

Effect of Anaerobic Co-digestion of Cow Dung and Maize Stubble on Biogas Production: Optimization and Economic Viability Analysis

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DECLARATION

I, Peter Bol Kuol Kiir, affirm that the ideas, concepts, and writings presented in this research dissertation are solely my own. As far as I am aware, no part of this research dissertation has been submitted to Gulu University or any academic institution for a degree award.

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APPROVAL

This dissertation titled "Effect of Anaerobic Co-Digestion of Cow Dung and Maize Stubble on Biogas Production" has been developed with our supervision and guidance and therefore, approved for submission.

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DEDICATION

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LIST OF ACRONYMS

AcoD	Anaerobic Co-Digestion
AD	Anaerobic Digester
ANOVA	Analysis of variance

APHA	American Public Health Association
ARR	Account Rate of Return
ASTM	American Society for Testing and Materials
CCD	Central Composite Design
CD	Cow dung
COD	Chemical Oxygen Demand
DOE	Design of Experiment
FC	Fixed Carbon
GHG	Greenhouse Gas
HHV	Higher Heating Values
LPG	Liquefied Petroleum Gas
LR	Loading Rate
MS	Maize Stubble
MSW	Municipal Solid Waste NPV
Net Present Value	
OLR	Organic Loading Rate
PP	Payback period
RSM	Response Surface Methodology
UNEP	United Nations Environment Program
VFAs:	Volatile fatty acids
VS	Volatile Solids
VTR	Volumetric Treatment Rate

ABSTRACT

The production of biogas depends on factors like temperature and substrate mixing ratios. This study involved the characterization of cow dung and maize stubble, as well as their anaerobic co-digestion to optimize biogas volume and methane yield while minimizing carbon dioxide concentration. An economic assessment was also conducted to evaluate the viability of anaerobic co-digestion. Feedstock characterization included proximate and ultimate analyses, along with determination of higher heating values. Process conditions for anaerobic co-digestion were optimized using Response Surface Methodology based on Central Composite Design (CCD). The independent variables were mixing ratio and temperature, while the dependent variables were biogas volume, methane yield, and carbon dioxide concentration. Thirteen experimental conditions were generated using the Design Expert software version 13. 1.5 L plastic bottles with a working volume of 1.2 L, were loaded with a mixture of cow dung (CD) and maize stubble (MS) in ratios of 100:0 (CD: MS), 0:100 (CD: MS), 40:60 (CD: MS), and 70:30 (CD: MS), and submerged in a water bath operated at temperatures ranging from 25–45 °C. Daily readings were taken to measure the biogas volume using the water displacement technique for 26 days, and a 3-day gas composition analysis was performed using a portable biogas analyzer (Geotech, 5000). The carbon content of CD and MS was found to be 32.87% db and 31.55% db, respectively. The C/N ratios of CD and MS were found to be 21.54 and 29.84, respectively, which lay within the range of 20–30 ideal for anaerobic digestion. pH levels for CD and MS were found to be 8.6 and 5.46, respectively. Higher heating values of CD and MS were found to be 13.63 and 16.05 MJ/kg, respectively, suggesting a higher energy potential of MS compared to CD. Low sulfur levels of 0.09% in CD and 0.11% in MS suggest minimal emissions due to sulfur oxides. The optimal conditions were found to be a mixture ratio of 40:60 (CD: MS), and a temperature of 35 °C, resulting in a maximum biogas volume and methane yield of 5.35 L/kgVS and 95.20 %/kgVS, respectively, with a minimum CO₂ concentration of 15.24 kgVS. An economic analysis revealed a positive net present value (NPV), confirming the project's economic viability. The payback period was found to be 4 years. Based on the accounting rate of return (AAR), the project results in a 27% annual return, which is economically attractive. Additionally, a profitability index (PI) greater than 1 confirms that the future value exceeds the initial investment, highlighting the strong economic viability of anaerobic co-digestion of cow dung with maize stubble.

Keywords: Cow dung, maize stubble, anaerobic co-digestion, optimization, biogas.

CHAPTER ONE: INTRODUCTION

1.1 Background

Energy is a key driver of progress, though global dependence on fossil fuels has contributed to climate change, environmental degradation, and negative health impacts. The depletion of non-renewable fossil fuel reserves, coupled with environmental damage, has prompted the shift toward renewable and sustainable energy alternatives (Okewale et al., 2018). Biogas production has emerged as a globally recognized renewable energy source, addressing energy shortages and environmental issues (Black et al., 2021). It is produced through anaerobic digestion via four stages, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Animal manure, crop residues, and organic materials serve as substrates for biogas generation (Rojas et al., 2016). This process not only provides a sustainable alternative to fossil fuels but also plays a crucial role in reducing greenhouse gas (GHG) emissions, a key driver of climate change.

Significant advancements in biogas technology have been made in developed countries, where organic waste is increasingly utilized to generate energy and reduce environmental pollution (Ibro et al., 2022). Uganda, like many developing nations, grapples with challenges related to energy accessibility and environmental sustainability (Singh et al., 2021). However, the heavy dependence on biomass is driven by cultural practices, low costs, and the wide availability of these resources (Mohammed et al., 2013). Adopting biogas production through anaerobic digestion is becoming increasingly important as a renewable energy technology, especially in agricultural regions like northern Uganda. In this area, both livestock farming and crop production are prominent, generating a significant amount of organic waste. Cow dung, a byproduct of livestock farming, is rich in organic matter and is a promising substrate for biogas production. It is abundant due to the large livestock population, including over 13 million cattle, vital for the rural economy (FAO, 2021). Each cow produces an estimated 1512 kg of dung

daily, representing a significant resource for biogas (Vasanth et al., 2022). Similarly, maize stubble, often left as waste after harvesting, has significant potential for green energy production. It creates substantial agricultural waste, with approximately 1.4-1.9 tons remaining for every ton of maize produced (Amon et al., 2007). Effectively using these materials in co-digestion could generate considerable biogas, providing an energy source and waste management solution (Achinas et al., 2017). The combination of agricultural waste with animal manure in anaerobic co-digestion has shown promising results in enhancing biogas yield compared to mono-digestion of a single substrate (Murphy et al., 2015).

Furthermore, there are considerable effects on the overall environmental values of the process (Dębowski et al., 2022). Co-digestion offers several advantages, including improved overall biodegradability of substrates, balanced nutrient content, and a more stable digester environment (Santpoort, 2020). Developing efficient biogas systems could bolster energy security, decrease reliance on wood and charcoal, and address waste management issues in the country (Mukhuba et al., 2018). The success of anaerobic co-digestion depends on several key factors, including careful selection of substrates, precise temperature control, assessment of waste properties, regulation of pH levels, and maintaining a balanced mix of carbon- and nitrogen-rich materials. Additionally, effective mixing techniques and rigorous microbiological monitoring are essential to optimize the process (Pakvilai, 2021).

Response Surface Methodology (RSM) is a statistical technique used to analyze interactions among multiple parameters and determine optimal conditions for desired outcomes. It enhances product yield, minimizes variability, and improves system performance by identifying the best operating parameters (Mkruqulwa et al., 2020). RSM develops empirical models to estimate responses from various factor combinations, aiding decision-making in process optimization. This study employed RSM to optimize process conditions for anaerobic co-digestion of cow dung and maize stubble for biogas production. The study also involved carrying out an economic analysis of the biogas system, based on economic indicators like

Net Present Value (NPV), Payback Period and Profitability Index PI (Liu, 2022).

1.2 Problem Statement

Despite Northern Uganda's significant agricultural potential and the abundance of residues and animal waste suitable for biogas production, only a small fraction is utilized as manure. Most of these resources are either burned in the fields or left to decompose, which releases unmanaged methane emissions. Previous studies show that cow dung is an effective biogas feedstock due to its abundant microbial content and balanced nutritional composition. Similarly, studies on maize stubble have shown that it can serve as a valuable carbon source in anaerobic digestion. However, biogas production from these substrates has largely been limited to mono-digestion, limiting the yield and quality. This study set out to optimize the anaerobic co-digestion of cow dung and maize stubble to maximize biogas yield and quality, minimize CO₂ concentration, and conduct an economic analysis to ascertain the economic viability of anaerobic co-digestion of the substrates.

1.3 Objectives

1.3.1 Major Objective

The main objective of this study was to evaluate the potential of anaerobic co-digestion of cow dung and maize stubble for biogas production and to assess its economic viability through financial analysis.

1.3.2 Specific Objectives

- i. To characterize cow dung and maize stubble feedstocks for biogas production.
- ii. To optimize the conditions for anaerobic co-digestion of cow dung and maize stubble to maximize biogas production.
- iii. To assess the economic viability of anaerobic co-digestion of cow dung and maize stubble for biogas production.

1.4 Research Hypothesis

- i. What physicochemical characteristics of cow dung and maize stubble influence their suitability and performance in biogas production?
- ii. What combination of operating conditions (e.g., temperature, substrate ratio) optimizes biogas yield during the co-digestion of cow dung and maize stubble?
- iii. To what extent is the anaerobic co-digestion of cow dung and maize stubble economically viable based on key financial indicators (NPV, IRR, and payback period)?

1.5 Research Justification

Anaerobic co-digestion is a promising approach to maximize generation of biogas from various substrates and enhance its quality. Despite its potential to enhance biogas production per unit of energy and improve the quality of digestate, anaerobic co-digestion utilizing the substrates of cow dung and maize stubble is yet to be explored. Previous studies have demonstrated that biogas production is significantly increased by co-digestion of cow dung with other substrates as compared to mono-digestion. Cow dung can be co-digested with maize stubble in appropriate amounts, providing sufficient co-substrate, including structural carbohydrates, minimal hemicellulose, and lignocellulosic composition. It also offers a favourable carbon to nitrogen (C/N) ratio, micronutrients, phosphorus, and essential nutrients, which optimize digestion in biogas digesters. This process enhances stability and offers a low-cost method for biogas production. In Northern Uganda, the rural population lacks access to off-grid electricity, forcing them to rely on traditional biomass energy sources, such as animal manure, agricultural waste and wood for cooking. However, the region faces a challenge as the availability of wood fuel has reduced due to rising demand from a growing population and ineffective afforestation programs. The abundant cow dung from smallholder farms can be utilized in biogas digesters to enhance biogas production capacity. Additionally, maize stubble increases significantly

during the harvest season, given the surge in maize production and remains available in large quantities throughout the year.

1.6 Limitations of the Research

The composition of cow dung is significantly influenced by the specific type of feed given to the animals. This variation not only alters the nutritional profile of the dung but also affects the quality and quantity of biogas produced during anaerobic digestion. Similarly, the composition of maize stubble can be shaped by factors such as the specific maize variety cultivated, soil health, and particular agronomic practices used, including irrigation methods and fertilization strategies. These variations ultimately impact the biochemical properties of the stubble, which, in turn, influences the quality of biogas generated through the codigestion process. However, it is vital to note that these critical factors were not monitored in this study, creating a gap in the understanding of their potential effects on biogas production results.

1.7 Conceptual Framework

This study uses a conceptual framework that links two independent variables, co-digestion ratio and temperature, to dependent variables: biogas volume, methane yield, and carbon dioxide concentration. The relationship is affected by contextual factors such as pH, total solids, volatile solids, moisture content, C/N ratio, and inoculum characteristics. These factors shape microbial activity and substrate degradation during anaerobic digestion, which then determines biogas production efficiency and its economic and environmental values. Figure 1.1 illustrates the interrelationships among variables in the anaerobic co-digestion of cow dung and maize stubble for enhanced biogas generation.

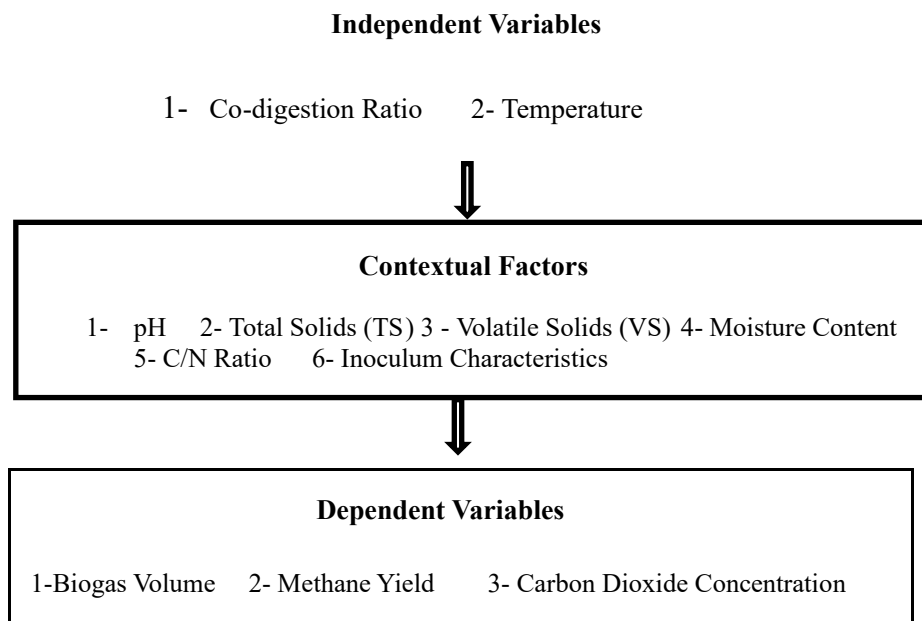


Figure 1.1: Conceptual Framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Background

Biogas technology was initially introduced to Uganda in the early 1950s with the development of fixed-dome digesters constructed with cement and a metal inlet tube topped with a gas holder (Obileke et al.2021). Over time, cement in place and floor digester designs were introduced and widely adopted by farmers due to their practicality and economic feasibility (Usack et al., 2014). Today, new designs are being promoted by groups such as the Association for the Advancement of the Biodigester Sector in Uganda (AABReg), offering a range of options (Benti et al., 2021). Despite the availability of various biogas construction technologies, challenges persist in the dynamics of applied materials, physical size, and optimizing conversions. Cow dung is often recognized for its high biogas production and widespread availability, it has not been deemed profitable due to its slow digestion rate, low methane content, and susceptibility to methane production inhibition (Ona et al., 2019).

These factors are due to cow manure's high ratio of the mass of carbon to that of nitrogen in organic matter and low nutrient content (Prasad, 2012). Maize is the most prominent cereal crop, playing a vital role in local food security. However, its utilization is limited as the burning of maize stubble is a significant source of atmospheric pollution, posing public nuisances and potential hazards (Herrmann, 2013). Anaerobic digestion-produced biogas can significantly meet the local energy demand while providing environmental benefits. However, the lack of suitable substrates for biogas production remains a major challenge. In remote rural areas like northern Uganda, where open firewood or woody biomass is used for pottery and food processing, fuel shortages are becoming a critical issue (Meegoda et al., 2018). Anaerobic co-digestion may present an alternative by combining the advantages of increased methane yield, improved final product quality, and complexity using organic residues (Hagos et al., 2017).

2.2 Biogas Composition

Biogas is a renewable energy source produced from organic waste materials using a process called anaerobic digestion (Liu et al., 2018). Biogas typically consists of methane (CH₄) at 35-75%, carbon dioxide (CO₂) at 15-65%, hydrogen sulfide (H₂S) at 0.3%, hydrogen (H₂) at 0-7%, ammonia (NH₃) at 0-2%, nitrogen (N₂) at 1-10%, oxygen (O₂) at 0-2%, water vapour (H₂O) at 1-7%, and traces of other gases (Mamun et al., 2015). It is an odourless, flammable gas created through the biological decomposition of organic material without oxygen. It results from the anaerobic digestion of biodegradable substances such as biomass, cow manure, green waste, and agricultural byproducts like cassava and sugarcane (Jameel et al., 2024). Table 2.1 shows the biogas composition from agricultural, waste and landfill. It shows that the ranges, medians, and means of CH₄ and CO₂ for agricultural and waste biogas are similar. However, the wide range indicates that biogas utilization techniques, such as fuel cells, need to consider significant variations in CH₄ content and heating value from site to site. Landfill gas exhibits even greater variability, with older landfills producing gas with lower CH₄ fractions (Cambry et al., 2022). Conversely, the anaerobic digestion of lignocellulosic biomass often requires a longer HRT due to its low digestibility and the resilient nature of lignin (Shi et al., 2017).

Table 2.1: Comparison of biogas compositions from agricultural, Solid waste, and landfill

%V	Agricultural			Solid waste			Landfill	
	Range	Median	Mean	Range	Median	Mean	Range	Median
CH ₄	49-69	56	56	44-67	57	56	40-70	53
CO ₂	29-44	39	40	30-40	38	37	25-40	35
N ₂	0.6-13	3	3	0.1-6	1	1	0-17	<5
O ₂	0.2-3	0.6	0.6	0.1-3	0.4	0.6	0-3	<1

(Cambry et al., 2022).

2.3 Anaerobic digestion

Anaerobic digestion (AD) is a biological process that decomposes organic material in the absence of oxygen to produce biogas and digestate (Liew et al., 2015). The process is carried out by a group of microorganisms that convert complex organic compounds into simpler

molecules, including methane (CH_4) and carbon dioxide (CO_2), thereby forming biogas (Khalid et al., 2011). However, Mutnuri. ,2016), described the anaerobic digestion process as a naturally occurring and synergistic biotic process involving microbial decomposition in an oxygen-free environment. The anaerobic digestion (AD) process consists of four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis and each stage requires unique microbial populations and distinct ecological conditions, which collectively break down organic matter into biogas, primarily methane and carbon dioxide.

2.3.1 Hydrolysis Stage

In the first stage, hydrolysis breaks down macromolecules like proteins, fats, and carbohydrates into monomers that microorganisms can absorb and consume (Jayaraj, 2014). Vorgelegt (2017) and Ravi (2018) highlight the significance of the step, particularly when using fibre-rich feedstock. (Saady,2015) describes hydrolysis as the process that converts polymeric materials into fatty acids, amino acids, and sugars, with carbohydrates rapidly transformed into simpler sugars that are further fermented to volatile fatty acids (VFAs) during hydrolysis.

2.3.2 Acidogenesis Stage

In acidogenesis, the second stage of anaerobic digestion, fermentative bacteria, either strictly or facultatively anaerobic, consume the monomers and oligomers produced during hydrolysis. This process generates three main categories of end products: (1) acetate, (2) hydrogen and carbon dioxide, and (3) non-acetate short-chain fatty acids, alcohols, and methylated substrates. Specifically, monomeric glucose is converted into acetate and propionate, among other intermediates, which serve as substrates for the subsequent stages of the digestion process (Insam et al., 2010). Fermentative bacteria, including *Clostridium*, *Lactobacillus*, and *Enterobacter*, overlap with hydrolytic bacteria, and their products are excreted. Maintaining a balance between acidogenic and acid-scavenging microbes is essential for the stability of the

anaerobic digestion process, as a decrease in pH is a common cause of reactor failure Rasouli et al., 2015.

2.3.3. Acetogenesis Stage

Anaerobic digestion produces acetate, molecular hydrogen, and carbon dioxide. Acetogenic bacteria oxidize these byproducts, but the accumulation of hydrogen can inhibit them. (Satpathy et al., 2017). Maintaining a low partial pressure of molecular hydrogen is crucial for efficient digestion, which is facilitated by H₂-producing acetogenins and H₂-scavenging methanogens. (Raphela et, al, 2021).

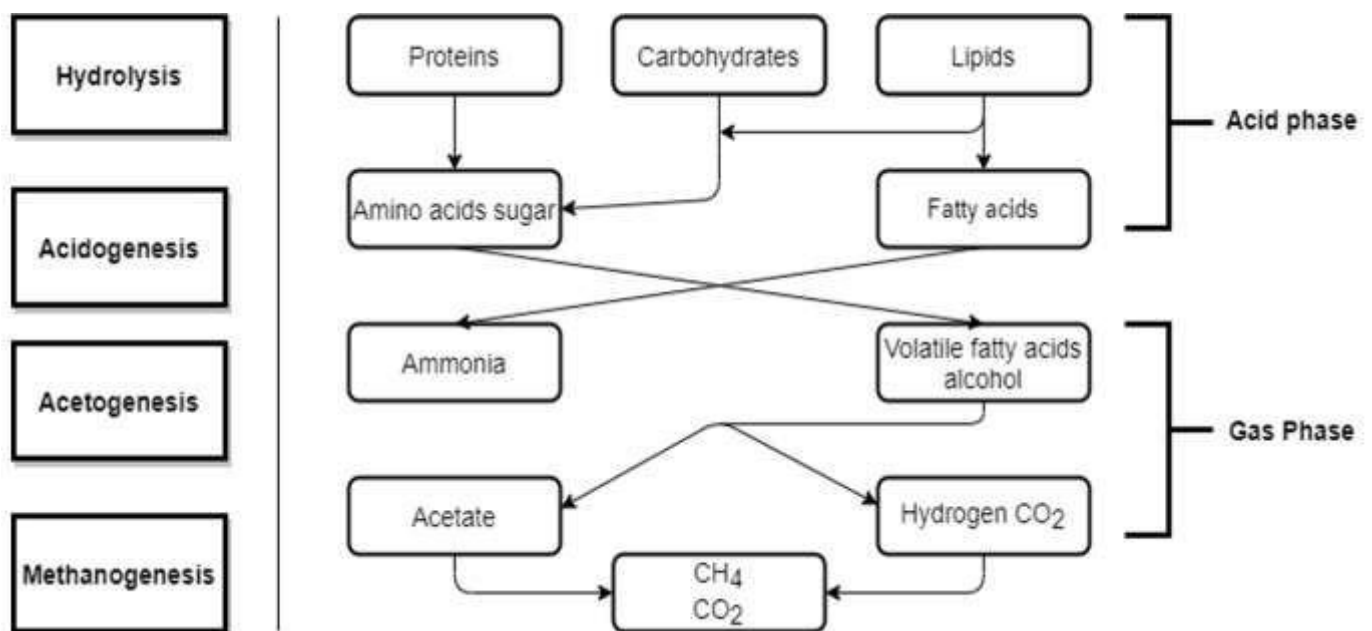


Figure 2.1: Stages of complex organic matter conversion to methane (Raphela et, al, 2021)

2.3.4 Methanogenesis Stage

Methanogens, are crucial microorganisms in (AD) bioreactors and pose challenges for study due to their sensitivity and the difficulty in employing culture-based methods. (J. Li et al., 2016). The methanogenic population significantly influences the AD process, using a polymerase chain reaction (Székely et al., 2013) ,(Prabhudessai 2013). This population includes acetogens, following either the acetolactic or hydrogenotrophic pathway (Prabhu 2016).

2.4 Microbial Communities in Anaerobic Digestion

Microbial communities play a pivotal role in anaerobic digestion by facilitating the different stages of the process. Acidogenic bacteria are responsible for the breakdown of complex organic compounds into simpler molecules, primarily volatile fatty acids. Subsequently, acetogenic bacteria convert these acids into acetate, hydrogen, and carbon dioxide.

The methanogenic archaea play a crucial role in methane production, as they utilize the substrates produced by the preceding microbial groups and convert them into methane and carbon dioxide through biochemical reactions. They are highly specialized and adapted to thrive in low-oxygen environments, such as the digester, and their activity is vital for efficient biogas production (Batstone et al., 2002).

Operating conditions, pH, temperature, and substrates are only a few of the variables that affect the variety and composition of microbial communities. To maximize the efficiency of anaerobic digestion, maintain steady biogas production, and reduce methane emissions, it is crucial to comprehend and regulate these microbial populations (Weiland, 2010). On the other hand, microbial interactions are the intricate driving force behind anaerobic digestion. By using the cooperative abilities of diverse microorganisms, it offers a viable approach to managing organic waste, producing biogas, and reducing methane emissions.

2.5 Sources of Biogas

The selection of feedstock significantly influences biogas quality, production efficiency, and process stability. Variations in energy content, moisture levels, and contaminants affect digestion performance and may necessitate gas treatment or system adjustments.

2.5.1 Animal Manure

Animal manure is one of the most used feedstocks, particularly in agricultural settings. It is readily available and contains substantial organic content. Its relatively stable composition

supports a consistent digestion process. Co-digestion with other substrates, such as food waste or crop residues, is often employed to enhance biogas yield (Vasantha et al., 2022).

2.5.2 Food Waste

Food waste possesses a high methane potential due to its rich organic composition and rapid biodegradability. It is widely sourced from households, commercial kitchens, and food processing industries. However, it typically requires preprocessing to remove contaminants and improve suitability for digestion. Its utilization also contributes to reducing landfill emissions (Saji Ananthu, 2019).

2.5.3 Agricultural Residues

These include crop by-products such as straw, corn stover, and grass silage. Although their individual gas yield may be lower compared to more energy-dense materials, they are abundant and cost-effective. Their use supports sustainable practices by converting agricultural by-products into energy without compromising food production (Nkoa, 2014).

2.5.4 Sewage Sludge

Sewage sludge from wastewater treatment facilities provides a consistent and centralized source of organic material. While widely utilized in anaerobic digestion systems, the resulting biogas may require purification due to impurities such as sulfur compounds and siloxanes before it can be used effectively (Potdukhe et al., 2021).

2.5.5 Organic Industrial Waste

Industries such as breweries, dairies, and food processing plants generate organic waste streams rich in sugars, fats, and starches. These materials offer high biogas yields and can be efficiently integrated into existing industrial processes, enhancing energy recovery and waste management (Khalid et al., 2011).

2.6 Importance of Biogas Production

Biogas is a renewable energy source generated through the anaerobic digestion of organic waste by microorganisms in an oxygen-free environment. It primarily consists of methane, a flammable gas that can be utilized for cooking, heating, and lighting (Mahanta et al., 2005). Effective biogas production requires a consistent supply of organic materials, preferably in moist form, to ensure optimal microbial activity and gas yield (Kasinath et al., 2021). Such feedstocks, including animal dung and other organic residues, not only support energy generation but also contribute to improved sanitation and waste management practices (Müller, 2011).

This is particularly significant in countries like Uganda, where access to modern energy remains limited. With rural electrification rates at approximately 10%, many communities—especially in northern regions continue to rely heavily on traditional energy sources such as firewood, charcoal, and crop residues. These areas are often characterized by inadequate infrastructure, limited access to clean cooking technologies, and persistent energy poverty (Kakumba, 2021). Consequently, biogas presents a viable and sustainable alternative for addressing both energy and environmental challenges in such contexts.

2.7 Application of Biogas

Biogas, a sustainable and renewable energy source, is widely applied across multiple sectors for heating, lighting, electricity generation, cooking, and as a substitute for natural gas in industrial processes. It can also be upgraded to biomethane, enabling its injection into natural gas pipelines or use as a transport fuel for buses and trucks (Kabeyi et al., 2022). In practical applications, biogas is utilized in radiant heaters—commonly used in livestock farming—where it fuels burners that heat ceramic elements to temperatures of 600–800°C to provide warmth for animals, requiring proper handling and maintenance for safe and efficient operation. It is also used in specially designed water heaters for domestic, commercial, and industrial

purposes, where it efficiently transfers heat to water for space heating (Torres et al., 2024). Additionally, absorption refrigerators operating on ammonia–water systems can be powered by biogas, with a typical 100-litre unit consuming approximately 2,000 litres of biogas per day depending on ambient conditions (Bochmann, 2013). Furthermore, biogas can be used as a fuel in modified diesel and four-stroke engines, demonstrating its versatility in mechanical and energy generation applications (Herrera et al., 2024).

2.8 Anaerobic Co-digestion Process

Anaerobic co-digestion involves the simultaneous anaerobic digestion (AD) of various organic biomass substrates in a single reactor (Hagos et al., 2017). This process relies on a series of biological processes carried out by different groups of microorganisms, including hydrolytic bacteria, acidogenic or fermentative bacteria, acetogenic bacteria, and methanogenic bacteria. Hydrolytic bacteria initially break down complex organic molecules into simpler compounds, while fermentative bacteria convert these compounds into volatile fatty acids (VFA) through hydrolysis and acidogenesis (Wang, 2014). Acetogenic bacteria transform VFA into acetate, carbon dioxide, and hydrogen through acetogenesis. Finally, methanogenic bacteria convert acetate, carbon dioxide, and hydrogen into biogas through methanogenesis (Collins et al., 2018).

2.8.1 Effects of Anaerobic Co-Digestion on Methane Production

It involves the combination of different materials with varying nutritional compositions and the subsequent use of such a mixture as a substrate in a digester. This approach is primarily employed to dilute toxic substances present in certain materials or to correct imbalances in the carbon-to-nitrogen (C/N) ratio, which is critical for efficient anaerobic digestion (Alvarez et al., 2014; Yen 2007). Co-digestion is a well-established technology that utilizes multiple organic wastes to enhance microbial activity and increase methane production (Li et al., 2011). Among various combinations studied, the co-digestion of potato waste and pig manure has been

reported to yield higher methane production, whereas the co-digestion of cow manure and chicken waste results in comparatively lower methane yields under mesophilic conditions (Li et al., 2013). However, careful selection of co-substrates, appropriate mixing ratios, and controlled operational conditions are essential to avoid process instability and reduced methane generation (Zhang et al., 2014).

2.8.2 Economic Advantages of Anaerobic Co-digestion

The utilization of digestate, a by-product of anaerobic digestion (AD), presents opportunities for extracting valuable resources and maximizing economic benefits. Selective separation processes have been explored to extract humus, organic acids, and nutrients from the liquid portion of digestate, in addition to biomethane production. These processes enhance the economic value of digestate and improve the stability of AD by removing inhibitory compounds. Electrode ionisation can be used to separate organic acids, mitigating the accumulation of volatile fatty acids that can hinder the AD process. The separated organic acids can be recycled for various applications, contributing to a circular economy. The economic feasibility of manufacturing short-chain carboxylic acids as a substitute for biomethane production has been demonstrated by recent studies. These acids are attractive for carbon reduction purposes since their potential energy outputs are either higher or equivalent to those of biogas.

2.8.3 Anaerobic Co-digestion, Progress and Challenges

Anaerobic co-digestion refers to the simultaneous breakdown of different organic materials, leading to enhanced biogas production, greater process stability, and the capacity to handle various types of waste. However, grasping these elements is essential for effective and sustainable waste management. The Loading Rate (LR) and pH levels play a significant role in anaerobic digestion, with appropriate pH control being vital for maintaining process stability (Ayeni et al., 2022). Hydraulic Retention Time (HRT) is a critical factor in the degradation of

organic matter and the production of biogas, as it provides the necessary duration for effective microbial activity. Bacterial communities are instrumental in enhancing anaerobic digestion through the decomposition of organic material. Additionally, the carbon-to-nitrogen ratio plays a vital role in optimizing biogas production by ensuring balanced microbial activity and maintaining stable digestion processes. However, while advancements have been made, there are still challenges to address in the co-digestion process (Shah et al., 2019). Below is the progress of this technology:

1. The anaerobic Co-digestion allows for the immediate breakdown of several substrates, resulting in an increment of biogas production compared to digesting a single substrate and diverse organic materials to achieve a balanced nutrient composition, which is vital for optimal microbial activity and biogas production (Hagos et al., 2017).
2. It provides an effective solution for managing several types of waste, such as agricultural remains, diet leftovers, and wastewater sludge. It combines different substrate processes to enhance the digestion performance by improving stability and efficiency in anaerobic digestion, leading to higher rates of organic matter degradation (Valenti et al., 2018a). Subsequently, there are also challenges apart from progress, which is the availability and quality of feedstock and ensuring a consistent and reliable supply of suitable feedstock for co-digestion are the most consistent challenges and require proper sourcing, handling, and preprocessing of diverse organic materials and the involvement of complex interactions between different substrates, necessitating careful process optimization to achieve optimal biogas production. Digestion performance is the main challenge that affects the anaerobic digestion process (Hagos et al., 2017).

2.9 Substrate Potential

Co-digestion of both substrates, cow dung and maize stubble, offers several potential advantages for biogas production. These advantages play a significant role in sustainable

energy production. Initially, the co-digestion of cow dung and maize stubble holds promise for enhancing biogas yield. Nevertheless, cow dung, renowned for its high organic matter content, creates an optimal environment for the proliferation and metabolic activity of anaerobic bacteria (Makaj et al., 2022). Conversely, maize stubble brings a high carbon-to-nitrogen C/N ratio to the digestate, effectively balancing the nutrient composition. Symbiotic interaction between these substrates manifests in a synergistic effect, culminating in a notable increase in biogas (Valenti et al., 2018). In addition, the co-digestion process allows for the utilization of multiple waste materials, thus promoting waste management and environmental sustainability. Codigestion of cow dung, which is a common agricultural waste, and maize stubble that is continually generated during harvest, is a valuable resource for biogas production, reducing the need for landfilling or open burning. This approach aligns with the principles of the circular economy and contributes to the reduction of greenhouse gases. Moreover, the co-digestion of cow dung and maize stubble exhibits the potential to enhance the stability and reliability of the biogas production process. Cow dung, characterized by its elevated moisture content, contributes to optimizing the water balance within the digester, thereby mitigating the risk of desiccation and fostering favourable conditions for bacterial activity. Conversely, the fibrous structure of maize stubble facilitates improved mixing and prevents sedimentation within the digester, thereby ensuring efficient biogas production.

2.10 Factors Affecting Biogas Production

The production of biogas through anaerobic digestion depends on several key factors that influence the generation process, including the following:

2.10.1 Temperature

The production of biogas is significantly influenced by the temperature of the digester (Luo et al., 2010). Microbial activity in the digestion process involves various types of microorganisms, including psychrophilic (10 °C), mesophilic (37 °C), thermophilic (50 °C), and extremophilic

(>65 °C) organisms, each of which has specific temperature ranges for optimal growth and function. The temperature requirements of these microorganisms are closely associated with their native environments (Jarvis et al, 2010). For instance, microorganisms indigenous to human or animal intestines are adapted to thrive at approximately 37 °C, while those originating from septic tanks are more suited to lower temperatures around 10 °C.

Biogas production generally increases with temperature, provided the microorganisms are adapted to those conditions. Schnurer and Jarvis (2010) note that mesophilic (37 °C) and thermophilic (55 °C) temperatures are typically used for biogas production. Studies by Dobre et al. (2014) show that optimal production occurs at mesophilic temperatures of 32 to 40 °C. Most anaerobic digesters operate at mesophilic conditions, as psychrophilic conditions are less economically viable and thermophilic conditions are less common.

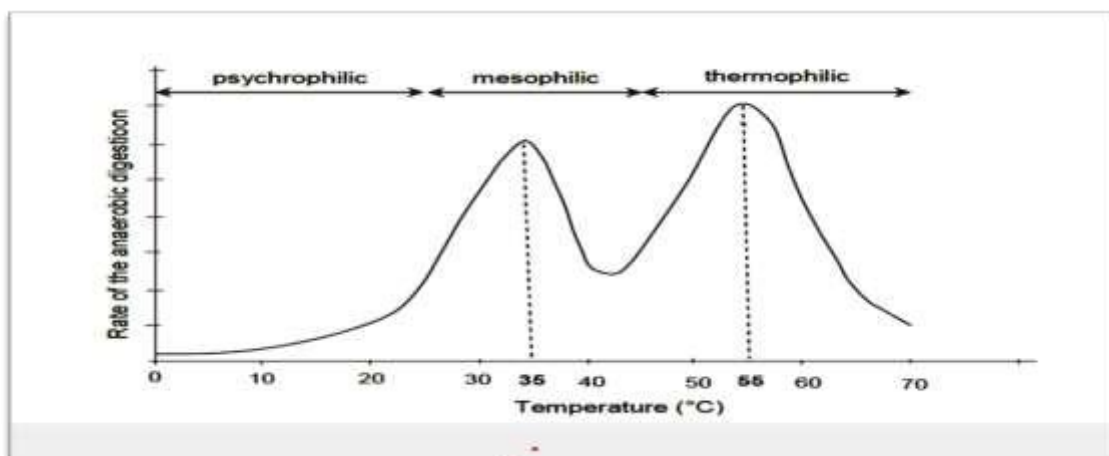


Figure 2.2: A relationship between temperatures and the rate of AD (Dobre et al., 2014)

2.10.2 C/N Ratio

The C/N ratio is crucial in maximizing biogas production and assessing feedstock nutritional content. A high C/N ratio results in nitrogen shortage, while a low ratio indicates poor carbon content. Adding nitrogenous substitutes like animal dung or carbon-rich substrates like kitchen waste can improve biogas production. Co-digesting substrates with the appropriate C/N ratio, such as rice straw and *Hydrilla verticillate*, have been shown to provide necessary nutrition for

microbial activities and result in the best biogas yields Wang et al., 2014). The study revealed that substrates with a higher carbon-to-nitrogen C/N ratio resulted in increased methane production. This indicates that the feedstock composition, particularly the C/N ratio, can impact the efficiency of methane generation. However, determining the optimal feedstock ratios is essential for maximizing biogas production. Researchers have conducted various studies to identify the perfect feedstock ratios for enhanced biogas production.

2.10.3 pH

According to Gerardi (2003), the continuous production of CO₂ during the acidogenesis phase significantly affects the pH of the digestate. An increase in CO₂ concentration during the acidogenesis phase leads to pH reduction, as excessive CO₂ can dissolve in the digestate. Simultaneously, the VFAs generated during the process contribute to further pH reduction, potentially dropping below 6.0. However, Kim et al. (2003) noted that the decline below 5.0 would have adverse effects on the hydrolysis and acidogenesis stages. Hence, accurate monitoring and adjustment of the digestive pH are crucial to ensure optimal and sustainable performance in the anaerobic digestion process.

2.10.4 Loading Rate (LR)

The loading rate in anaerobic co-digestion digesters is the degree to which organic feedstock is introduced into the digester. It plays a vital role in the performance and efficiency of the AD process. However, the loading rate is a significant factor that affects the performance of the process in anaerobic co-digestion digesters (Smith et al., 2018). It determines the amount of organic feedstock that can be effectively digested within a specified period, which influences the biogas production and inclusive efficiency of the system. Moreover, the low loading rate in anaerobic co-digestion digesters results in underutilization of the digester capacity and inefficient biogas production. On the other hand, operating at a loading rate that is too high can lead to the accumulation of volatile fatty acids, ammonia, and other inhibitory compounds,

causing process instability and decreased biogas yield (Li et al., 2019) Conversely, the determination of the suitable loading rate for anaerobic co-digestion digesters considers factors such as the characteristics of the feedstock, digester design, and microbial community composition (Zhang et al., 2021). It is very crucial to optimize the loading rate based on these factors to achieve maximum biogas production and maintain stable digester operation.

2.10.5 Hydraulic Retention Time (HRT)

The average duration that liquid and soluble compounds remain within a reactor or tank is referred to as HRT. According to Karnaros et al. (2014), shortening the HRT allows for faster turnover of wastewater in the reactor, promoting better mixing and preventing the settling of solids. Additionally, increasing the organic loading rate can enhance digester efficiency by providing more substrate for microbial activity, leading to improved degradation of organic matter.

2.10.6 Mixing Ratio

A methane digester requires a mixing device to improve contact between substrates and microorganisms, with propellers and paddles being the most common. While mixing is not essential for methanogenesis (Demuynck et al., 1984), it helps achieve homogeneity, reduce scum formation, and keep contents in suspension (Demuynck et al., 1984).

2.10.7 Substrate Size Reduction

Size reduction is a simple yet effective technique utilized to enhance the efficiency of biomass use in anaerobic digestion. Decreasing the particle size of the substrates, such as lignocellulosic materials or various organic waste, this approach increases the specific surface area accessible to microbial enzymes. The sampler surface area promotes more efficient enzymatic hydrolysis, speeding up the degradation of organic materials into digestible parts

(Czekala et al., 2023). One of the key effects of the size reduction process is to increase biogas production. The reduction in particle size exposes more biomass to microbial activity, boosting both the rate and volume of biogas generation (Bochmann, 2013). However, Smaller particles improve fluid dynamics within digesters, leading to a decrease in the viscosity of the substrate mixture. This results in more efficient mixing and improved contact between the substrate and microbes. Lastly, lowering particle size helps prevent the creation of floating layers. These layers, usually made up of undigested material, can block gas outlets, hinder gas release, and decrease reactor efficiency by making some biomass unavailable for digestion. Size reduction is an effective method that enhances biomass efficiency in anaerobic digestion. By reducing the particle size of feedstocks such as lignocellulosic materials and organic waste, the specific surface area accessible to microbial enzymes increases, promoting more efficient enzymatic hydrolysis and accelerating the breakdown of organic materials (Habib et al., 2024).

2.10.8 Influence of Substrate Ratios

The composition of the substrates also plays a crucial role in methane generation during anaerobic digestion. Research conducted by Jin et al. (2015) examined the influence of feedstock composition on methane production in a pilot-scale anaerobic digester. A study by Zeng& Li (2015) examined the co-digestion of different ratios of grass and manure. They found that a specific ratio of grass to manure resulted in the highest biogas yield. The study suggests that a certain feedstock ratio can optimize biogas production. Nonetheless, the choice and composition of feedstock, as well as the ratios used in anaerobic digestion, have a significant impact on biogas production. Understanding the influence of different feedstock ratios on biogas production and methane generation is crucial for optimizing the anaerobic digestion process and maximizing biogas yield.

2.11 Energy Access Challenges in Rural Areas

The utilization of cow dung-based biogas production is prevalent among most households in Uganda due to the widespread practice of cattle-rearing through traditional and small-scale production systems. According to Organic Catalyst Uganda Ltd., an estimated 8000 standard plastic biogas digesters are currently operational in the region, indicating the substantial reliance on biogas in Ugandan society. With an average household size of 7 people in Northern Uganda, a significant portion of the population utilizes biogas, addressing waste management challenges and providing primary energy for cooking. Despite having one of the highest rural access to electricity in Uganda, with an average of only 9% in the entire region, it remains unlikely that grid electricity will reach the poorest hinterlands. The development of stand-alone cow dung biogas digesters has received support from local and national government initiatives in Northern Uganda. Efforts are underway to maximize the biogas potential of natural cattle manure for broader use in the region's primary energy supply. Biogas energy, accessible to farmers and utilized in agriculture and agro-processing units near cow dung biogas energy plants, is just one of the renewable energy sources that should be harnessed in an area where agricultural and livestock waste is often managed inadequately.

Today, approximately 8 million out of 40 million people in Uganda are connected to the electricity grid, leaving about 15 million without access to electricity (Kavuma et al., 2021). Rural electrification is currently not feasible, and there is no framework to support the provision of solar home systems in rural areas. The expensive nature of solar technology and its limited reach in the country have left most of the rural population without its benefits (Pillot et al., 2017). The challenge of rural electrification persists due to the inability of the electricity grid to extend connections to remote areas. Not only has electricity access declined in rural areas, but the electricity system itself is currently overwhelmed, resulting in an unreliable power supply, which in turn hampers the prospects of sustainable and reliable energy for the rural poor

(Assefa et al., 2022). The reliance on traditional energy sources has led to the rapid depletion of forest reserves. While the introduction of energy-saving stoves is a positive step, charcoal will remain a primary energy source until alternative cooking fuels become more widely available. Unfortunately, rural electrification via the existing grid is currently infeasible due to urban and political interests dominating policy development, resulting in a disproportionate budget allocation favouring urban areas. This situation not only hinders progress but also contributes to an increase in rural poverty in the North (Bergland et al 2024).

2.12 Key Issues Arising from the Literature Review

The literature identifies several key issues affecting biogas production through anaerobic codigestion. Substrate limitations, particularly the imbalance in the carbon-to-nitrogen (C/N) ratio between cow dung and maize stubble, reduce efficiency and require careful optimisation. Process parameters such as temperature, pH, hydraulic retention time, and loading rate also play a critical role, with poor control leading to instability and lower methane yields.

In addition, technological and operational constraints, including digester design and inadequate substrate preprocessing, limit performance. Economic challenges, such as high costs and scalability issues, further hinder adoption despite the potential benefits of digestate utilisation. Moreover, limited energy access and environmental concerns in rural areas emphasise the need for sustainable alternatives.

While anaerobic co-digestion has strong potential, its success depends on proper substrate management, optimised operating conditions, and overcoming economic and technical barriers

CHAPTER THREE: METHODOLOGY

3.1 Study Area

The experiments on anaerobic co-digestion of cow dung and maize stubble were set up in the Soil Laboratory at the Faculty of Agriculture, Gulu University. The university is found in Gulu City, in the northern region of Uganda, between 30–32°E longitude and 2–4°N latitude. It is bordered by Amuru and Nwoya districts to the west and southwest, Lamwo to the northeast, Pader to the east, Lira to the southeast, and Oyam to the south. The total area of Gulu is 1,872 km², with a projected population of 325,600 (Midamba et al., 2023). The city is located 332 km north of Kampala, and the Great North Road provides access to South Sudan and the Democratic Republic of the Congo. For cooking, most residents continue to utilize biomass with improved stoves, while unreliable grid power is anticipated to drive the further adoption of standalone solar systems.

3.2 Sample Collection and Preparation

Fresh cow dung and maize stubble were collected from Lujor Awinyi village, located on the outskirts of Gulu City. This area is significant due to extensive maize cultivation and the presence of grazing cows owned by small-scale farmers. The collection methods for cow dung and maize stubble varied for each type. For maize stubble, 20 kg were collected from the field and placed in a plastic bag, while for cow dung, 15 kg of cow dung was collected and put in plastic buckets. Shovels, pitchforks, and gloves were used for gathering cow dung, while sickles and scythes were used for maize stubble samples. Plastic bags and plastic buckets were used as containers for both types of samples. The samples were carefully purified by removing the impurities. It was evenly spread and dried under controlled conditions to minimize moisture content by 6.73% for cow sand and 4.85% for maize stubble, and to inhibit microbial degradation. After achieving a safer state of moisture content, both samples were securely stored in airtight plastic containers to preserve their integrity and prevent contamination.

Finally, the samples were maintained at room temperature until its utilization in the experiment (Mkruqulwa et al., 2020). The analysis of these samples was carried out in the Soil and Water laboratory in the Faculty of Agriculture and Environment at Gulu University

3.3. Pre-treatment Procedures

To efficiently convert maize stubble (MS) and cow dung (CD) into biogas, it was essential to pre-treat the feedstocks. The feedstocks were sun-dried to reduce their moisture content, preventing microbial growth and spoilage. Figure 3.2 shows the feedstocks dried in the sun. Dried cow dung was then crushed into small particles using a grinder, followed by sieving with a 2 mm mesh to enhance the surface area and accelerate its decomposition. For maize stubble, chopping knives and cutting mills were used to process the material, followed by drying and grinding to achieve a particle size of approximately 2 mm before use. These preparations were essential to enhance substrate digestibility and optimize the anaerobic codigestion process.



Figure 3.1: Sundried substrates of CD & MS

3.4 Proximate and Elemental Analyses

The substrates used in this study consisted of maize stubble (MS) and cow dung (CD). Proximate, ultimate, and nutrient analyses were conducted to provide further insight into the characteristics of the substrates. The detailed characterization is as follows:

3.4.1 Proximate Analysis

The proximate analysis involved the determination of the moisture content, volatile matter, and fixed carbon and ash contents of each of the feedstocks. The methods followed to determine each parameter are described below.

3.4.1.1 Determination of Moisture Content

The term moisture content refers to the amount of water present in a substance. (Hussien et al., 2018) . It was determined using the APHA 2540 B, 2012 standard method. A sample weighing 3 grams from each initial substrate, specifically maize stubble (MS) and cow dung (CD), was measured for analysis to ensure uniformity in experimental conditions. These samples were subsequently placed in a convection oven (MKR-136L). The samples were dried for 4 h at 105 °C. Following the drying process, the moisture content was computed using Equation (3.1).

$$MC \% = \frac{W_i - W_f}{W_f} \times 100 \quad (3.1)$$

where: MC: is the moisture content in dry matter (%), W_i : is the weight of the sample before drying and W_f : the weight of the sample after drying. Figure 3.3 shows the feedstocks after oven drying in three replicates.



Figure 3.2: Substrates after oven drying

3.4.1.2 Determination of Total Solids (TS)

Total Solids (TS) refers to the amount of dry matter in the material (Bochmann et al., 2013). Three grams of each sample of maize stubble (MS) and cow dung (CD) were weighed using a scale balance (JA4000C, 201601203), with a readability of 0.1 g. The samples were put in pre-dried aluminium weighing dishes and placed in a convection oven (MKR-136L) according to the APHA 2540 B 2012 standard method. The oven was set at $105 \pm 3^{\circ}\text{C}$ for 4

h. Subsequently, the dishes were cooled in a desiccator. The pre-dried aluminium dishes were then weighed to the nearest 0.1 mg using the analytical balance. After that, the oven-dried sample contained within the dish was weighed to the nearest 0.1 mg, and the mass was recorded. The dish and sample were then returned to the convection oven, maintained at $105 \pm 3^{\circ}\text{C}$, and dried until a constant weight was achieved in three different replicates. The TS content was calculated using Equation (3.2).

$$\text{TS}\% = \frac{W_d - w_w}{W_d} \times 100 \quad (3.2)$$

where: W_d is the initial weight in (g) of the sample before drying and w_w is the weight of the sample (g) after drying to constant weight.

3.4.1.3 Determination of Volatile Matter (VM)

The volatile solids (VS) content of the CM and MS was measured by analyzing the total solids (TS) content. Both samples were heated in an inert environment using a furnace (Thermo Scientific, M-104), set at 900°C for 7 min (Okalebo et al., 2013). After heating, the crucible containing the samples was allowed to cool for at least 10 min in a desiccator before being weighed (Peces et al., 2014). The VS content was determined using Equation (3). Figure 3.4 presents images of the substrates after ignition.

$$VM\% = \frac{W_i - W_a}{W_i} \times 100 \quad (3.3)$$

where, W_i is the initial weight of the sample in g before ignition and W_a is the weight of ash residue (g) after ignition.



Figure 3.3: Substrates after drying in the muffle furnace at 900 °C

3.4.1.4 Determination of Ash Content

The ash content of the dried biomass samples was determined using the gravimetric method according to the APHA 2540 B 2012 standard method. For each sample, 3 g of the sample was weighed and placed into a crucible. The crucibles were then subjected to a 5-hour heating in a furnace under air at a temperature of 550°C (Thermo Scientific, M-104). Following the test, the crucibles with the samples were cooled in desiccators, and the weight of the crucible and the sample was then recorded. The percentage of ash was calculated using the recorded data and Equation (3.4). The results for the samples of the ash are presented in Figure 3:3 after ignition at 550 C°.

$$\text{Ash}\% = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \%$$

(3.4) where: W_1 : is the crucible weight, W_2 : is the combined weight of the dry sample of feedstocks and the crucible and W_3 : is the weight of the ash along with the crucible after drying. Figure 3.5 shows the results



Figure 3.4: Substrates after drying in the muffle furnace at 550 C° of the ash.

3.4.1.5 Determination of Fixed Carbon % FC

The FC was calculated using the moisture content, volatile matter, and ash content by difference, using the appropriate Equation (3.5).

$$\%FC = 100 - (\% \text{ ash} + \% \text{ VM} + \% \text{ MC}) \quad (3.5)$$

where FC is the fixed carbon and %VM is the volatile matter, % ash is the ash content, and MC% is the moisture content.

3.4.1.6 Determination of pH

A 5 g sample of each substrate was carefully weighed and combined with 75 mL of distilled water to create a homogeneous solution. This mixture was thoroughly stirred to ensure even distribution and dissolution of soluble components. The pH of the resulting solution was then measured using a precision pH meter (ORP 920) to assess its acidity or alkalinity. This step was crucial for evaluating the initial chemical characteristics of the feedstocks, as pH plays a significant role in the anaerobic digestion process. Proper calibration of the pH meter was performed before measurement to ensure accuracy and reliability (Valenti et al., 2018).

3.4.2 Elemental Analysis

Carbon and hydrogen were primarily analyzed as part of the elemental analysis, with sulfur as a minor component. This analysis followed the Environmental Protection Agency (EPA) Method 440 (EPA, 1997). The elemental analyzer (ELTRA, CHS 580) available at Makerere University was used for the analysis.

3.4.3 Total Carbon Determination

Organic carbon was determined using a mixture of sulfuric acid and potassium dichromate ($K_2Cr_2O_7$). After complete oxidation from the solution, heat and external heating, the remaining $K_2Cr_2O_7$ was titrated with ferrous ammonium sulfate. The difference between the added and remaining $K_2Cr_2O_7$ was used to determine the organic carbon content in the substrate (Zhong et al, 2014).

Procedure:

- 0.5 g of (2 mm) feedstock was weighed and placed in a block digester tube. Then, 5 ml of potassium dichromate solution and 7.5 ml of concentrated H_2SO_4 were added.
- The sample was weighed and placed in a tube in a pre-heated block at 145-155°C for 30 minutes, then cooled. The digested material was placed in a 100 ml conical flask, and 0.3 ml of indicator solution was added. After thorough mixing with a magnetic stirrer, the digest was titrated with ferrous ammonium sulfate until a colour change from greenish to brown indicated the endpoint. The recorded titre was corrected for the mean of two reagent blanks, and Equations 3.6 and 3.7 were used to calculate the organic carbon.

$$\text{Organic Carbon (\%)} = \frac{T \times 0.2 \times 0.3 \times \text{Sample weight}}{V_b - V_s} \quad (3.6)$$

$$\text{Organic carbon C \%} = \frac{0.003 \times 0.3 \times V_b - V_s \times 100}{W} \quad (3.7)$$

where, V_b : represents the volume, measured in millilitres, of 0.2 M ferrous ammonium sulfate utilized for titrating the reagent blank solution, V_s : Volume (ml) of 0.2 M ferrous ammonium sulfate used to titrate sample: 12/4000; 0.003 is the milliequivalent weight of C in grams. and W : is the sample weight.

3.4.4 Total Nitrogen Determination

Organic carbon was determined using the dichromate oxidation method, where a mixture of sulfuric acid and potassium dichromate ($K_2Cr_2O_7$) was used to oxidize the sample. The excess dichromate was then titrated with ferrous ammonium sulfate, and the difference between the initial and residual dichromate was used to calculate the organic carbon content (Zhong et al., 2014). Briefly, 0.5 g of 2 mm feedstock was digested with potassium dichromate and concentrated H_2SO_4 at 145–155°C for 30 minutes, followed by titration with ferrous ammonium sulfate using an indicator to determine the endpoint. The titre was corrected using reagent blanks, and the organic carbon content was calculated accordingly.

$$\%N = (V_1 - V_2) \times N \times F \times 100 / V \times (0.014) \times (100 / W) \quad (3.8)$$

where: N represents the total nitrogen ratio, V_1 is the sample volume in mL, N is the normality of the standard HCl, V_2 is the volume of the blank (mL), V is the volume used for distillation, F is the factor of the standard HCl and W : Weight of the sample (g).

3.4.5. Determination of the C/N Ratio

The carbon and nitrogen content of each substrate was determined, and their contributions were calculated and summed to obtain total carbon and nitrogen. The C/N ratio was then calculated by dividing total carbon by total nitrogen.

3.5 Optimization of Biogas Production

After conducting proximate and ultimate analyses, as well as determining heating values, optimization of anaerobic conditions for biogas production was performed at the laboratory scale using RSM based on CCD. More details are given in the subsequent sections.

3.5.1 Experiment Design

Response surface methodology based on central composite design (CCD) was used to optimize the anaerobic digestion process. Design Expert software was used to design the experiments in which 13 experimental runs were arrived. This approach optimized two key process parameters: the co-digestion ratio of cow dung to maize stubble (A) and temperature (B), as shown in Table 3.1. The CCD method allowed for the exploration of a range of values for each parameter, helping to identify the optimal levels for maximizing biogas production. ANOVA was employed to analyze the statistical significance and interactive effects of these parameters. These levels were designated as -1, 0, and +1. The response variable measured was the gas volume in mL, methane CH₄%, and carbon dioxide in CO₂%. Thirteen experimental runs were organized in random order to minimize the impact of uncontrolled variables. These comprised four factorial points, four axial points, and five central replicates, as derived from Equation (3.9).

$$N = 2N_f + 2N_a + N_c \quad 3.9$$

where N denotes the total number of experimental runs needed, while n represents the number of factors. Additionally, N_c indicates the number of replicates at the centre points. The central points were used to assess experimental error and ensure data reproducibility. Table 3.1 presents an overview of the experimental levels, describing both the coded values and their corresponding actual values.

Table 3.1 Experimental Levels

Variable	Parameter	Unit	Level		
			Low (-1)	0	High (+1)
A	Co-digestion Ratios	%	40:60	70:30	100
B	Temperature	°C	25	35	45

3.5.2 Experimental Procedures

The experimental setup involved the preparation of substrates by combining 100 grams of cow dung and maize stubble in specific ratios to assess their co-digestion performance. The mixtures were formulated in the following proportions of cow dung to maize stubble: 100:0, 0:100, 40:60, and 70:30. To enhance microbial activity and accelerate the anaerobic digestion process, 200 mL of inoculum sourced from an active cow dung biogas digester was introduced into each digestion bottle. Additionally, 950 mL of distilled water was added to each bottle to achieve the desired moisture content and facilitate microbial metabolism. This standardized approach ensured consistency across all experimental conditions, promoting reliable and reproducible results, following the method outlined by Amon et al. (2007). According to the method. The anaerobic digestion process is most effective at 6-10% TS for wet digestion, so the proportions of feedstock and water were modified to achieve 8% TS. This composition aimed to create optimal conditions for anaerobic digestion and efficient biogas production. The experiments utilized 1.5 L plastic bottles with a working volume of 1.2 L, submerged in a water bath (WNE10-230V-50/60Hz). The bottles were incubated at controlled temperatures of 25 °C, 35 °C, and 45 °C to examine the effect of temperature on biogas production. Thirteen bottles were filled with acidified water containing 0.5 parts of HCl per litre to reduce the risk of gas backflow into the biogas collection. Graduated bottles were used to collect water, which was used to determine the biogas volume by applying the water displacement method. The bottles were interconnected with a hose pipe to transfer gas from the first to the second bottle, serving as a water tank, and then to the third graduated bottle functioning as a water collector (Ingabire et al., 2023). Oxygen was removed from the digester bottles by bubbling nitrogen gas for three

minutes. After filling with inoculum and substrates according to experimental specifications, the bottles were sealed immediately (Mkruqulwa et al., 2020). The bottles were manually shaken daily to ensure reactor homogeneity, release trapped gases, and prevent scum build-up (Singh et al., 2021). Figure 3.7 shows the experimental setup involving anaerobic co-digestion of cow dung and maize stubble.



Figure 3.5: Experimental setup of anaerobic co-digestion of CD and MS

3.6 Measurement of Biogas Volume

Daily gas volume readings were carefully recorded by measuring the volume of water displaced due to biogas production, with results expressed in millilitres. Biogas was effectively captured in a specially designed container connected to an inverted graduated bottle filled with distilled acidified water. As the biogas accumulated in the container, it displaced the water in the inverted bottle, allowing for precise measurement of the biogas volume. The use of distilled acidified water ensured that any potential reactions or contaminations would not interfere with accurate readings, facilitating reliable monitoring of biogas production over time. Each reading involved meticulous observation and timing to ensure precision in the recorded volume (Ingabire et al., 2023).



Figure 3.6: Biogas Analyzer GA5000

3.7 Measurement of Biogas Composition

To analyze the composition of biogas, an additional set of thirteen bottles was prepared as replicates. These bottles were placed in a controlled temperature water bath (25, 35, and 45 °C) with different substrate ratios (0:100, 40:60, and 70:30). They were sealed with septa rubber stoppers and each fitted with a hose pipe. The portable biogas analyzer (Geotech, GA 5000) was used to determine the biogas composition. Each sample was connected to the gas inlet port, as shown in Figure 3.8. The equipment identifies and quantifies various gases, including methane, carbon dioxide, and hydrogen sulfide. Gas composition was monitored every three days throughout the experimental period.

3.8 Economic Analysis

An economic analysis was conducted to evaluate the viability of anaerobic co-digestion of cow dung and maize stubble for biogas production. Capital budgeting techniques were applied, including Net Present Value (NPV) to assess profitability, Internal Rate of Return (IRR) to evaluate investment attractiveness, Payback Period to estimate the time required to recover initial costs, and Profitability Index (PI) to determine cost-effectiveness (Herbes et al., 2020).

In addition, a Cost-Benefit Analysis (CBA) compared total costs comprising capital investment, operation, and maintenance with projected revenues from biogas utilization (Liu, 2022).

The assessment further examined the potential of biogas to replace conventional cooking fuels for a household of 4–5 members. In Northern Uganda, LPG usage is limited due to high costs and restricted availability, while fuelwood and charcoal remain more accessible (Crawford et al., 2015). Based on Alamu (2014), a household of this size requires approximately 1–2 m³ of biogas per day for cooking. A 1 m³ biodigester, capable of producing about 1.5 m³ of biogas daily under optimal conditions, was therefore considered appropriate.

The estimated construction cost of a 1 m³ biodigester in Gulu City was calculated using an exchange rate of 365,000 UGX per \$100 (Economy, 2024), as presented in Table 4.5. For comparison, household energy costs were also evaluated. LPG, priced at \$3 per kg, results in a monthly cost of \$36 for a 12 kg cylinder, while charcoal, at \$20 per sack, amounts to approximately \$40 per month for two sacks. These estimates indicate that LPG is relatively more affordable than charcoal.

Annual savings were determined by multiplying the monthly savings (\$36) by 12 months, providing an estimate of total yearly cost savings, as summarized in Table 4.6. Annual Savings = Monthly Savings × 12 = \$36 × 12

This annual savings figure of \$432 was subsequently utilized to evaluate economic indicators, such as the profitability index and payback period, to assess the project's financial viability.

Table 3.2: Estimated Cost for the Construction of the 1m³ Digester

Items	Total Price in USD
Construction of a biogas 1 m ³ digester	900
Labour cost	400
Cost of a gas stove	50
Additional cost (operation cost)	250
Total	1600

The study also considered the viability of commercial-scale implementation by assessing key factors such as substrate availability and logistical considerations as follows:

3.8.1 Payback Period

An inclusive analysis was conducted using various economic indicators to evaluate the potential profitability, including Payback Period, which refers to the amount of time required for an investment to generate sufficient cash flow to recover its initial cost. Generally, projects with shorter payback periods are considered more appealing, as they indicate faster returns and lower financial exposure. Equation 4.4 was used to determine the payback period.

(Herbes et al., 2020).

$$\text{Payback period} = \frac{\text{Initial investement}}{\text{Annual saving}} \quad (3.4)$$

3.8.2 Net Present Value (NPV)

NPV refers to the total present value of net cash flows for each year, calculated at a specified discount rate during the project's calculation period. Equation 4.5 was used to calculate it.

$$\text{NPV} = \sum_{t=1}^n \frac{K_t}{1+r^t} - \text{Initial Investement} \quad (4.5)$$

where: K_t : net cash flow in the year, r : discount rate (10%), t : is the year (1- n) and the initial investment accounts for 1600 USD.

From the results, the sum of all discounted cash inflows over 10 years is calculated as \$2,654.45 USD, after subtracting the initial investment of \$ 16,00 USD, the final NPV is 10,054,45 USD

The project exhibits a Net Present Value (NPV) of USD 1,054.45, signifying financial viability and potential profitability under the current expectations. However, ensuring longterm sustainability requires a comprehensive risk assessment. This should include sensitivity analyses on critical variables, such as discount rates and operational costs, to assess the impact of fluctuations on financial performance. A detailed understanding of these factors will enable us to make informed decisions and implement risk-mitigation strategies, thereby enhancing the study overall resilience and financial sustainability.

3.8.3 Profitability Index (PI)

The Profitability Index (PI) measures the ratio of the present value of future cash flows to the initial investment, serving as a useful tool for ranking investment projects. It highlights the value generated for each unit of investment. (Kengatharan, 2016). Equation 4.6 was used to compute the PI.

$$PI = \frac{\text{Present value of future cash flow}}{\text{Initial Investment}} \quad (4.6)$$

3.9 Statistical Analysis

Statistical analysis was conducted using Central Composite Design (CCD) within the framework of Response Surface Methodology (RSM) to optimize biogas production. This approach enables the systematic evaluation of multiple process variables at different levels and facilitates the development of a predictive model for methane yield. To determine the significance of individual factors and their interaction effects, Analysis of Variance (ANOVA) was employed.

Furthermore, CCD under RSM utilizes ANOVA to assess the adequacy and statistical significance of the fitted quadratic model. The analysis provides key statistical indicators, including the model F-value, p-value, coefficient of determination (R^2), adjusted R^2 , predicted R^2 , and lack-of-fit test, which are used to evaluate the reliability and predictive capability of the model. These results also support the identification of optimal conditions for efficient co-digestion, thereby enhancing both the yield and quality of biogas.

3.9.1. Model Significance

- F-value (Model F-test): Indicates whether the model is statistically significant.
- p-value (Prob > F): A p-value less than 0.05 indicates that the model is statistically significant.

3.9.2 Significance of Model Terms

Each term in the model is tested individually:

- Linear terms (A, B, C)
- Interaction terms (AB, AC, BC)
- Quadratic terms (A^2 , B^2 , C^2)

Each has its own F-value and p-value to determine significance.

3.9.3 Lack of Fit Test

- Evaluates whether the model fits the data well.
- Non-significant lack of fit ($p > 0.05$) is desirable → indicates good model fit.

3.9.4. Coefficient of Determination

- R^2 : Proportion of variance explained by the model.
- Adjusted R^2 : Adjusted for the number of predictors.
- Predicted R^2 : Indicates predictive ability of the model.
 - These values should be reasonably close.

3.9.5. Adequate Precision

- Measures signal-to-noise ratio.
- A value > 4 indicates adequate model discrimination.

3.9.6. Coefficient of Variation (CV%)

- Indicates model reliability and precision.
- Lower CV% suggests better reproducibility.

3.9.7 Sum of Squares (SS) and Mean Squares (MS)

- Used to compute F-values. Includes:
- Model SS
- Residual SS
- Pure error SS
- Lack of fit SS

CHAPTER FOUR: RESULTS

4.0 Characteristics of cow dung and maize stubble

This chapter provides a detailed characterization of the feedstock, including proximate and ultimate analyses, along with the heating value of the substrates. These evaluations are essential for understanding the biochemical properties that influence anaerobic digestion efficiency and biogas production.

4.1 Proximate Analysis Composition

A notable difference in the moisture content between cow dung and maize stubble was observed. Specifically, the moisture content of cow dung was recorded at 6.73% (db.), which is slightly higher than that of maize stubble, measured at 4.85% db. Consequently, this leads to a higher total solids content in maize stubble, noted at 95.15 % (db.), compared to cow dung at 93.27% db. Furthermore, the volatile solids content of cow dung, at 59.45 % db., is lower than that of maize stubble, which stands at 70.85% db. Additionally, the ash content for cow dung was found to be greater, measuring 21.4% db., in contrast to maize stubble, which exhibited an ash content of only 9.19% db. Finally, the fixed carbon content in cow dung was also lower at 12.38% db., whereas maize stubble demonstrated a slightly higher fixed carbon content of 15.14% db.

Table 4.1: Proximate composition of the Substrates

substrate	MC (db. %)	TS (db. %)	VS (db. %)	AC (% db.)	FC (%) db.
Cow dung	6.73	93.27	59.45	21.4	12.38
Maize stubble	4.85	95.15	70.85	9.15	15.14
Inoculum	84.1				

Table 4.1 shows results obtained from proximate analysis of the feedstocks.

4.2 Elemental Composition and Heating Value

The carbon content of cow dung was slightly higher (32.87) compared to maize stubble (31.55%), affecting organic matter degradation. The hydrogen content in both substrates was similar (cow dung 1.39%, maize stubble 1.37), contributing equally to organic compound reduction during digestion. Maize stubble had a higher sulfur content (0.11) than cow dung, which can pose corrosion risks in biogas applications due to hydrogen sulfide formation. **Table 4:2 Elemental Compositions, Heating value**

Sample Type	C (%db.)	H (% db)	S (%db)	HHV MJ/kg
Cow dung	32.87	1.39	0.08	13.630
Maize stubble	31.55	1.37	0.11	16.050

Table 4.2 shows the elemental composition and heating value of the feedstocks.

4.3 Nutrient Composition and pH

Table 4:3 shows the nutrient composition of the feedstocks in terms of total nitrogen, total carbon and C/N ratio, as well as pH. Maize stubble exhibited 56.46% total carbon (TC), which was higher than that of cow dung, 51.71%. This higher carbon content suggests greater energy potential for microbial degradation and could enhance the carbon-to-nitrogen (C/N) ratio in co-digestion.

Table 4:3 Nutrient Composition of the feedstock and pH

Sample Type	TN (db.)	TC (db.)	pH	C/N Ratios
Cow dung	2.4	51.71	8.6	21.54
Maize Stubble	1.9	56.46	5.46	29.84

Cow dung exhibited a nitrogen content of 2.4, compared to maize stubble's 1.9, making it a better nitrogen source for anaerobic digestion. The lower nitrogen content in maize stubble results in a higher C/N ratio, potentially affecting its biodegradability. Cow dung exhibited

more nitrogen (2.4) than maize stubble (1.9), translating to C/N ratios of 21.54 and 29.84, respectively. Thus, there is a need for co-digestion to enhance maize stubble's biodegradability.

4.3 Response Surface Methodology

4.3.1 Biogas Volume, Methane Yield, and CO₂ Concentration

The reactors were set up with the following ratios of cow dung (CD) to maize stubble (MS): 100:0, 0:100, 40:60, and 70:30, each with an inoculum volume of 200 mL. The temperature was maintained within a range of 25-45 °C. Samples 1, 2, 4, 8, and 13 at the centre point were used to determine the experimental error and the reproducibility of the data. The results of the experiment are summarized in Table 4.4, which provides details on the cumulative gas volume, methane yield and carbon dioxide concentration for the period of the experiment. **Table 4:4**

Experimental conditions and results

Std	Run	A: Co-digestion Ratio %	B: Temperature °C	Response 1 Gas Volume L biogas/KgVS	Response 2 Methane Yield CH ₄ % /kg VS	Response 3 Carbon Dioxide Concert. CO ₂ % kgVS
8	1	70	45	4.39	50.93	26.13
9	2	70	35	5.19	82.56	36.44
6	3	100	35	3.99	82.24	44.00
2	4	100	25	4.80	66.54	27.95
5	5	40	35	5.35	93.68	30.91
3	6	40	45	5.09	55.80	28.75
13	7	70	35	5.25	76.06	28.79
11	8	70	35	4.99	95.20	37.45
12	9	70	35	4.71	54.26	32.82
7	10	100	25	4.20	57.09	31.29
10	11	70	35	4.83	86.56	27.67
1	12	40	25	3.70	64.07	15.24
4	13	100	45	2.02	30.48	24.84

Table 4:4 demonstrates that co-digestion at 35°C significantly enhances both biogas yield and methane content. The highest gas production, measured at (5.35 L/kg VS), occurred with a 40:60 ratio of cow dung to maize stubble (Run 5, 35°C). Conversely, the highest methane yield, at (95.20 % kg/Vs), was achieved with a 70:30 co-digestion ratio (Run 8, 35°C). In contrast, mono-digestion of maize stubble, particularly at 45°C, resulted in lower methane yields and higher carbon dioxide (CO₂) concentrations, indicating inefficiencies (Run 13).

4.3.1.1 ANOVA Quadratic Model Fitting for Gas Volume

The statistical significance of the quadratic models was assessed by analyzing the lack of fit and the significance of the coefficients. The model's F-value of 24.45 indicates that the model is significant (Table 4.5). Given the presence of noise, there is only a 0.03% chance that an F-value this large could occur purely by chance; values less than 0.0500 suggest that the model terms are significant. In this case, the terms A, AB, A², and B² are considered significant.

Conversely, values greater than 0.1000 indicate that the model terms are not significant. The Lack of Fit F-value is 0.87, which suggests that the lack of fit is not significant when compared to the pure error. There is a 47.39% chance that a Lack of Fit F-value this large could arise due to noise. A non-significant lack of fit is desirable as it indicates that the model effectively fits the data.

Table 4.5 Analysis of Variance (ANOVA) for the Fitted Quadratic Model for Gas Volume

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	9.22	5	1.84	24.45	0.0003	Significant
A-Co-digestion Ratio	2.38	1	2.38	31.56	0.0008	Significant
B-Temperature	0.2545	1	0.2545	3.38	0.1088	Insignificant
AB	4.35	1	4.35	57.68	0.0001	Significant
A ²	0.4961	1	0.4961	6.58	0.0373	Significant
B ²	0.9532	1	0.9532	12.64	0.0093	Significant
Residual	0.5278	7	0.0754			
Lack of Fit	0.1363	2	0.0682	0.8703	0.4739	
Pure Error	0.3915	5	0.0783			
Cor Total	9.74	12				

A quadratic model was developed using CCD data and fitted in Design-Expert 13, with ANOVA. The results were adjusted to a second-order polynomial equation in coded terms, as presented in Equation (4.1).

$$Y1 = +5.04 - 0.6017A - 0.9724AB - 0.4866A^2 - 0.6634B^2 \quad (4.1) \text{ where } Y1 \text{ is}$$

the gas volume (response), A is the co-digestion ratio (CD: MS) B is the Temperature °C.

4.3.1.2 ANOVA Quadratic Model Fitting for Methane Yield

In Table 4:6, the Model's F-value of 4.04 indicates that the model is significant, with only a 4.80% probability of observing such a large F-value due to random noise. P-values below 0.0500 suggest that the model terms are significant, and in this case, B² stands out as a significant term. Conversely, values exceeding 0.1000 indicate that the model terms are not significant. If numerous insignificant model terms are present (excluding those necessary for maintaining hierarchical structure), reducing the model may enhance its performance.

F-value of 0.33 suggests that the Lack of Fit is not significant when compared to the pure error, with a 73.53% likelihood that such a Lack of Fit F-value could arise from noise. A nonsignificant Lack of Fit is a positive indication, reinforcing that the model fits well.

Table 4.6 Analysis of Variance (ANOVA) for the Fitted Quadratic Model of Methane Yield CH₄%

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	3258.22	5	651.64	4.04	0.0480	Significant
A-Co-digestion Ratio	280.78	1	280.78	1.74	0.2284	Insignificant
B-Temperature	333.49	1	333.49	2.07	0.1935	Insignificant
AB	150.25	1	150.25	0.9323	0.3664	Insignificant
A ²	28.33	1	28.33	0.1758	0.6876	Insignificant
B ²	1850.72	1	1850.72	11.48	0.0116	Significant
Residual	1128.11	7	161.16			
Lack of Fit	130.53	2	65.27	0.3271	0.7353	Significant
Pure Error	997.57	5	199.51			
Cor Total	4386.32	12				

A quadratic model using CCD data was fitted in Design-Expert 13, with ANOVA results, utilized and generated the coded Equations (4.2).

$$Y_2 = +80.46 - 7.94A - 29.23B^2 \quad (4.2)$$

where Y₂ is the methane(responses), A is the co-digestion ratio (CD: MS), B represents the Temperature (°C)

4.3.1.3 ANOVA Quadratic Model Fitting for Carbon dioxide (CO₂) Concentration

Table 4.6 shows that the Model F-value of 5.79 indicates statistical significance, suggesting that the regression model explains a substantial portion of the variability in the response variable. The observed F-value has a probability of occurring by chance at only 1.98%, which is well below the 5% threshold. This suggests that the model's analysis of p-values is significant. Specifically, the terms with values below 0.0500, particularly terms A and B², have a significant influence on the model. In contrast, terms with p-values above 0.1000 are statistically insignificant and may not meaningfully contribute to the model. Thus, including many insignificant terms can lower performance and interpretability, suggesting that a model reduction could enhance precision and simplicity. The Lack of Fit F-value of 0.89 shows that the lack of fit is not statistically significant when compared to pure error, with a 46.84% probability of this result occurring due to random variation. This indicates that the model captures data variation well and reliably represents the observations, supporting its validity in explaining and predicting system behaviour. A negative predicted R² value indicates that the current model may not perform better than the overall mean in predicting the response variable. This suggests potential weaknesses in the model's predictive abilities, and it may be necessary to explore alternative approaches, such as incorporating higher-order terms, to enhance model performance.

Table 4.7 Analysis of Variance (ANOVA) for the Fitted Quadratic Model Carbon Dioxide (CO₂) Concentration

Source	Sum of Squares	df	Mean Square	Fvalue	p-value	Remarks
Model	463.12	5	92.62	5.79	0.0198	Significant
A- Co-digestion Ratio	95.53	1	95.53	5.97	0.0445	Significant
B-Temperature	42.25	1	42.25	2.64	0.1482	Insignificant
AB	89.13	1	89.13	5.57	0.0503	Insignificant
A ²	25.65	1	25.65	1.60	0.2460	Insignificant
B ²	291.26	1	291.26	18.20	0.0037	Significant
Residual	112.02	7	16.00			
Lack of Fit	29.31	2	14.66	0.8860	0.4684	
Pure Error	82.71	5	16.54			
Cor Total	575.14	12				

A quadratic model using CCD data was fitted in Design-Expert 13, with ANOVA results.

The results were adjusted to the second polynomial equation in coded terms. The derived Equation in its coded form is shown in Equation (4.3).

$$Y_3 = +33.01 + 3.81A^2 - 11.60B^2 \quad (4.3)$$

where, Y₃ is carbon dioxide concentration, A is the co-digestion ratio (CD: MS), B is the Temperature (°C).

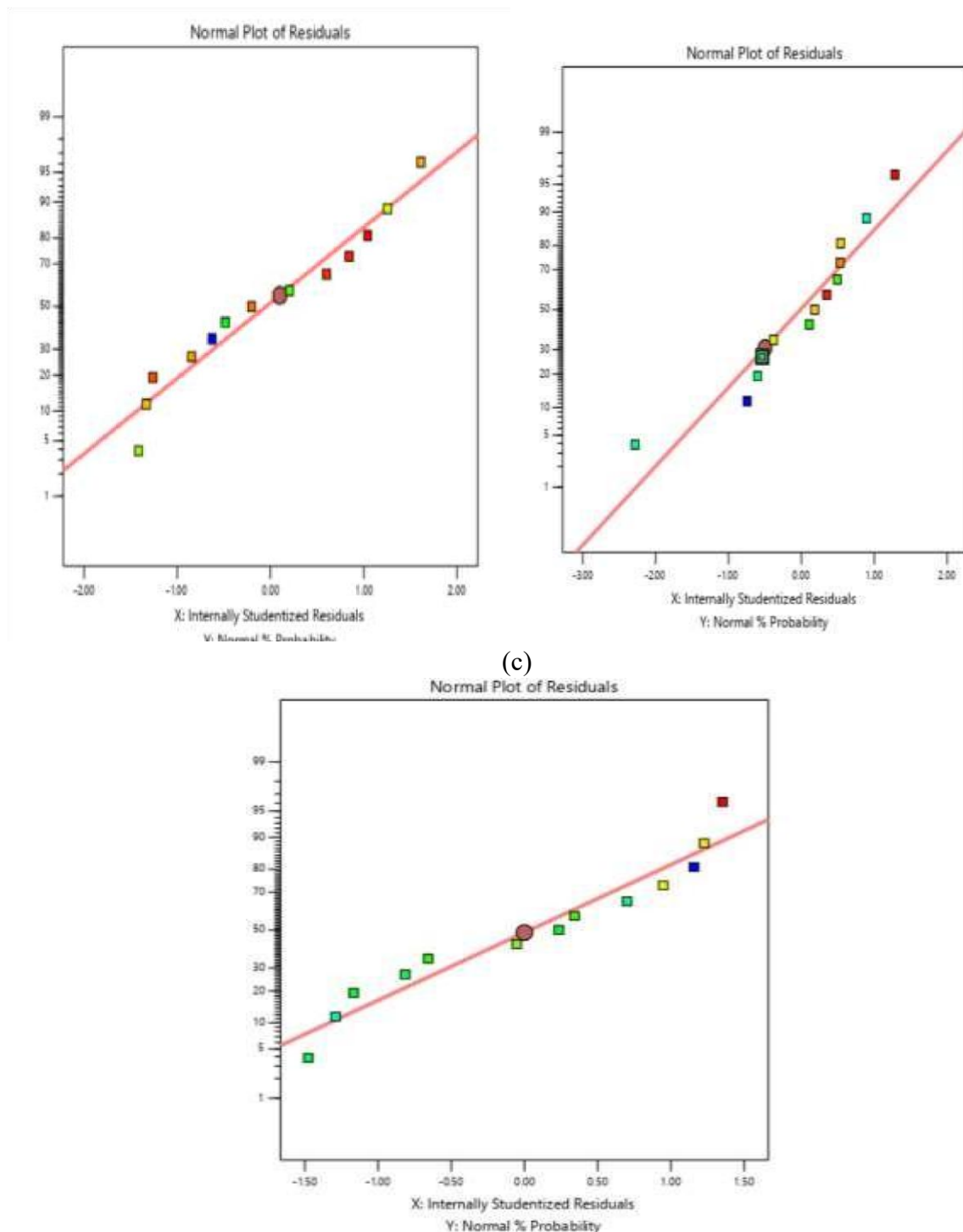
4.4 Diagnostics and Adequacy Assessment

4.4.1 Normal Probability Plot of Residual Values

The suitability of the fitted model was assessed to confirm its validity. A model is considered robust when its residuals are normally distributed. Figure 4.1 shows the normal probability plots of residue values for gas volume, methane yield and carbon dioxide. The plots demonstrate that the points on the normal probability plots closely align on a straight line, confirming the normal distribution of the data.

(a)

(b)



(c)

Figure 4.1: Normal Probability Plot of Residues for (a) Gas Volume, (b) Methane Yield, (c) Carbon Dioxide CO₂ Concentration

4.4.2 Responses Residuals Versus Predicted Values

The plots of externally standardized residuals versus the predicted values are shown in Figure 2. The plots do not exhibit any distinct patterns, suggesting that the residuals are randomly distributed, which is an essential characteristic of a good model.

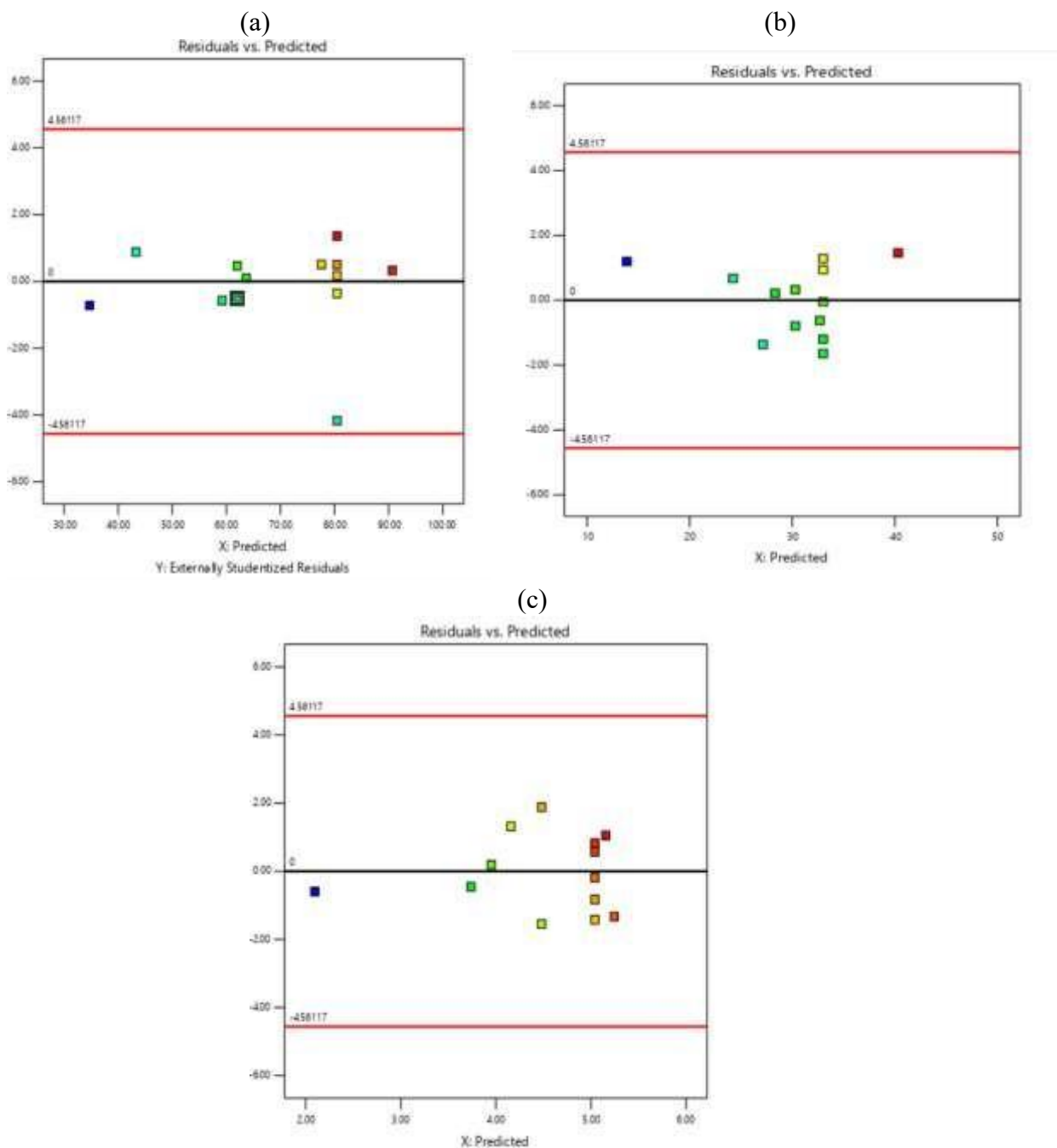


Figure 4.2: Residuals Versus Predicted Values for Gas Volume (a), Methane Yield (b), and Carbon Dioxide CO₂ Concentration (c)

4.4.3 Predicted Versus Actual Values

The plots that compare the predicted values to the actual values for methane yield, gas volume, and carbon concentration exhibit minimal deviation from the straight line. This indicates that the response surface models developed are effective in representing the relationship between the experimental factors and the response variables.

4.4.3 .1 Predicted Versus Actual Value Gas Volume

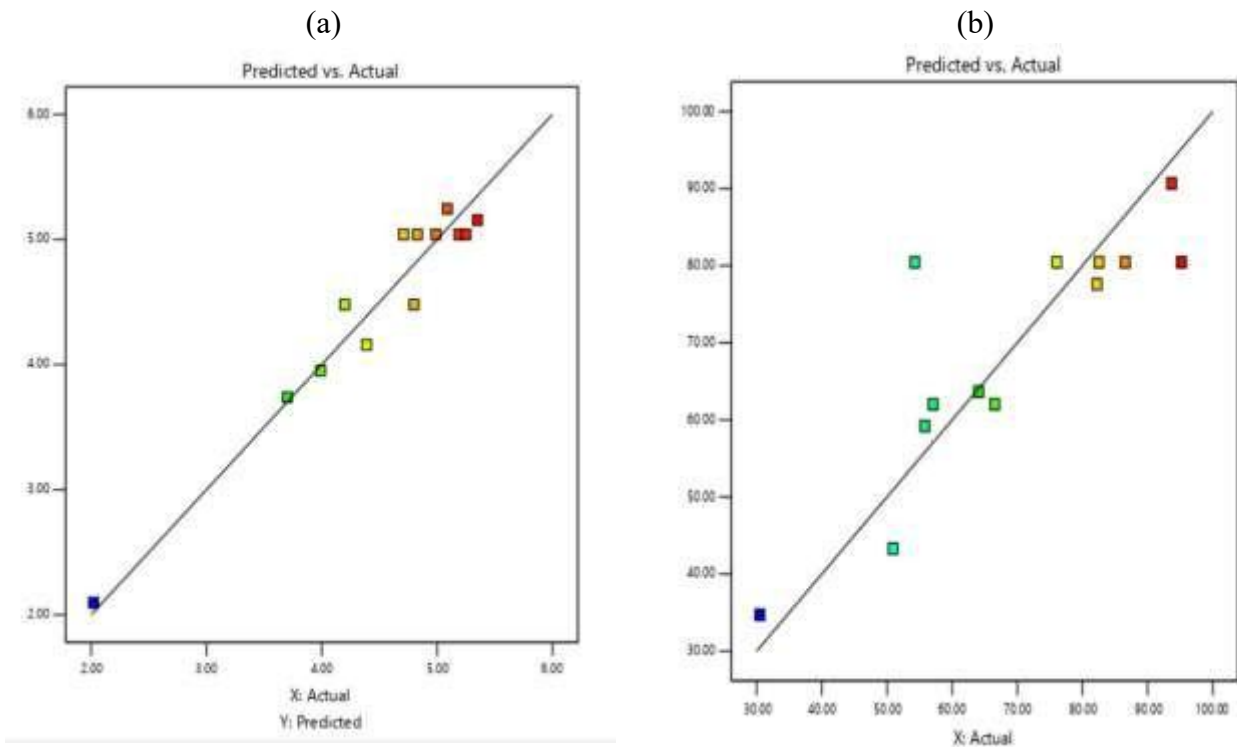
Figure 4.3(a) shows a close match between predicted and actual gas volume, confirming the model's accuracy. The predicted R^2 value of 0.7547 was found to be close to the adjusted R^2 value of 0.9071, with a difference below the acceptable threshold of 0.2. This indicates the strong predictive capability of the developed model. The adequate precision value, at 16.876, supports a strong signal-to-noise ratio, as values greater than 4 are desirable. Thus, the model is effective for navigating the design space and making reliable predictions. Additionally, the coefficient of variation (CV) at 6.10% reflects low residual variation, confirming the experiment's reliability.

4.4.3.2 Predicted Versus Actual Value Methane Yield

Figure 4.3(b) shows predicted vs. actual values of methane yield. The Predicted R^2 of 0.3643 shows reasonable agreement with the Adjusted R^2 of 0.5591, since the difference between the two was found to be less than 0.2. Adequate precision, which measures the signal-to-noise ratio. The ratio of 6.488 demonstrates an adequate signal, confirming that this model can effectively guide you through the design space. The coefficient of variation (CV) at 18.43% reflects low residual variation, confirming the reliability of the experiment.

4.4.3.3 Predicted Versus Actual Value Carbon Concentration CO₂

Figure 4.3(c) shows the plot of predicted versus actual carbon dioxide concentration, demonstrating model reliability. The adequate precision value, which assesses the signal-to-noise ratio, was calculated to be 9.732. Since a ratio greater than 4 is considered acceptable, this result shows that the model had a sufficient signal relative to the noise. Therefore, the model was deemed suitable for exploring and navigating the design space. The coefficient of variation (CV) at 13.26% reflects low residual variation, confirming the reliability of the experiment.



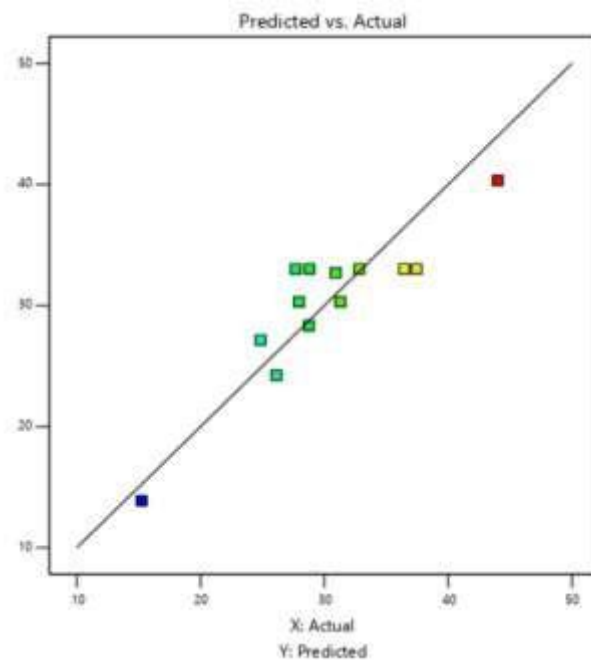


Figure 4.3: Predicted And Actual Values for Gas Volume(a), Methane Yield(b), And CO₂ Concentration(c)

4.5 Response Surfaces

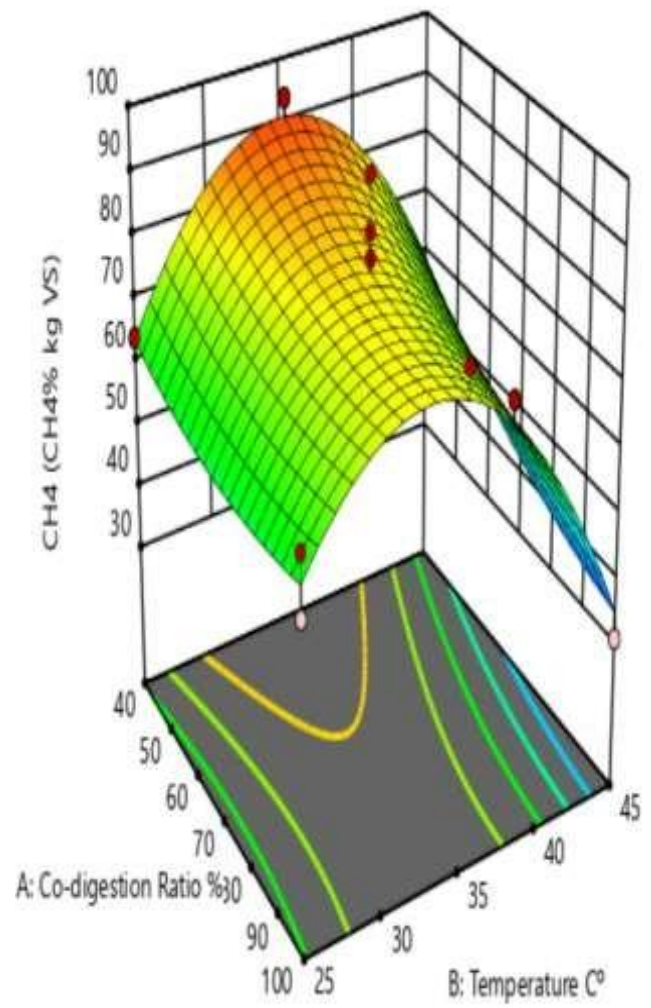
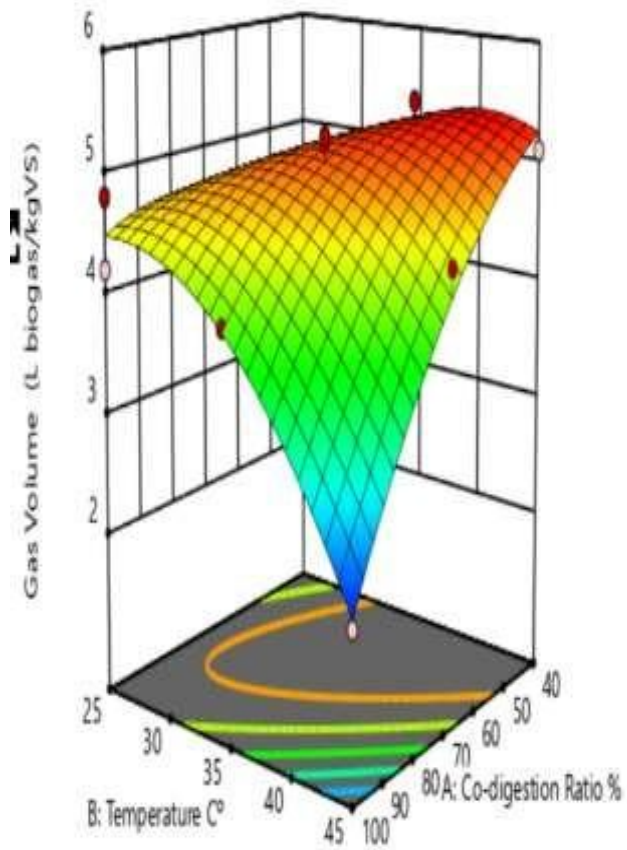
3D plots in Response Surface Methodology are graphical representations that illustrate how the response variable changes with two independent variables, while keeping other variables constant (Isa et al., 2011). These plots help visualize interactions between variables and the curvature of the response surface. They are useful for identifying the optimal levels of the independent variables to achieve the best response. Figure 4.4 (a), (b), and (c) show 3D response surface plots depicting how substrate ratio (40–100%) and temperature (25–45°C) affect gas volume, methane yield, and CO₂ concentration.

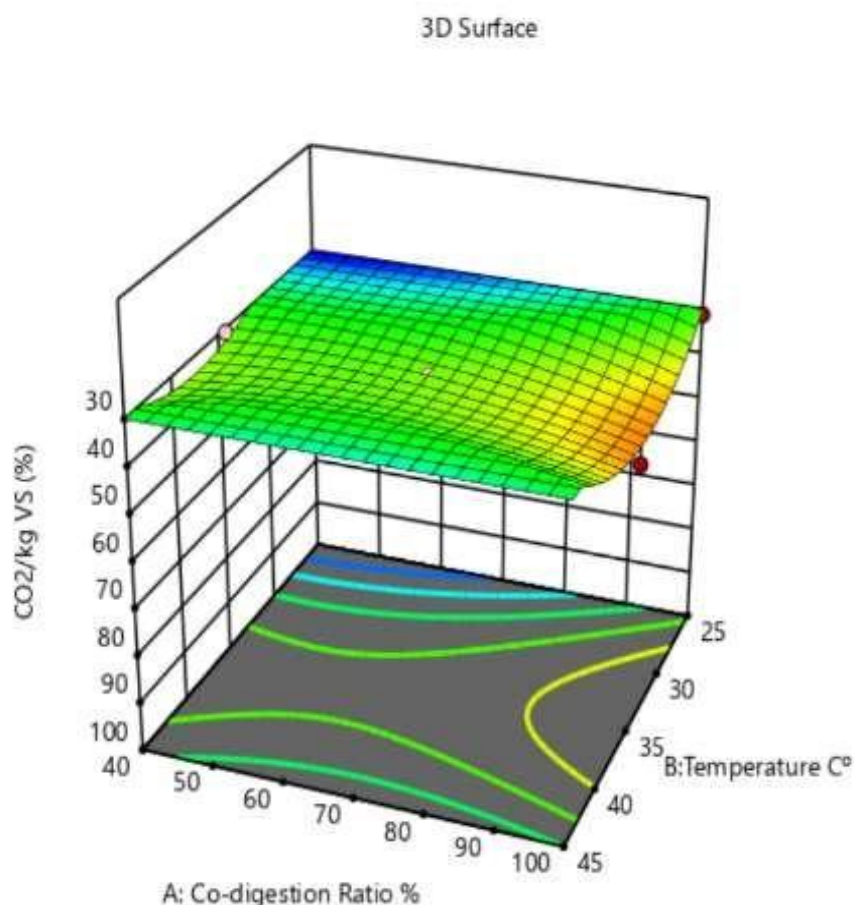
(a)

(b)

3D Surface

3D Surface





(c)

Figure 4.4: (a), (b), and (c): 3D Response Surface Plots Illustrating the Effects of Substrate Ratio (40–100%) and Temperature (25 °C to 45 °C) on the Responses

4.6 Economic Viability of Co-Digesting Cow Dung and Maize Stubble for Biogas

Production

4.6.1 Economic Viability of Biogas Production for Household Energy Needs

The results of the economic analysis for biogas production, including annual savings, payback period, Net Present Value (NPV), and Profitability Index (PI), are presented in Table 4:8. These indicators provide a comprehensive assessment of the project's financial performance and viability. Annual savings reflect the reduction in household energy expenditure achieved through the adoption of biogas technology. The payback period indicates the time required to recover the initial investment cost. The Net Present Value (NPV) evaluates the profitability of the project over

its lifetime by accounting for the time value of money, while the Profitability Index (PI) measures the return per unit of investment. Collectively, these indicators demonstrate the economic feasibility and attractiveness of the biogas production system.

Table 4:8 Shows the Economic Volatility Results

S/No	Economic Indicator	Value
1	Annual Savings	\$432
2	Payback Period	3.7 years
3	Net Present Value (NPV)	\$1,054.45
4	Profitability Index (PI)	1.66

CHAPTER FIVE: DISCUSSIONS

5.1 Introduction

This Chapter presents an elaborate discussion of the results generated from the effect of optimization of the factors on response variables, starting with proximate composition, ultimate analysis, heating value, and total carbon. Lastly, it discusses the effects of variables on the responses.

5.1 Proximate and Elemental Composition

5.1.1 Proximate Composition

Cow dung was found to have a higher moisture content of 6.73 db compared to maize stubble's 4.85, indicating its greater water retention capacity. This characteristic could significantly impact microbial activity in processes like anaerobic digestion. Maize stubble, with a total solids content of 95.15%, db contains more solid matter and less water than cow dung, which had a total solids content of 93.27 db. Due to its low water content and higher total solids content, maize stubble may pose challenges for biological decomposition or processing without additional water (Detman et al., 2021). With a volatile solids percentage of 70.85 db, maize stubble is a better candidate for biogas production due to its higher biodegradable content, in contrast to cow dung's 59.45 db. Maize stubble exhibits a notably higher fixed carbon content of 15.14 db. However, the biogas potential is influenced by the efficiency of its breakdown in comparison to cow dung, which has a fixed carbon content of 12.38 db. The greater presence of volatile organic matter in cow dung, which contains more accessible volatile solids, enhances the co-digestion process, resulting in increased methane production. Containing more readily available volatile solids enhances the co-digestion process, leading to improved methane production (Li et al., 2018). It implies that maize stubble has a greater amount of non-volatile organic material, contributing to long-term carbon stability in digestion efficiency.

Additionally, cow dung has a higher ash content of 32.43 db than maize stubble, which is 9.15 db, indicating potentially greater mineral residue after combustion or decomposition.

5.1.2 Elemental Composition

A comparative analysis revealed that cow dung had a slightly higher carbon content, 32.87 % db. than maize stubble 31.35% db, enhancing its energy potential for microorganisms during anaerobic digestion. Both materials have similar hydrogen content, with maize stubble at 1.38% db and cow dung at 1.39% db, indicating comparable energy density for biogas production. Valenti et al. (2018) reported similar results of hydrogen levels in cow dung of around 1.3% to 1.4%. db Similarly, Chen et al (2008) reviewed various agricultural residues for biogas production and noted that the hydrogen content in crop residues typically ranged from 1.3% to 1.4% db. This similarity indicates that both substrates are viable options for maintaining a consistent energy density in biogas. Maize stubble contains more sulfur (0.11%) db than cow dung (0.085%) db, which can result in hydrogen sulfide (H_2S) production, negatively affecting biogas quality. The reduced sulfur content in cow dung may lead to cleaner biogas and less equipment maintenance, although both substrates maintain manageable sulfur levels. From Table 4.2, maize stubble has a higher heating value of 16.50 MJ/kg, compared to that of cow dung at 13.36 MJ/kg. This is due to maize stubble's better chemical composition, higher volatile matter, and lower moisture content, resulting in quicker ignition, steady flames, and efficient combustion with minimal residue (Anwar et al., 2024). In contrast, cow dung contains more ash and moisture and less fixed carbon, leading to a lower calorific value and energy output (Vasanth et al., 2022). This means that more cow dung is needed for the same heat output as maize stubble. However, blending cow dung with other biomass can enhance its heating value for improved fuel efficiency (Achinas et al., 2017).

5.1.3 C/N Ratio Analysis

The C/N ratio of the substrate was 21.45 for cow dung and 29.71 for maize stubble. This ratio is considered suitable for proper digestion, as reported by Mishra et al. (2015). According to their findings, a C/N ratio below 20 may result in ammonia inhibition due to excessive nitrogen, while a ratio above 30 can impede microbial growth due to nitrogen deficiency. The relatively low C/N ratio of cow dung could cause problems with ammonia if used alone. Maize stubble's higher C/N ratio provides a better balance, making the combination of cow dung and maize stubble ideal for effective digestion. Rabii et al. (2019) found that blending organic waste with a low C/N ratio with organic waste having a high C/N ratio can reduce the levels of inhibitory substances and achieve the optimal C/N for the digesters.

5.2 Effect of Substrate Ratio and Temperature on Gas Volume

Figure 4.4 (a) illustrates 3D response surfaces showing the interactive effects of co-digestion ratio and temperature on gas volume. The plots reveal that changes in these variables significantly influence gas volume, with a noticeable impact as the co-digestion ratio and temperature interact. This suggests that optimizing these parameters could significantly enhance gas yield. Statistical analysis confirms that the interaction between substrate ratio and temperature has a significant effect on gas volume. It was observed that gas volume reached its maximum level at 35 °C. The optimal biogas volume was 5.35 (L biogas/kgVS), with a (40:60) CD: MS ratio at 35°C, while the minimum was 2.02 (L biogas/kgVS) at 25°C with a (0:100) CD: MS ratio, as shown in Figure 4.4: (a).

This indicates that co-digesting substrates with a low C/N ratio, such as poultry manure, cow manure, and kitchen waste, with agricultural waste, which has a higher C/N ratio, can enhance biogas production. However, lignocellulosic materials in agricultural waste can complicate anaerobic co-digestion due to their long retention times and low biodegradability, resulting in reduced biogas. Rodriguez et al. (2017) reported that the highest biogas production was

achieved with a 50:50 mixture. Co-digestion generates more methane than mono-digestion of individual substrates, supporting this study's finding that a 40:60 mixture yields optimal gas volume. However, this contradicts the 40:60 mixture ratio of the study, as it demonstrates that a 50:50 ratio is more effective. Detman et al. (2021) reported that codigestion systems generally benefit from a balanced carbon-to-nitrogen (C/N) ratio, which enhances microbial activity and maximizes gas production. In this study, the lowest gas volume was observed with a substrate ratio of 0:100, meaning 100% maize stubble, at a temperature of 25 °C. This poor performance can be attributed to two key factors: first, maize stubble contains phenolic compounds that are known to inhibit methanogenesis, as noted by Khalid et al. (2011). Furthermore, the low temperature of 25 °C significantly reduced methanogenic activity. Methanogens generally operate less efficiently at temperatures below the mesophilic range, as demonstrated by Carotenuto et al. (2016) and Lin et al. (2016).

5.3 Effect of Co-digestion and Temperature on Methane Yield

The interaction effect of temperature and co-digestion on biogas yield was significant ($P < 0.05$), as shown in Table 4.5. However, ANOVA results indicated that the quadratic and linear terms of temperature and co-digestion significantly influenced biogas yield ($P < 0.05$). Figure 4.5 (b) illustrates the relationship between temperature and co-digestion on biogas generation. The methane yield was highest at a temperature of 35 °C. When the temperature was 25 °C, the methane yield was at its lowest level. Conversely, a slight inhibition in production was observed when the temperature was 45 °C. At a temperature of 35 °C with ratios of CD: MS of 100:0, 70:30, and 40:60, respectively, the results showed that the highest methane yield occurred at the ratio of 40:60 with a maximum methane yield of 95.20

CH₄%/kgVS. While the lowest value was recorded at a ratio of 100% pure maize stubble, with 30.48 CH₄%/kgVS. It indicates that using only cow dung or maize stubble resulted in lower methane yields, indicating that co-digestion enhances methane production by optimizing

nutrient balance and the carbon-to-nitrogen ratio, as reported by Zhang et al. (2014). Furthermore, the interaction between temperature and co-digestion ratio reveals that at 35 °C, the methane production peaked at a co-digestion ratio of approximately 40:60 and remained stable as the temperature increased, which agrees with the findings by Bochmann, 2013). The interactive effects of substrate ratios and temperature are shown in Figure 4.5 (b), demonstrating that both factors directly influence methane yield. An increase in the cow dung (MS) to cow dung (CD) ratio, combined with mesophilic temperatures, significantly boosted biogas production. In contrast, lower proportions of cow dung and lower temperatures led to a decline in methane yield. The substrate ratio had a statistically significant effect on methane production ($p < 0.05$), with higher proportions of maize stubble contributing to greater methane yields.

Table 4.6 shows that the model is statistically significant ($F = 4.04$, $p = 0.0480$), confirming its suitability for predicting methane yield. However, the linear effects of co-digestion ratio (A) and temperature (B), including their interaction (AB), are not significant ($p > 0.05$), while only the quadratic term of temperature (B^2) is significant ($p = 0.0116$)

These findings agree with previous studies that report temperature as a critical factor affecting anaerobic digestion, particularly under mesophilic conditions where microbial activity is optimized within a narrow range (Ward et al., 2008; Appels et al., 2008). The results emphasize that methane yield is primarily governed by the nonlinear effect of temperature, reinforcing the need for precise temperature control to achieve optimal biogas production.

5.4 Effect of Co-digestion Ratio and Temperature on CO₂ Concentration

The interaction between temperature and co-digestion had a significant effect on carbon concentration ($p < 0.05$), as presented in Table 4.6. Nevertheless, the ANOVA findings showed that both the quadratic and linear effects of temperature and co-digestion had a significant influence on the concentration of carbon dioxide ($p < 0.05$). The relationship between

temperature and co-digestion on biogas production is depicted in Figure 4.4 (c). The 3D representation illustrates how the co-digestion ratio and temperature influence the concentration of biogas production with minimum values. During the initial stages of the experiment, specifically within the first two weeks, the samples of co-digested materials cow dung (CD) and maize stubble (MS) exhibited a high percentage of CO₂, this can be attributed to the initial breakdown of organic matter, characterized by the slow degradation of carbon sources, particularly those with a higher carbon-to-nitrogen (C/N) ratio, such as maize stubble (Wang et al., 2014) found that carbon-rich substrates break down slowly during the acidogenesis phase, leading to an early release of CO₂. Their research also indicated that nitrogen-rich materials, like cow dung, help maintain a stable pH and provide essential nutrients that support microbial growth, which is crucial for the subsequent methane production stage. During the hydrolysis and acidogenesis phases, the organic materials are converted into volatile fatty acids, resulting in the production of a by-product. (Detman et al., 2021) noted that carbon-rich substrates decompose rapidly during the acidogenesis phase, which contributes to the early release of CO₂ as organic compounds are transformed into volatile fatty acids and alcohols. The early spike in CO₂ levels is a natural part of the anaerobic digestion. This spike occurs as carbon-rich compounds break down. The type of substrate used during the co-digestion process. For instance, maize stubble, with a high carbon-to-nitrogen C/N ratio, produces more CO₂ initially as microbes consume the available carbon. Meanwhile, cow dung, which has a low C/N ratio, supplies the necessary nitrogen for microbial activity.

5.5 Optimization and Validation of the Process

After simulating the model using the numerical optimization method of the Design Expert model, the optimum conditions were determined as follows: a co-digestion ratio of CD: MS of 40:60 at a temperature of 35°C. The predicted values from the developed model for gas volume, maximum methane yield, and carbon concentration are 5.35 (L biogas/kgVS), 90.67 CH₄

(kgVS), 32.69 (kgVS), respectively. To verify the predictive capability of the developed models, co-digestion was conducted under the suggested optimum conditions, and values for gas volume, methane yield, and carbon concentration were assessed. As a rule of thumb, the mean experimental values should lie within the range defined by the 95% prediction interval (PI) low and 95% PI high for the predictive capability of the models to be considered reliable. The experimental results were found to lie within the range, validating the developed models.

Table 5.1: Experimental Validation of the Proposed Models

Run 5 Response	Predicted Mean	Predicted Median	Observed	Std Dev	n	SE Pred	95% PI low	Data Mean	95% PI high
Gas Volume	5.16	5.16	5.35	0.27	1	0.34	4.35	5.35	5.96
CH4 %kgVS	90.67	90.67	93.68	12.69	1	15.75	53.44	93.68	127.90
CO2/kg VS%	32.697	32.697	30.91	4.00	1	4.96	20.96	30.91	44.43

5.6 Economic Analysis

The project's financial metrics analysis reveals a positive Net Present Value (NPV). According to Liu (2022), a project with a positive NPV is considered profitable and acceptable, indicating its feasibility for the company. Conversely, a negative NPV suggests that the project may not be viable, as it is unlikely to generate sufficient returns. With a payback period of 4 years, this project shows a relatively rapid return on the initial investment, particularly in comparison to energy or infrastructure initiatives, which typically have longer return periods.

The Profitability Index (PI) of 1.66 signifies that the project generates US \$1.66 in present value for every US \$1 invested. Since the PI is greater than 1, this indicates that the project is financially viable, with discounted cash inflows surpassing the initial investment. A PI of 1.66 implies that the project yields a 66% surplus in value over its cost, positioning it as a promising opportunity for investment.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusions

This study investigated the anaerobic co-digestion of cow dung (CD) and maize stubble (MS) to enhance biogas production, with emphasis on methane yield and total gas volume. A quadratic response surface model developed using Central Composite Design (CCD) and analyzed in Design-Expert Version 13 demonstrated high predictive accuracy. ANOVA results confirmed that mixing ratio and temperature significantly influenced biogas production. Co-digestion consistently outperformed mono-digestion, with optimal CD:MS ratios improving methane yield.

Substrate characterization showed that maize stubble had higher total solids (95.15% db) and volatile solids (70.85% db), indicating greater biodegradability, while cow dung had higher moisture content and nitrogen, supporting microbial activity. The balanced C/N ratio achieved through co-digestion enhanced process stability and performance. However, excessive maize stubble led to instability due to its higher C/N ratio and acidity.

The optimal conditions were observed at 35°C, with a 40:60 CD: MS ratio yielding the highest biogas production (5.35 kg/kg VS), and a 70:30 ratio producing the highest methane yield (95.25 kg/kg VS). Overall, co-digestion improved nutrient balance and significantly enhanced biogas production efficiency.

6.2 Recommendations

1. Cow dung and maize stubble are suitable substrates for biogas production owing to their favourable physicochemical and biochemical properties

2. For optimal biogas production, cow dung and maize stubble should be co-digested anaerobically at a mixing ratio of 70:30 (cow dung to maize stubble) under mesophilic conditions at an operating temperature of 35°C.
3. The anaerobic co-digestion of cow dung and maize stubble is economically viable, provided the Net Present Value (NPV) is positive, and the Profitability Index (PI) is greater than 1.
4. Further investigation is required to understand how particle size and mixing speed affect biogas production efficiency.
5. Further research is needed on the co-digestion of multiple substrates such as cow dung, agricultural residues, and wastewater, to enhance biogas yield and optimize resource utilization.
6. Incorporating additional economic indicators, such as the Accounting Rate of Return (ARR), would enhance the financial analysis of the economic viability of anaerobic co-digestion.

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APPENDICES

APPENDIX A: ETHICAL APPROVAL



02/08/2024

To: PETER KIIR

+211921312034/+256779754291

Type: Initial Review

Re: GUREC-2023-760: Potential of Anaerobic Co-digestion of Cow Dung and Maize Stubble for Biogas Production in Northern Uganda

I am pleased to inform you that at the **111th** convened meeting on **14/03/2024**, the Gulu University REC meeting voted to approve the above referenced application.

Approval of the research is for the period of **02/08/2024** to **02/08/2025**.

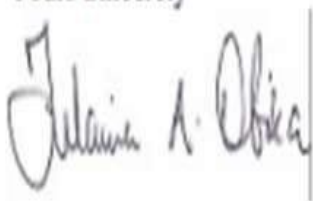
As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **02/08/2025** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:

No.	Document Title	Language	Version Number	Version Date
1	Protocol	English	Version 2.0	2024-07-29
2	'COVID-19 risk management plan'	English	Version 2.0	2024-07-22
3	Informed Consent Waiver	English	Version 2.0	2024-06-04
4	Data collection tools	English	version 2.0(1)	2024-05-08

Yours Sincerely



Julaina A. Obika (PhD)

For: Gulu University REC

APPENDIX B: EXPERIMENT DESIGN FOR OPTIMIZATION OF TEMPERATURE AND CO-DIGESTION RATIO (DATA COLLECTION TOOLS)

				Coded values and (Temperature CD/MS)		Actual values		Response 1 Gas volume (ml)	Response 2 Methane CH ₄ %	Response 3 Carbon dioxide CO ₂ %
Std	ID	Run	Space Type	A	B	A	B	R1	R2	R3
8	8	1	Axial	0	1	70	45			
9	9	2	centre	0	0	70	35			
6	6	3	Axial	1	0	100	35			
2	2	4	Factorial	1	-1	100	25			
5	5	5	Axial	-1	0	40	35			
3	3	6	Factorial	-1	1	40	45			
13	9	7	Centre	0	0	70	35			
11	9	8	Centre	0	0	70	35			
12	9	9	Centre	0	0	70	35			

7	2	10	Factorial	1	-1	100	25			
10	9	11	Centre	0	0	70	35			
1	1	12	Factorial	-1	-1	40	25			
4	6	13	Axial	1	1	100	45			

APPENDIX C: ORIGINALITY REPORT

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P.O. Box 166
Gulu, Uganda



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URL: www.gu.ac.ug

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PLAGIARISM CHECK

Declaration for Originality Form

This form must be completed and signed for all scholarly work produced.

Name: **PETER BOL KUOL KIIR (MR.)**

Personal File No.(staff) Reg. (student). **REG. No. 22/X/1975/MBE/PS**

Faculty/Institute: **AGRICULTURE AND ENVIRONMENT, GULU UNIVERSITY**

Department: **BIOSYSTEMS ENGINEERING**

Title and bibliographic description of the work

**POTENTIAL OF ANAEROBIC CO-DIGESTION OF COW DUNG AND MAIZE
STUBBLE FOR BIOGAS PRODUCTION IN NORTHERN UGANDA**

Declaration.

1. I understand what plagiarism is and I am aware of the University policy on this regard,
2. I declare that this ..., **Dissertation** ... (thesis, Proposal, essay, assignment, paper, report etc.) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my work has been used, this has been properly acknowledged and referenced in accordance with Gulu University requirements,
3. I have not sought or used the services of any professional agency to produce this work,
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own,
5. I understand that any false claim of this work shall result in disciplinary action in accordance with University Policy on Plagiarism.

Signature 

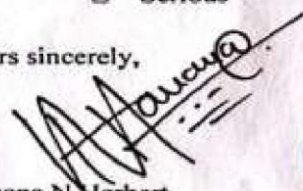
Date **25th/11/2024**

Originality Report

The article/thesis/document titled above have been subjected to plagiarism check and the level was found to be.....17% (percent) this is classified as:

- ☒ Minor
- ☐ Moderate
- ☐ Serious

Yours sincerely,


Manana N Herbert
Ag. University Librarian

