Total	11	167.3084917			

R-Square	Coeff Var	Root MSE	CM_Volatile_solids Mean
0.150300	14.48659	4.867615	33.60083

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	24.07381667	12.03690833	0.51	0.6254
Var	3	1.07262500	0.35754167	0.02	0.9972

Table D5-b: t Tests (LSD) for Chicken Manure Volatile Solids

t Tests (LSD) for CM_Volatile_solids

TE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	23.69368
Critical Value of t	2.44691
Least Significant Difference	9.725

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
Â	34.103	3	M
Â	33.550	3	T
Â	33.390	3	N
Â	33.360	3	H

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D6-a: Dependent Variable – Goat Manure Volatile Solids

Dependent Variable: GM_Volatile_solids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6.56675000	1.31335000	3.75	0.0693
Error	6	2.10215000	0.35035833		
Corrected Total	11	8.66890000			

R-Square	Coeff Var	Root MSE	GM_Volatile_solids Mean
0.757507	3.400809	0.591911	17.40500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.93705000	0.46852500	1.34	0.3309
Var	3	5.62970000	1.87656667	5.36	0.0392

Table D6-b: t Tests (LSD) for Goat Manure Volatile Solids

t Tests (LSD) for GM_Volatile_solids

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	0.350358
Critical Value of t	2.44691
Least Significant Difference	1.1826

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	18.0267	3	M
A	17.7200	3	H
A	17.6267	3	N
B	16.2467	3	T

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D7-a: Dependent Variable - Chicken Manure Density

Dependent Variable: CM_Density

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	207123.1135	41424.6227	71.35	<.0001
Error	6	3483.6099	580.6017		
Corrected Total	11	210606.7235			

R-Square	Coeff Var	Root MSE	CM_Density Mean
0.983459	3.107449	24.09568	775.4167

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	3805.8451	1902.9226	3.28	0.1091
Var	3	203317.2684	67772.4228	116.73	<.0001

Table D7-b: t Tests (LSD) for Chicken Manure Density

t Tests (LSD) for CM_Density

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	580.6017
Critical Value of t	2.44691
Least Significant Difference	48.141

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	999.03	3	H
B	717.75	3	M
B	710.85	3	N
B	674.05	3	T

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D8-a: Dependent Variable – Goat Manure Density

Dependent Variable: GM_Density

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	86891.55510	17378.31102	33.81	0.0003
Error	6	3084.33687	514.05614		
Corrected Total	11	89975.89197			

R-Square	Coeff Var	Root MSE	GM_Density Mean
0.965720	2.655979	22.67281	853.6517

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	33.30407	16.65203	0.03	0.9683
Var	3	86858.25103	28952.75034	56.32	<.0001

Table D8-b: t Tests (LSD) for Goat Manure Density

t Tests (LSD) for GM_Density

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	514.0561
Critical Value of t	2.44691
Least Significant Difference	45.298

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	999.40	3	H
B	817.03	3	M
B	813.46	3	N
B	784.71	3	T

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal pretreatment

Table D9-a: Dependent Variable: Chicken Manure C/N ratio

Dependent Variable: CM_CN_ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.14131667	0.02826333	114.32	<.0001
Error	6	0.00148333	0.00024722		
Corrected Total	11	0.14280000			

R-Square	Coeff Var	Root MSE	CM_CN_ratio Mean
0.989613	0.115783	0.015723	13.58000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	0.00845000	0.00422500	17.09	0.0033
Var	3	0.13286667	0.04428889	179.15	<.0001

Table D9-b: t Tests (LSD) for Chicken Manure C/N ratio

t Tests (LSD) for CM_CN_ratio

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha		0.05
Error Degrees of Freedom		6
Error Mean Square		0.000247
Critical Value of t		2.44691
Least Significant Difference		0.0314

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	13.66667	3	N
B	13.63000	3	M
B	13.62333	3	H
C	13.40000	3	T

Key: H = Hydro, M = Mechanical, N = Non pretreatment, T = Thermal

Table D10-a: Dependent Variable: Goat Manure C/N ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.25035833	0.05007167	2.85	0.1172
Error	6	0.10533333	0.01755556		
Corrected Total	11	0.35569167			

R-Square	Coeff Var	Root MSE	Goat_Manure_CN_ratio Mean
0.703863	0.704555	0.132497	18.80583

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Rep	2	0.14426667	0.07213333	4.11	0.0752
Var	3	0.10609167	0.03536389	2.01	0.2135

Table D10-b: t Tests (LSD) for Goat Manure C/N ratio

Alpha		0.05
Error Degrees of Freedom		6
Error Mean Square		0.017556
Critical Value of t		2.44691
Least Significant Difference		0.2647

t Grouping	Mean	N	Var
A	18.8933	3	H
A	18.8633	3	N
A	18.8167	3	T
A	18.6500	3	M

Means with the same letter (a) are not significantly different.

Key: H = Hydro, M = Mechanical, N = None, T = Thermal pretreatment

Table D11-a: Dependent Variable – Control experiment biogas yield rate

Dependent Variable: Biogas_yield_rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	1.10667692	0.07377846	4.68	<.0001
Error	36	0.56729231	0.01575812		
Corrected Total	51	1.67396923			

R-Square	Coeff Var	Root MSE	Biogas_yield_rate Mean
0.661109	36.83764	0.125531	0.340769

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	12	0.58096923	0.04841410	3.07	0.0045
Var	3	0.52570769	0.17523590	11.12	<.0001

Table D11-b: t Tests (LSD) for Control biogas yield rate

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	0.015758
Critical Value of t	2.02809
Least Significant Difference	0.0999

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	0.49077	13	D
B	0.37154	13	B
C	0.26846	13	C
C	0.23231	13	A

Key: A = Goat manure, B = Cow manure, C = Chicken manure, D = Goat + Chicken + Cow manure

Table D12-a: Dependent Variable – Hydro pretreatment biogas yield rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.36961389	0.03360126	109.72	<.0001
Error	24	0.00735000	0.00030625		
Corrected Total	35	0.37696389			

R-Square	Coeff Var	Root MSE	Hydro_pretreatment_biogas_yield_rate Mean
0.980502	3.164239	0.017500	0.553056

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Rep	8	0.34113889	0.04264236	139.24	<.0001
Var	3	0.02847500	0.00949167	30.99	<.0001

Table D12-b: t Tests (LSD) for Hydro pretreatment biogas yield rate

Alpha	0.05		
Error Degrees of Freedom	24		
Error Mean Square	0.000306		
Critical Value of t	2.06390		
Least Significant Difference	0.017		
<i>t Grouping</i>	<i>Mean</i>	<i>N</i>	<i>Var</i>
A	0.593333	9	K
B	0.563333	9	L
C	0.536667	9	J
D	0.518889	9	M

Means with the same letter (s) (a, b, c, d) are not significantly different.

Key: J = 0 hrs (control), K = 6 hrs, L = 12 hrs, M = 18 hrs

Table D13-a: Dependent Variable – Mechanical pretreatment biogas yield rate

Dependent Variable: Biogas_yield_rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.44229444	0.04020859	70.30	<.0001
Error	24	0.01372778	0.00057199		
Corrected Total	35	0.45602222			
	R-Square	Coeff Var	Root MSE	Biogas_yield_rate Mean	
	0.969897	3.971346	0.023916	0.602222	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	8	0.38387222	0.04798403	83.89	<.0001
Var	3	0.05842222	0.01947407	34.05	<.0001

Table D13-b: t Tests (LSD) for Mechanical pretreatment biogas yield rate

t Tests (LSD) for Biogas_yield_rate

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000572
Critical Value of t	2.06390
Least Significant Difference	0.0233

Means with the same letter are not significantly different.

t Grouping	Mean	N	Var
A	0.64444	9	C
B	0.62222	9	D
B	0.60556	9	B
C	0.53667	9	A

Key: A= Control, B = 2 mm, C = 3 mm, D = 4 mm

Table D14-a: Dependent Variable – Thermal pretreatment Biogas yield rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.34883333	0.03171212	111.02	<.0001
Error	24	0.00685556	0.00028565		
Corrected Total	35	0.35568889			

R-Square	Coeff Var	Root MSE	Thermal_pretreatment_Biogas_yield_rate Mean
0.980726	2.942169	0.016901	0.574444

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Rep	8	0.31778889	0.03972361	139.06	<.0001
Var	3	0.03104444	0.01034815	36.23	<.0001

Table D14-b: t Tests (LSD) for Thermal pretreatment biogas yield rate

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000286
Critical Value of t	2.06390
Least Significant Difference	0.0164

t Grouping	Mean	N	Var
A	0.615556	9	L
B	0.585556	9	M
C	0.560000	9	K

D	0.536667	9	J
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Means with the same letter (s) (a, b, c, d) are not significantly different.

Key: J = Control, K = 60 °C, L = 80 °C, M = 100 °C

Table D15-a: Dependent Variable – Comparative pretreatment biogas yield rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.37721667	0.03429242	165.15	<.0001
Error	24	0.00498333	0.00020764		
Corrected Total	35	0.38220000			
<i>R-Square</i>	<i>Coeff Var</i>	<i>Root MSE</i>	<i>Thermal_pretreatment_Biogas_yield_rate Mean</i>		
0.986961	2.542885	0.014410	0.566667		
Source	DF	Type III SS	Mean Square	F-value	Pr > F
Rep	8	0.35515000	0.04439375	213.80	<.0001
Var	3	0.02206667	0.00735556	35.42	<.0001

Table D15-b: t Tests (LSD) for Comparative pretreatment biogas yield rate

Alpha	0.05		
Error Degrees of Freedom	24		
Error Mean Square	0.000208		
Critical Value of t	2.06390		
Least Significant Difference	0.014		
<i>t Grouping</i>	<i>Mean</i>	<i>N</i>	<i>Var</i>
A	0.602222	9	M
B	0.575556	9	T
C	0.552222	9	H
D	0.536667	9	C

Means with the same letter(s) (a, b, c, d) are not significantly different.

Key: *M* = Mechanical pretreatment, *T* = Thermal pretreatment, *H* = Hydro pretreatment, *C* = Control

Table D16-a: Dependent Variable - Inoculums level Biogas yield rate

<i>Source</i>	<i>DF</i>	<i>Sum of Squares</i>	<i>Mean Square</i>	<i>F Value</i>	<i>Pr > F</i>
Model	12	0.31118111	0.02593176	45.59	<.0001
Error	32	0.01820145	0.00056880		
Corrected Total	44	0.32938256			
<i>R-Square</i>	<i>Coeff Var</i>	<i>Root MSE</i>	<i>TS_Biogas_yield_rate Mean</i>		
0.944741	4.211184	0.023849	0.566336		
<i>Source</i>	<i>DF</i>	<i>Type III SS</i>	<i>Mean Square</i>	<i>F-value</i>	<i>Pr > F</i>
Rep	8	0.28416508	0.03552063	62.45	<.0001
Var	4	0.02581920	0.00645480	11.35	<.0001

Table D16-b: t Tests (LSD) for Inoculums level Biogas yield rate

Alpha	0.05		
Error Degrees of Freedom	32		
Error Mean Square	0.000569		
Critical Value of t	2.03693		
Least Significant Difference	0.0229		
<i>t Grouping</i>	<i>Mean</i>	<i>N</i>	<i>Var</i>
A	0.60660	9	L
B	0.57661	9	M
BC	0.56513	9	K
CD	0.54698	9	N
D	0.53636	9	J

Means with the same letter (s) (a, b, c, d) are not significantly different.

Key: S/I for J = 6:1, K = 5:1, L = 4:1, M = 3:1 and N = 2:1

Table D17-a: Dependent Variable - TS Biogas_yield_rate

<i>Source</i>	<i>DF</i>	<i>Sum of Squares</i>	<i>Mean Square</i>	<i>F Value</i>	<i>Pr > F</i>
Model	14	0.60809841	0.04343560	73.30	<.0001
Error	48	0.028444444	0.00059259		
Corrected Total	62	0.63654286			
<i>R-Square</i>	<i>Coeff Var</i>	<i>Root MSE</i>	<i>TS_Biogas_yield_rate Mean</i>		
0.955314	4.166322	0.024343	0.584286		
<i>Source</i>	<i>DF</i>	<i>Type III SS</i>	<i>Mean Square</i>	<i>F-value</i>	<i>Pr > F</i>
Rep	8	0.51280000	0.06410000	108.17	<.0001
Var	6	0.09529841	0.01588307	26.80	<.0001

Table D17-b: t Tests (LSD) for Total Solids biogas yield rate

Alpha	0.05		
Error Degrees of Freedom	48		
Error Mean Square	0.000593		
Critical Value of t	2.01063		
Least Significant Difference	0.0231		
<i>t Grouping</i>	<i>Mean</i>	<i>N</i>	<i>Var</i>
A	0.63000	9	M
BA	0.61667	9	N
BA	0.61667	9	L
B	0.59889	9	K
C	0.56778	9	P
D	0.53333	9	J
D	0.52667	9	Q

Means with the same letter (s) (a, b, c, d) are not significantly different.

Key: J = 7.5%, K = 8.0%, L = 8.5%, M = 9.0%, N = 9.5%, P = 10.0%, Q = 10.5% total solids

Appendix E: Digester Design based on Field conditions

I. Digester variables and their relationships

Plate B presents an impression of a fixed dome digester and was used for the design,

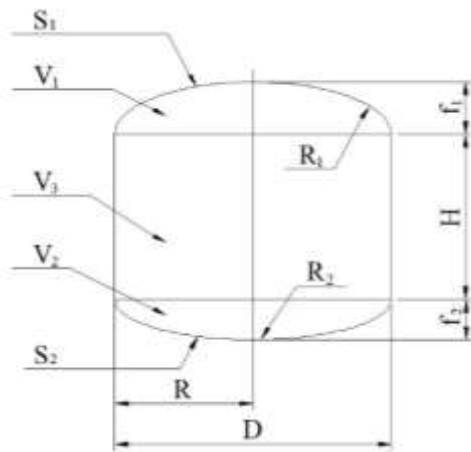


Plate E: A Design Impression of a Fixed dome Digester

Where; V_1 -Volume of the Dome, V_2 -Volume of the Bottom, V_3 -Volume of the fermentation chamber, f_1 -Vector rise of the dome, f_2 - Vector rise of the bottom, R_1 -Curvature radius of the dome, R_2 - Curvature radius of the Bottom, D_0 -Internal diameter of the digester , R - Internal radius of the digester, H -Height of the digester wall. Relationships between digester variables were based on Thenabadu (2010) and SLS 1246:2003 standards (2007) and are as in Table D1

Table E1: Digester variables and their relationships

Equation number	Digester variable	Equation
A	Height of the digester wall, H_0	$H_0 = \frac{D_0}{2.5}$
B	Vector rise of the dome, f_1	$f_1 = \frac{D_0}{5}$
C	Vector rise of the bottom, f_2	$f_2 = \frac{D_0}{8}$
D	Internal radius of the digester, R	$R = \frac{D_0}{2}$
E	Volume of the Dome, V_1	$V_1 = 0.0827D_0^3$
F	Volume of the Bottom, V_2	$V_2 = 0.0501D_0^3$
G	Volume of the fermentation chamber, V_3	$V_3 = 0.3142D_0^3$
H	Total volume, V_{total}	$V_{total} = (0.4470)D_0^3$
I	Internal diameter of the digester, D_0	$D_0 = 1.3078V^{\frac{1}{3}}$

II. Digester measurements

Values of digester parameters were computed using Equations A to B above;

Volume of the dome (V_1) was therefore = volume of the gas = $0.78 \text{ m}^3/\text{m}^3\text{d}$ (peak daily biogas production rate from 3mm mechanical mincing on the 6th day as in Table 4.8 was used) and from equation E,

$$0.78\text{m}^3 = 0.0827D_0^3$$

$$\text{Therefore, internal diameter, } D_0 = \sqrt[3]{\frac{0.78}{0.0827}} = 2.11\text{m}$$

$$\text{Volume of dome } V_1 = 0.0827 \times 2.11^3 = 0.78\text{m}^3$$

$$\text{Volume of the bottom, } V_2 = 0.0501 \times 2.11^3 = 0.47\text{m}^3$$

$$\text{Volume of the substrate holding chamber, } V_3 = 0.3142 \times 2.11^3 = 2.95\text{m}^3$$

Total digester volume (V) is therefore given by equation 8, and
 $V = (0.0827 + 0.0501 + 0.3142)2.11^3 = 4.21\text{m}^3$

$$\text{Dome height, } f_1 = \frac{D_0}{5} = \frac{2.11\text{m}}{5} = 0.42\text{m}$$

$$\text{Base depth, } f_2 = \frac{D_0}{8} = \frac{2.11\text{m}}{8} = 0.26\text{m}$$

$$\text{Wall height, } H_0 = \frac{D_0}{2.5} = \frac{2.11\text{m}}{2.5} = 0.84\text{m}$$

$$\text{Total height} = f_1 + f_2 + H_0 = 0.42 + 0.26 + 0.84 = 1.52\text{m}$$

$$\text{Internal radius of digester, } R_0 = \frac{D_0}{2} = \frac{2.11\text{m}}{2} = 1.06\text{m}$$

Therefore when constructing a field digester to give similar results, technicians would require the measurements as presented in Table E2;

Table E2: Digester measurements

Equation number	Digester variable	Measurements
A	Height of the digester wall, H_0	0.84 m
B	Vector rise of the dome, f_1	0.42 m
C	Vector rise of the bottom, f_2	0.26 m
D	Internal radius of the digester, R	1.06 m
E	Volume of the Dome, V_1	0.78 m ³
F	Volume of the Bottom, V_2	0.47 m ³
G	Volume of the fermentation chamber, V_3	2.95 m ³
H	Total volume, V	4.21 m ³
I	Internal diameter of the digester, D_0	2.11 m

From Table E2, it is evident that, a fixed dome batch digester with volume of 4.21m³ working under uncontrolled field conditions would be required to give the similar biogas production rates (for this case 0.78 m³/m³d) as a 1 m³ fixed dome batch laboratory digester working under controlled conditions of operation (mesophilic temperature of 35 ± 0.5° C and stirring). This is when co-digesting chicken-goat manure minced to 3 mm effective particle sizes with untreated cow manure, mixed at ratios 1:1:1, volatile solids basis.

Appendix F: Optimization

Table F1: Analysis of Variance table for the Response Surface Quadratic Model

Source	Sum of squares	df	Mean Square	F – Value	p-value Prob> F
Model	2730.57	9	303.40	7.99	0.0016
A-S/I	0.24	1	0.24	0.006236	0.9386
B-TS	132.46	1	132.46	3.49	0.0913
C-Size	58.71	1	58.71	1.55	0.2420
AB	75.15	1	75.15	1.98	0.1897
AC	30.26	1	30.26	0.80	0.3929
BC	12.01	1	12.01	0.32	0.5862
A ²	1916.20	1	1916.20	50.49	< 0.0001
B ²	301.62	1	301.62	7.95	0.0182
C ²	558.94	1	558.94	14.73	0.0033
Residual	379.55	10	37.96		
Cor Total	3110.12	19			

Other statistics: Std. Dev = 6.16, Mean = 71.72, C.V. % = 8.59, R² = 0.8780, Adjusted R² = 0.7681, Predicted R² = 0.0869, Adequate Precision = 7.538

Table F2: Adequacy of the model tested

Source	Sum of Squares	D f	Mean Square	F Value	p-value Prob> F	Comment
Mean vs Total	102900	1	102900			
Linear vs Mean	191.41	3	63.80	0.35	0.7899	
2FI vs Linear	117.42	3	39.14	0.18	0.9070	
Quadratic vs 2FI	2421.74	3	807.25	21.27	0.0001	Suggested
Cubic vs Quadratic	369.22	4	92.31	53.63	< 0.0001	Aliased
Residual	10.33	6	1.72			
Total	100600	20	5299.84			

Table F3: Verified biogas production data

Replication	Biogas production rate		Difference from predicted value	
	m ³ /m ³ d		m ³ /m ³ d	%
1	0.61		0.02	3.38
2	0.60		0.01	1.69
3	0.60		0.01	1.69
Predicted	0.59		-	-

Appendix G: Research Permit from NACOSTI


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Appendix H: Relevant Publications based on this Work

Appl. Res. J. Environ. Eng.
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 International Institute for Applied Research
Skies Educational

Co-digestion of Pretreated Chicken - Goat and Untreated Cow Manure at Different Substrate to Inoculums Ratios and Total Solids for Biogas Production

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ABSTRACT

Biogas production can be greatly affected by inoculum addition and total solids. The effect of substrate to inoculum ratios and total solids of chicken, goat and cow manure on biogas production was studied using a 0.15m³ laboratory scale batch digester at a constant temperature of 35°C. Feed stocks were mechanically minced to 3 mm effective particle sizes prior to co-digesting with untreated cow manure from a free-range dairy rearing system. Different amounts of cow substrate inoculum were used at ratios of 2:1, 3:1, 4:1, 5:1 and 6:1, while total solid levels between (7.5% and 10.5%) at intervals of 0.5% were used to study their effects on biogas production. Increasing inoculums and total solids resulted in increased biogas production with peaks at a substrate to inoculum ratio of 4:1 (20% inoculum addition) and 9% total solids. Biogas production rates of 0.61 and 0.63m³/m³d were realized respectively.

Keywords: Biogas Production, Chicken-Goat-Cow Manure, Substrate to Inoculum Ratios, Total Solids

INTRODUCTION

Countries are moving away from fossil to renewable fuel-based economies because of increased negative environmental effects of the latter, thus biomass production and consumption chains are being promoted in generation of eco-friendly and sustainable fuels [1]

Substrate to inoculum (S/I) ratio is the quantitative relation of the amount of volatile solids in the substrate per the amount of volatile solids contained in the inoculum at the start of each batch digestion process [2; 3]. Each feed stock has its suitable substrate to inoculum ratio [4], because of the material-specific quantity of volatile fatty acids and its capacity to buffer against the VFAs that accumulate throughout its biodegradation process. Chicken-goat-cow manure mixture can provide the right C/N ratio for anaerobic digestion and re-feeding of spent slurry (contains washed out microbes) into the digester can also be a way of improving biogas production [5].

Total solids (TS) content is the dry matter of a feedstock or substrate and includes both volatile and dissolved solids. The role total solids (TS) on activities of anaerobic bacteria are always important in order

EFFECT OF CO-DIGESTING PRETREATED CHICKEN - GOAT AND UNTREATED COW MANURE ON BIOGAS PRODUCTION IN FIXED DOME LABORATORY DIGESTER

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ABSTRACT:

The effect of hydro, mechanical and thermal pretreatment of chicken and goat manure on biogas production was done using a 0.15m³ laboratory scale batch digester. The feedstocks were subjected to 6, 12 and 18 hours of soaking, 2 mm, 3 mm and 4 mm of mechanical mincing, and 60°C, 80°C and 100°C of heating. Pretreated feed stocks were co-digested with untreated cow manure from an extensive dairy rearing system. Experiments were done at 8.9% substrate total solids and a constant temperature of 35°C. Averagely, mechanical pretreatment resulted in the highest increase in mean biogas production rate with 11.11%, over the co-digestion control (0.54 m³/m³d), followed by thermal and hydro by 5.56% and 1.85%. Maximal increase in production for each pretreatment was at 6 hour soaking time (9.30%), 3 mm effective feed stock particle sizes (18.52%) and 80°C of heating (14.81%). Co-digestion increased mean biogas production rate over mono-digestion by 68.97% (chicken), 81.84% (goat) and 8.8% (cow manure). Superior outer cell wall and cover disruption of feed stocks for easy hydrolysis advantaged mechanical pretreatment.

Keywords: *pretreatment, chicken-goat-cow manure, biogas production, fixed dome, laboratory digester*

1.0 INTRODUCTION

In Kenya, the government's effort towards provision of food and nutrition security for its population has rapidly grown poultry and livestock industries; consequently, increasing their wastes. Improper manure management may degrade the general environment (Sakar *et al.*, 2013), while uncontrolled greenhouse gases (CH₄ and others) emitted from waste storage units contribute to global warming (Yetilmezsoy *et al.*, 2008). Anaerobic digestion (AD) can solve this emissions problem while generating

clean energy for the population (Atilade *et al.*, 2014). The country's economy is agriculture-based and hence biogas substitutes use of fuel wood (Arthur *et al.*, 2011).

Chicken and goat manure uptake as AD substrates is low due to their low Carbon to Nitrogen (C/N) ratios of about 5 to 10 (Yangin-Gomoc *et al.*, 2013) and an average of 18 (Zhang *et al.*, 2013) respectively, low specific surface area (SSA) and high dry matter that inhibits the anaerobic process (Chen *et al.*, 2008). In this study, the effect of hydro, thermal and mechanical