CO-DIGESTION OF A MIXTURE OF COW DUNG AND MAIZE STALK RESIDUES AND ITS INFLUENCE ON BIOGAS PRODUCTION

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A thesis Submitted to the Department of Mechanical and Industrial Engineering in Partial Fulfillment of the requirement for the Award of the degree of Master of Science in Industrial Engineering and Management

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DECLARATION

This thesis is my original work prepared with no other than the indicated sources and has not been presented for a degree or any award in any other university or any other institution.

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CERTIFICATION

The undersigned certify that we have read and hereby recommend for acceptance by Masinde Muliro University of Science and Technology a thesis entitled "**Co-digestion of a mixture of cow dung and maize stalk residues and its influence on biogas production.**"

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DEDICATION

This work is devoted to my family, with the intention of inspiring and blessing not just them but also those in their vicinity. I express gratitude for their unwavering support and encouragement. May God bless all of you.

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I want to praise God for His guidance and the strength that has allowed me to reach this point. I appreciate Masinde Muliro University of Science and Technology for accepting me as a student at their institution for my academic pursuits. This study was successful due to the guidance of my competent supervisors, Dr. Barasa H. Masinde, PhD and Dr. Peter T. Cherop, PhD. I am immensely grateful to all of my colleagues who provided me with moral support. Thank you and may God bless all of you.

ABSTRACT

The rapid exhaustion of finite non-renewable energy sources like fossil fuels has resulted in adverse effects on human health, environmental deterioration, and global climate change. Two primary factors dominate Kenya's energy scene: a heavy dependence on declining biomass energy resources for the rural household to meet energy needs and a significant reliance on petroleum imports to meet contemporary economic needs. The expanded use of renewable energy was one of the factors needed to drive sustainable development. Production of biogas energy from cow dung in Kenya using agricultural residues because of the high C/N ratio is one option for producing energy derived from renewable resources. The altered C/N ratio is partially responsible for the creation of biogas by co-digestion. The study utilized response surface approach to maximize the critical parameters that influence biogas output from co-digestion and Na_2CO_3 pre-treatment using a digester. The parameters studied were substrates (cow dung and maize residues) and percentage soda ash. The study's objective was to examine the influence of the duration of pretreatment of maize stalks regarding biogas production when cow dung and maize stalks were co-digested in an anaerobic digestion process. The maize residue was prepared based on the design of the experiment where different samples were pretreated at different Na_2CO_3 concentrations and varied duration. Under different Na_2CO_3 concentration, the maize stalk pretreated by 7% Na_2CO_3 concentration for 4 days achieved the highest total solid of 15.15% which was 7.26% higher than that of untreated one, and was subsequently used to carry out co-digestion with the core substrate which was the cow dung. The study found that the optimum substrate ratio for biogas production by the co-digestion of cow dung, pre-treated maize stalks, and dilution was 1:1:3, with cow dung and maize stalks being in equal proportions, these optimum substrate ratios and dilution made favorable conditions for the multiplication of bacteria. The daily average biogas yield produced by the above-mentioned ratio was 203.64mL which is 2.12 times greater than that of cow dung mono-digestion as per the experiment. Co–digestion bioreactors operating with low cow dung concentration vis a vis maize stalks, yielded low biogas compared with the ones with balanced substrate ratios or cow dung being slightly higher. It was deduced that co-digestion improves the effectiveness of the digester, resulting in a higher yield of biogas. The findings demonstrated that higher dilution levels resulted in greater material degradation, hence enhancing the production of biogas and methane. Conclusions were made based on proximate analysis, the proportion of biogas produced, and effects of soda ash as pretreatment media. Therefore, co-digestion of cow dung and maize stalk residues typically improves the biogas yield compared to digesting cow dung alone. This is because maize stalk residues add additional organic matter and increase the carbon-to-nitrogen (C/N) ratio, which can optimize the anaerobic digestion process. It was recommended that there should always be ensured that the carbon-to-nitrogen (C/N) ratio is balanced between cow dung and maize stalk residues and by regular monitoring of this ratio can improve microbial activity and enhance biogas yield. Furthermore, more research to determine the ideal mixing ratios of cow dung and maize stalk residues for specific local conditions to maximize biogas yield and ensure consistent production.

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LIST OF ABBREVIATIONS AND ACRONYMS

AD	Anaerobic Digestion
BOD	Biological Oxygen Demand
C	Carbon
C/N	Carbon-To-Nitrogen
COD	Chemical Oxygen Demand
CD	Cow dung
DOE	Design of experiments
GWh	Gigawatt hours
MoE	Ministry of Energy
MS	Maize stalk
MW	Mega Watts
TS	Total solids
VS	Volatile solids
W	Water

LIST OF SYMBOLS

CO_2	Carbon dioxide
CH_4	Methane
H_2	Hydrogen
H_2s	Hydrogen sulfide
N_2	Nitrogen
NH_3	Ammonia
O_2	Oxygen
V	Operating volume
Q	Volume flow rate

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The rapid exhaustion of finite non-renewable energy sources like fossil fuels has resulted in adverse effects on human health, environmental deterioration, and global climate change. Consequently, the adoption of renewable energy options like biogas and solar power at the household level is driving the development of the economy. (Kellner *et al.,* 2015; Sathaye *et al.,* 2011). Biogas can be used as a source of fuel, energy, and heat. It is highly desirable to establish an energy system that is sustainable, renewable, world-class, and produces zero carbon emissions. Biomass fermentation enhances the need for biogas as a reliable energy source and effectively addresses waste management issues.

The main materials used are animal manure, food waste, municipal sewage sludge, vegetable and fruit waste, and municipal solid waste. Municipal solid waste, food waste, and vegetable/fruit waste generate more methane than municipal sewage sludge and animal waste. Therefore, co-digestion is necessary to improve the effectiveness of biogas generation. There is a significant rise in the utilization of agricultural residues as substrate in biogas production, which results in trace element deficiencies in manures from cattle, chickens, or pigs. The process of co-digestion, which involves combining manures with other substrates, results in an increase of the carbon-to-nitrogen (C/N) ratio and the concentration of micro- and macronutrients. This, in turn, leads to a significant rise in the production of biogas (Adghim *et al.,* 2022; Ma *et al.,* 2020).

Energy demand has been consistently rising as a result of population development, expansion of industrial activity, and an uptick in the number of automobiles in the country. The power generation rose to 10,205 GWh in the 2016/17 year, up from 9,817 GWh in last year. The growth is associated with the favorable expansion of commercial and industrial electricity demand. Similarly, the highest level of electricity demand increased from 1,586MW to 1,656MW by June 2017 and reached 1,710MW by the conclusion of the 2017 calendar year. According to the Ministry of Energy (MoE), The demand for biomass energy is growing at a rate of 2.7% annually, while the sustainable supply is expanding at an imperceptive rate of 0.6% annually. The Ministry of Environment (MoE) has calculated that the current biomass shortfall is 60%. This has resulted in the loss of more than 75% of the overall forest cover in some sub-Saharan nations, thereby causing a significant increase in environmental disasters. The demand projection has been conducted in three scenarios: reference, high, and low. Each scenario is based on unique assumptions regarding the evolution of the demand drivers.

From the simulation results, the predicted peak demand for the years 2017–2037 falls into three categories: 1,754MW to 9,790MW in the high case, 1,754MW to 6,638MW in the reference case, and between1754MW in the low case scenario is 4,763MW in 2037 from 2017 levels. The projected energy growth is expected to reach 10,465 gigawatt-hours (GWh) in 2017 and is projected to increase to 39,187 GWh by 2037 in the reference scenario. During the same time frame, the amount of energy generated rises from 10,465 gigawatt-hours (GWh) to 57,990 GWh in the high case scenario, and from 10,465 GWh to 27,945 GWh in the low case scenario. The difference between this year's load forecast and the load forecast done in the latest update of 2015-2035 is quite small, indicating a variation of only 0.02%.

Kenya’s economy is based on agriculture, which has good potential to enhance the generation of biogas from agricultural leftovers and agro-industrial byproducts such as maize residues. Anaerobic digestion (AD) is a desirable method regarding garbage management technology in rural areas because it provides a way of safely disposing of Organic waste, like cow dung and maize stalk, while at the same time producing a stable, useful end product. Co-digestion facilitates the maintenance of a steady pH level and promotes high efficiency in the process of digestion, while preventing the occurrence of excessive acidification. One factor contributing to the rise in biogas generation by co-digestion is the adjustment of the C/N ratio (Prapinagsorn *et al.,* 2017; Sfez *et al.,* 2017; Sidra *et al.,* 2018).

Trace elements in substrates such as cow dung components, including iron (Fe), nickel (Ni), zinc (Zn), and calcium (Ca) can either enhance or hinder biogas production. The disparity in nutritional composition between cow dung and maize residues results in reduced effectiveness in anaerobic digestion (AD). The high carbon-to-nitrogen (C/N) ratio of maize stalks makes them susceptible to acidification. The retention duration for maize residues is long because of the resistant composition of lignocelluloses (de Medeiros *et al.,* 2022; Kucharska *et al.,* 2018). Co-digestion is a process that has the ability to effectively utilize the nutrients included in different types of waste materials and maintain a balanced bacterial community, resulting in optimized digestion performance.

Co-digestion, for instance, can mitigate the inhibition of both cow manure and maize residues and improve the stability of biogas production, by doing so, the hydrolysis process is sped up, resulting in a shorter lag phase and decreased retention period for agricultural residues. Therefore, the co-digestion of crop residues with cow dung to improve biogas production has attracted great attention (Prapinagsorn *et al.,* 2017; Sfez *et al.,* 2017; Sidra *et al.,* 2018). The overall nitrogen concentration of cow dung is very high, making it suitable for adjusting the carbon-to-nitrogen (C/N) ratio of substrates with low nitrogen levels. In addition, cow dung aids in stabilizing the pH value during anaerobic digestion by utilizing the buffering effect of the ammonia it produces. Therefore, scientific evidence has demonstrated that cow manure is an excellent substance for co-digestion

Previous studies (Ghaleb *et al.,* 2021; Kainthola *et al.,* 2019) have largely attributed the enhancement in biogas production from co-digestion to achieving a balanced carbon-to-nitrogen (C/N) ratio. However, other factors influencing the performance of co-digestion are seldom discussed, and the extent to which initial pH impacts anaerobic digestion (AD) remains unclear. Additionally, the effect of ash pre-treatment—an abundant resource in many Kenyan households—has not been thoroughly investigated. This study conducted experiments using cow dung (as inoculum) and maize residue in co-digestion, with a focus on the effects of ash pre-treatment. The study also examined the impact of the duration of maize residue pre-treatment with ash. The primary objective was to evaluate the improvement in biogas production resulting from ash pre-treatment and co-digestion with maize residues on cow dung, a well-known substrate. The findings are expected to support further research and investment in maize cultivation and utilization in Kenya.

Kenya has a significant energy endowment, with wood fuel and other forms of biomass providing over 68% of the national energy consumption, serving as the primary energy source for much of the rural population. Imported petroleum accounts for 22% of the energy consumed, while 9% is sourced from electricity. Wind and solar power contribute less than 1% (4 Energy Scenarios Workshop, July 2013; Ministry of Energy, 2008 Economic Survey; Updated List of Power Development Costs, 2017-2032; Survey, June 2018). The main sources of electricity generation in the country are hydroelectric, geothermal, and thermal power.

Given these dynamics, immediate and efficient interventions are necessary not only to ensure fuel availability but also to promote the restoration of degraded areas. Alternative energy solutions are required, with a focus on technologies that are culturally acceptable, familiar, repeatable, cost-effective, regionally accessible, easy to produce, environmentally sustainable, and culturally relevant. One such solution is the fermentation of animal waste and agricultural byproducts in home biogas digesters, which provides renewable energy and organic manure for farming. This study aims to contribute valuable information on the application of anaerobic digestion technology using cow dung and maize stalks on farms, presenting a practical method for turning waste into usable energy and supporting sustainable agriculture.

1.2 Statement of the Problem

The energy accessibility for household consumption in Kenya faces significant challenges, with rural populations heavily reliant on firewood, which contributes to environmental degradation, and urban households burdened by the high costs of cooking gas and kerosene. Despite the abundance of agricultural wastes like maize stalks, these resources remain underutilized, while the availability of cow dung for biogas production has diminished due to limited land for cattle rearing. This situation underscores the urgent need for alternative, sustainable energy sources. Therefore, improving the utilization of agricultural wastes, such as maize stalks and cow dung, as viable alternatives for energy production is critical to addressing the country's energy deficit and reducing environmental impact. Furthermore, this study will come up with efficient co-digestion strategies that combine cow dung and maize stalk residues to enhance biogas production, offering a sustainable alternative energy source while addressing waste management challenges.

1.3 Objectives

1.3.1 Main objective

The main objective of this study is to determine the effect of co-digestion of cow dung and pretreated maize stalk residue on biogas production.

1.3.2 Specific objectives

i. To determine the effect of chemicals as a pre-treating medium on biogas yield.
ii. To examine the impact of maize residue pre-treatment duration before its addition to cow dung in anaerobic digesters.
iii. To assess the optimal ratio of cow dung to maize stalk residue for biogas production.
iv. To evaluate the impact of co-digestion on the quantity of biogas produced.

1.3.3. Research Questions

i. What is the effect of using chemicals as a pre-treatment medium on biogas yield?
ii. How does the duration of maize residue pre-treatment affect its performance when added to cow dung in anaerobic digesters?
iii. What is the optimal ratio of cow dung to maize stalk residue for maximizing biogas production?
iv. How does co-digestion influence the overall quantity of biogas produced?

1.4 Justification

Kenya produces over 14 million tonnes of crop residues annually (Kimutai, Muumbo *et al.*, 2014), with maize residues being particularly abundant, covering approximately 1,600,000 hectares of cultivated land. The prevalent practice of incinerating or disposing of these residues contributes to significant environmental challenges. Given that maize is a stable crop in the country, leveraging maize residues to enhance anaerobic digestion for biogas production presents a valuable opportunity. This initiative has important implications for policymakers, academia, and the broader community. Policymakers can play a crucial role in developing frameworks that promote the sustainable use of agricultural waste, aligning environmental objectives with energy needs. Supporting research and innovation in this field can lead to more effective utilization of lignocellulosic materials through advanced pre-treatment methods and co-digestion with nutrient-rich substrates (Grala et al., 2011; Pilarski *et al.*, 2016; Zaky *et al.*, 2022).

For academia, there is a pressing need for further research on the co-digestion of cow dung and maize residues in Kenya. This research can fill existing knowledge gaps and contribute to the development of best practices and guidelines for optimizing biogas production. For society and local communities, promoting the use of maize residues for biogas can lead to significant environmental benefits and enhance energy access. By turning waste into a resource, communities can improve their livelihoods, reduce dependence on traditional fuels, and mitigate environmental impacts. Overall, enhancing the efficiency of pre-treatment processes using available chemicals and implementing co-digestion strategies can provide sustainable solutions that benefit policymakers, academic institutions, and communities alike, ultimately fostering a cleaner and more energy-secure future for Kenya.

1.5 Scope

This study focused on the co-digestion of cow dung and maize stalk residues to assess their combined influence on biogas production. The study primarily involved cow dung as the inoculum and maize stalk residues as the co-substrate, exploring their interactions during anaerobic digestion. The research investigated various pre-treatment techniques for maize stalk residues, including chemical pre-treatment, to enhance biodegradability and optimize biogas yield. The study evaluated different ratios of cow dung to maize stalk residues to determine the optimal combination for maximizing biogas production. The study was conducted under controlled laboratory conditions using anaerobic digesters, where parameters such as temperature, pH, and retention time were monitored and analyzed. The research evaluated biogas production over a defined period, allowing for the assessment of both short-term and long-term effects of co-digestion on biogas yield.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Biogas is produced through the process of breaking down organic molecules without oxygen. These days, the use of this technology is spreading throughout the globe, and study into connected topics is becoming more and more important. The process's multifunctional character provides a plausible reason for its rapid proliferation. Its duties consist of: multifunctional nature of the process. Its functions include:

i. Production of renewable energy; methane, the primary ingredient in biogas, can be burned directly to produce heat or indirectly to replace fossil fuels and electric power or through upgrading to be injected into the natural gas distribution network or utilized as fuel for vehicles. Waste management; Anaerobic digestion (AD) technology offers a means of stabilizing several forms of organic waste, including sewage waste and industrial waste.

ii. Organic fertilizer production involves the retention of nutrients from organic waste in the residue (digestate). This residue can then be used as fertilizer on agricultural land, reducing the requirement for mineral fertilizers and consequently decreasing fossil fuel consumption associated with their production (Mao *et al.*, 2015)

Biogas can be generated from various organic resources, including municipal waste, food waste, sewage sludge agricultural waste (Lantz *et al.,* 2007). Approximately 75% of Kenya's population relies on the agriculture industry, either directly or indirectly.

Consequently, any alterations in this sector, given its dominant position, will have a ripple effect on the entire economy. Therefore, the reduction of forests may be minimized, and the leftover agricultural products can be effectively utilized (Ministry of Agriculture, 2013). Kenya has an annual availability of around 13,913,223 tonnes of agricultural residues, which are spread among the seven provinces. If completely utilized for biogas production, these residues have the potential to significantly enhance the existing traditional energy sources (Kimutai *et al*., 2014).

Materials rich in lignocelluloses, such as agricultural residues are the most promising sustainable organic feedstock for biogas generation because they don't compete with arable land for production (Awoyale & Lokhat, 2019). Unfortunately, lignocellulose materials' nature restricts how they can be used in anaerobic digestion. For instance, lignocellulose cannot be effectively broken down due to its insolubility and resistant structure, and its poor nutrient content which includes:(nitrogen, phosphorus and trace elements reduces the availability of nutrients for bacteria that break down lignocellulosic materials (Xu et al., 2019). The intricate microbiological process of anaerobic digestion of organic materials necessitates coordinated action from multiple populations of microbes with various metabolic capabilities.

Numerous efforts have been made to enhance biogas production efficiency by using lignocellulosic materials as substrates, including various pre-treatment methods (de Medeiros *et al.*, 2022; Xu *et al.*, 2019) and co-digestion with nutrient-rich materials (Kucharska *et al.*, 2018; Santibáñez *et al.*, 2011; Sulzenbacher *et al.*, 2023). However, there is still limited understanding of the microbial communities responsible for cellulose degradation during the biogas production process, despite decades of research in this area. Additionally, there has been even less focus on improving process efficiency by utilizing locally available resources, such as ash, during the pre-treatment phase.

2.2 Anaerobic Digestion

The anaerobic digestion (AD) system is a biological process that utilizes bacteria to decompose organic material in the absence of oxygen, resulting in the production of biogas. This technology is applicable to a wide array of biodegradable waste types, including industrial byproducts, agricultural residues, animal manure, vegetable scraps, and energy crops. Anaerobic digestion involves a complex consortium of microorganisms that work in synergy to break down organic matter. This includes hydrolytic bacteria, which degrade complex organic polymers into simpler compounds, followed by acidogenic bacteria that convert these compounds into volatile fatty acids, and finally methanogenic archaea that produce methane (CH_4) from the acids. The versatility of the AD system allows it to process various organic materials, making it an effective method for managing waste across multiple sectors. This capability contributes to a circular economy by transforming waste into valuable energy (Mao *et al.*, 2015).

2.3 Main products of anaerobic digestion

The anaerobic digestion (AD) system generates three main by-products: biogas energy, water, and digested sludge. Typically, the biogas produced during the AD process has the following composition: methane (40-75%), oxygen (0-2%), carbon dioxide (25-55%), hydrogen sulfide (less than 1%), ammonia (0-0.05%), nitrogen (0-2%), hydrogen (0-1%), and water vapor (2-7%) (Hamawand & Baillie, 2015).

The quality and composition of biogas are influenced by various factors, including the type of material used, the digestion process, temperature, dilution levels, retention time of the inoculum source, inoculum concentration, substrate ratios, and other parameters. Undigested solids, known as decomposers, remain from the original feedstock and are not utilized by microorganisms, including dead bacteria from the reactor. Additionally, organic fertilizer can be produced from waste materials or wastewater. The water content in the reactor originates from the moisture in the organic matter, with further water being generated through microbial activity during digestion. By removing moisture from the digested material, it becomes possible to extract and release this water (Mao *et al.*, 2015). The summary of biogas content is shown in **Error! Reference source not found.**

Table 0-1 : Biogas composition (Bharathiraja *et al.*, 2018)

Constituent	Symbols	Composition (Vol %)
Methane	CH_4	40-75
Oxygen	O_2	0-2
Carbon dioxide	CO_2	25-55
Hydrogen sulfide	H_2s	<1
Ammonia	NH_3	<1
Nitrogen	N_2	0-2
Hydrogen	H_2	0-1

2.4 Anaerobic Digestion Process

Anaerobic digestion (AD) is a complex biological process that involves the breakdown of organic matter by microorganisms in the absence of oxygen. This process is typically divided into four distinct stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each stage is characterized by specific microbial communities and metabolic pathways, and they all play a crucial role in the overall efficiency of biogas production. In the hydrolysis stage, complex organic materials such as carbohydrates, proteins, and lipids are broken down into simpler compounds. This step is primarily facilitated by hydrolytic bacteria, which secrete enzymes to degrade macromolecules into soluble sugars, amino acids, and fatty acids (Khalid *et al.*, 2011). The rate of hydrolysis can significantly influence the overall digestion process, as slow hydrolysis can limit the subsequent stages of AD (Zhang *et al.*, 2016).

Following hydrolysis, the acidogenesis stage occurs, where the soluble products generated in the first step are further fermented by acidogenic bacteria. These bacteria convert the simple compounds into organic acids, primarily volatile fatty acids (VFAs), hydrogen, and carbon dioxide (McCarty, 2001). Acidogenesis plays a critical role in the production of intermediates that are essential for the next stages of digestion, and the types of acids produced can be influenced by the composition of the feedstock (Ramsay *et al.*, 2006). In the acetogenesis phase, the organic acids and alcohols produced during acidogenesis are converted into acetate, hydrogen, and carbon dioxide by acetogenic bacteria. This stage is important because acetate serves as a primary substrate for methanogens, the bacteria responsible for methane production. Acetogenesis is also sensitive to environmental conditions such as pH and temperature, which can affect the activity and growth of acetogenic bacteria (Liu *et al.*, 2020).

The final stage of anaerobic digestion is methanogenesis, where methanogenic archaea convert acetate and hydrogen into methane and carbon dioxide. This process is vital for the overall energy recovery from organic matter. Methanogenesis can be inhibited by high concentrations of volatile fatty acids, low pH, and the presence of ammonia (Rivard *et al.,* 2013). Methanogens are typically classified into two groups: hydrogenotrophic methanogens, which use hydrogen and carbon dioxide, and acetolactic methanogens, which utilize acetate (Angelidaki & Sanders, 2004). The interdependence of these four stages is crucial for the efficient operation of anaerobic digesters. Any disruption in one stage can adversely affect the subsequent stages, thereby reducing the overall biogas yield. For example, if hydrolysis is slow, it can lead to an accumulation of complex substrates that hinder acidogenesis. Conversely, excessive accumulation of VFAs during acidogenesis can inhibit methanogenesis, illustrating the delicate balance required in anaerobic digestion systems.

Hydrolysis is a process in which an intricate macro-molecular organic substance consisting of carbohydrates, proteins, and fats are broken down by enzymes and converted to monosaccharaides, amino acids, and long-chain fatty acids.

Acidogenesis, commonly known as fermentation, is the subsequent stage in hydrolysis. It refers to an anaerobic microbial activity that generates acids in the absence of any extra electron acceptor or donor (Sikora *et al.,* 2017) . Monosaccharides and amino acids obtained from hydrolysis undergo decomposition into several simpler substances, including volatile fatty acids (VFAs) such as propionic acid (CH_3CH_2COOH), butyric acid carbohydrates Sugar Amino Acids Hydrogen, Acetic Acid, Carbon dioxide Biogas (CH_4, CO_2) and Long Chain Fatty Acids (LCFA) (Batstone et al., 2002). Methanogens are unable to directly utilize these products and hence they must undergo further degradation in a subsequent process known as acetogenesis (Krzysztof Ziemiński, 2012) . The anaerobic conversion of complex organics during liquid phase decomposition frequently occurs at the fastest rate during acidogenesis.

Acetogenesis is a metabolic process that occurs in various microorganisms including bacteria and archaea. This is a type of anaerobic(Wang et al., 2023) respiration in which organic compounds are converted to acetic acid (acetate) as the end product *(Wang et al.,* 2023). In the process of making acetone, microorganisms use organic substrates, such as sugars, alcohols, fatty acids, and amino acids, as electron donors (Schink, n.d.). These substrates are oxidized through a series of enzymatic reactions to produce Acetyl-CoA, which is then converted to acetate. The following is a representation of the overall response:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad \textbf{0-1}$$

Methane production is the process of converting fermentation products, such as acetate, H_2, and CO_2, into CH_4 and CO_2 by methanogenic archaea, which are anaerobic organisms that require specific conditions to survive.

2.5 Co-digestion

Co-digestion is the process of digesting a uniform combination of two or several substrates at the same time. Historically, anaerobic digestion was a method used exclusively for the pre-treatment of a single substrate with a single purpose. It has recently been discovered that anaerobic digestion becomes increased stability when a greater range of substrates are used simultaneously. The most frequent scenario is when a significant quantity of one primary substrate (such as sewage sludge or cow dung) is combined and digested with smaller quantities of one or more other substrates (Jie *et al.*, 2020; Kirchmayr *et al.*, 2007).

Co-substrates are typically used to improve biogas production from an anaerobic digester as a result of the co-substrates' provision of missing nutrients and the positive synergy built in the digestion medium (Chong, *et al*., 2020; Wu, 2007) . Lignocellulose materials are characterized by high C/N ratio, low buffering capacity and a lack of essential nutrients (de Medeiros *et al.,* 2022) . The mono-digestion of lignocellulose materials frequently leads to a slow process and low production of methane (Sawatdeenarunat *et al.,* 2016). One way to address this constraint is by use co-substrates, such as animal dung, in conjunction with lignocellulose biomass. This allows for the supplementation of macro- and micronutrients as well as buffering capacity. (Ma *et al*., 2020).

An example of co-digestion has been researched for combining wheat straw with cow dung and poultry manure (Wang *et al*., 2023), the combination of rice straw, kitchen garbage, and pig dung (Ye *et al.,* 2013), and oat straw with cow dung (L. Li & Zhou, 2015). These investigations indicate that the methane yields are larger (about 200-400 mL/g VS) when straw is used in combination with other materials, compared to using straw alone (around 120-200 mL/g VS). *Poulsen et al.,* (2017) conducted a study to examine the effects of co-digestion compared to mono-digestion on the production of biogas and CH_4 for five various types of biomass materials include vegetable food waste, cow dung, pig manure, grass clippings, and chicken manure.

The results revealed that CH_4 yields were notably higher during co-digestion compared to mono-digestion. In addition, there was a little but insignificant rise in the CH_4/CO_2 ratio of the gas generated when compared to mono-digestion. Therefore, co-digestion seems to have a mutually beneficial impact on the production of both biogas and CH_4. Dos Santos Ferreira *et al,* (2020), demonstrate that employing techniques to enhance the hydrolytic stage leads to an increase in biogas production by facilitating the breakdown and release of organic matter, hence making it more readily available for microbial activity during aerobic processes.

Co-digestion offers several benefits, such as enhanced nutrient balance and digestion, as well as the ability to equalize various types of waste through dilution with manure or sewage sludge. It also allows for increased biogas collection, potential gate fees for waste treatment, and the reclamation of additional fertilizer and renewable biomass for digestion in agriculture. The limitations of co-digestion include: higher volume of effluent from the digester, further pre-treatment needs, increased mixing demands, wastewater treatment requirements, high utilization degree necessary, decreasing availability and rates, hygienist requirements, restriction of land use for dig estate, and economic dependence on crop costs and yield.

2.5.1 Benefits of Co-digesting with Cow Dung

Co-digestion with cow dung has been proven to enhance biogas production in various studies. Firstly, the C/N ratio of the substrates is narrowed down to the optimum range between 20 and 30, which is required by the methanogens in biogas production (Schnürer, 2016). This is because the nitrogen richness of the cow dung balances the carbon richness of maize stalks, which is usually the limiting factor for biogas production. Secondly, the degradation of the complex structure of the lignocellulosic maize stalk is intensified. The lipids of the cow dung have been reported to generate a synergistic effect when codigested with lignocellulosic substrates (Agwa *et al.,* 2012).

This is a process known as "lipid inhibition," but the inhibition on the breakdown of complex structure is not caused by the long chain fatty acids as the term suggested. Instead, these fatty acids are the preferred substrates for the methanogenic microorganisms. The degradation of lignin, which is the most resistant polymer in the maize stalk, is enhanced by at least two times. On top of that, the lag phase required by the methanogens to acclimatize to the environmental changes is shortened thanks to the introduction of cow dung. The strong microbial networks of the cow rumen have been transported into the anaerobic digester in the form of spore, providing a head start for the cellulolytic and methanogenic activity (Saye *et al*., 2021; N. Singh *et al.*, 2021).

This means an overall higher biogas production and a faster rate of its production can be achieved. Also, the unstable process of biogas production at the initial stage, commonly known as the "acid crash," can be avoided. This is crucial as the biogas production does not have to halt for a period of time until the acetate-utilizing methanogens become dominant again. The possibility for the formation of volatile fatty acids, which is toxic to the microorganisms, in the digester is minimized during co-digestion with cow dung.

2.6 Factors Affecting Anaerobic Digestion

The anaerobic process allows for external control of environmental parameters that affect biological responses, including pH, temperature, nutrient levels, and inhibitor concentrations. (Vargas-Soplín *et al.,* 2022). Any significant alteration in these factors can affect biogas yield negatively. Therefore, it is necessary to adjust these parameters to ensure optimal operation of the biogas plant (Raju *et al.,* 2015).

2.6.1 pH

The pH level is a crucial factor that impacts the growth of microorganisms during the process of anaerobic digestion. The pH of the digester must be maintained within the specified range of 6.8-7.2 when operating it at the optimal loading rate. The digester liquor's pH is often reduced by the presence of acetate and fatty acids generated during digestion. Nevertheless, the equilibrium between ions and bicarbonate in carbon dioxide within the digester exerts significant resistance to alterations in pH.

The buffer capacity, which refers to the resistance to a change in pH, is measured by the quantity of strong acid or alkali needed to induce a pH alteration in the solution. Therefore, the inclusion of bicarbonate serves to mitigate the negative impact on microorganisms induced by acidic conditions resulting from excessive fatty acid synthesis during digestion. Proteins, along with other organic molecules and bicarbonate, contribute to the buffering capacity and the ability to withstand fluctuations in pH (Garbacz *et al.,* 2014). Optimal growth of most micro-organisms occurs at neutral pH settings, while different pH levels might negatively impact metabolism by disrupting enzymatic reaction balance or by causing enzyme destruction. Methanogenic bacteria are the most sensitive to changes in pH.

Acidic conditions can halt the series of biological processes in digestion. *Getachew et al.* (2006), proposed two primary operational approaches to rectify imbalanced, acidic conditions in the digester. One possible method is to halt the supply of food and give the methanogenic population sufficient time to decrease the concentration of fatty acids, resulting in an increase in pH to a minimum tolerable level of 6.8. Halting the supply of nutrients also decreases the rate of activity of fermentative bacteria, resulting in a reduction in the generation of acid (Sun *et al.,* 2020). Another approach entails the introduction of chemicals to elevate the pH level and enhance the buffer capacity. Chemical addition offers the benefit of rapidly stabilizing the pH and facilitating the prompt correction of imbalanced populations. Calcium hydroxide, sometimes known as lime, is frequently utilized. Sodium carbonate, often known as soda ash, is a costlier but effective means of preventing the formation of calcium carbonate precipitates.

2.6.2 Temperature

Anaerobic bacteria operate within three distinct temperature ranges. Bacterial activity is minimized at temperatures below 25ºC, which are considered psychrophilic (Linke, 1997). Mesophilic digestion occurs between 25ºC and 45ºC. Thermophilic digestion occurs between 45ºC and 71ºC. According to Marcham (1992), the most efficient digestion takes place at temperatures of approximately 35ºC (mesophilic) and 55ºC (thermophilic). However, digestive activity decreases at roughly 45ºC. Additionally, it was documented in the concluding biogas trainee's manual (Tasew, 2017) The optimal temperature for the functioning of digesters is approximately 35ºC. The biogas production decreases substantially as the temperature decreases, and the fermentation process ceases if the temperature falls below 10ºC.

Bacterial growth and waste breakdown occur at a higher rate in thermophilic environments. One benefit of thermophilic digestion is that it produces methane at a rate that is roughly double that of Mesophilic digestion. In contrast, thermophilic digestion generates malodorous effluent in comparison to mesophilic digestion. Typically, there is not enough heat to function within the thermophilic range. For instance, animal dung, while at the surrounding temperature, requires a significant amount of energy to increase its temperature to 55ºC (Chen & Chang, 2020; Nie *et al.,* 2021).

The enhancement of fruit and vegetable waste conversion to biogas was achieved by raising the temperature from psychrophilic to thermophilic settings. The experimental results demonstrated that biogas production is greater in thermophilic operations compared to psychrophilic and mesophilic operations under the given conditions. The rate of production of biogas was maximum when the temperature was thermophilic, and it was, on average, 144% and 41% greater compared to the biogas production rates from psychrophilic and mesophilic digesters, respectively. The biogas composition from thermophilic digesters ranged from 58 to 62% methane. Elevated temperatures enhance the process of anaerobic biodegradation of intricate organic substances (Bouallagui *et al.,* 2004). The biogas plants in Kenya are designed to run within the mesophilic temperature range of 25ºC- 45ºC, which is suitable for most regions in the nation.

2.6.3 Carbon to Nitrogen ratio (C/N ratio)

The carbon/nitrogen (C/N) ratio is a vital metric for evaluating the decomposition potential of materials. Microorganisms responsible for biogas production typically require carbon in a ratio that is 25 to 30 times greater than nitrogen, making the optimal C/N ratio for feedstock between 25:1 and 30:1 (Olanrewaju & Olubanjo, 2019; EREDPC, 2008). When substrates have an inadequate C/N ratio, they lead to increased ammonia production, which can be toxic and inhibit methane production. Conversely, a disproportionately high C/N ratio indicates a lack of nitrogen, adversely affecting protein synthesis and thus disrupting the energy and structural metabolism of microorganisms.

Elevated C/N ratios can also result in acidification and fermentation failure. To achieve the desired average C/N ratio in a composite feedstock, it is essential to mix materials with high C/N ratios with those that have low C/N ratios. Orhorhoro *et al.* (2016) suggested that when the C/N ratio is high, gas output can be improved by introducing nitrogen, such as animal urine, or by installing a latrine at the plant. Pig and cattle manure typically possess an optimal C/N ratio, while human and chicken waste often have a C/N ratio that is inadequate for efficient digestion. Fresh vegetation has a high

C/N ratio, which increases significantly in older plants. Therefore, combining these materials in the correct proportions is necessary to initiate the fermentation process and enhance biogas production. The carbon/nitrogen ratio of the feed material plays a direct role in biogas production (Gizaw Wakene Advisor & Zebene Kiflie, 2016). Yan *et al*. (2015) indicated that the ideal C/N ratio ranges from 20:1 to 30:1.

2.6.4 Type of Substrate

Cow dung possesses all the essential elements necessary for the growth of anaerobic microorganisms, exhibits a significant buffering capacity, and serves as a complete substrate for the synthesis of anaerobic methane (Regueiro *et al.*, 2012). Nevertheless, the relatively low concentration of total solids (TS) in animal wastes, often below 8%, reduces the economic viability of investing in biogas plants that exclusively process animal wastes. In order to address this problem, cow manure is frequently blended with crops. The inclusion of additional crops in the influent material results in an increased concentration of total solids and a corresponding enhancement in biogas generation due to their high methane potential. The optimal ratio for combining animal manure with crops, based on volatile solids (VS), is 60:40 (McEniry & O’Kiely, 2013).

2.6.5 Substrate Concentration

The substrate's content or composition can have various effects on anaerobic digestion (Camargo *et al*., 2023). The substrate concentration is often influenced by the cultural habitat, ambient circumstances, abiotic and biotic elements, as well as the geographical region (Sońta *et al.*, 2019). Understanding the concentration is crucial for predicting the trajectory and speed of the reaction, while also taking into account the quantity of biogas produced. The biogas generation rate, or bio-methanation potential, is determined by the concentrations of lipids, proteins, carbs, and cellulose. AD systems containing a significant amount of lipids often exhibit great efficiency in the production of bio-methane. However, due to their intricate structure, these systems require a long length of time for retention. Proteins have the shortest retention time span, followed by carbs and cellulose. (Dankawu *et al.*, 2022; Stanley, H.O., Okerentugba, P.O., Ogbonna, 2014). However, systems with an excessive number of proteins or lipids may contain inhibitory factors caused by the buildup of ammonium and nitrogen, which significantly impacts the bio-methanation output.

2.6.6 Retention Time

The retention period in anaerobic digestion (AD) systems refers to the duration that a feedstock, such as cow dung and maize stalk waste, remains inside the digester. The calculation is based on the number of days (d), as shown in the following equation.

$$\boldsymbol{d = {}^{V}/_{Q}} \qquad \textbf{0-2}$$

where,

d - Number of days

V- Operating volume

Q- Volume flow rate

The necessary duration for the organic matter to break down in the digester is dictated by the chemical oxygen demand (COD) of the entering influent or the particles present in it, along with the biological oxygen demand (BOD) of the liquid waste materials. Increased retention duration leads to greater breakdown of organic materials (XU *et al.*, 2009). The duration of retention of an anaerobic digestion (AD) system is influenced by the system's operating temperature and the content of the waste being processed.

The retention duration for dry systems or very solid wastes typically exceeds that of wet system liquid-type waste. Decreasing the duration of retention time reduces the volume needed for the reactor, which in turn lowers the initial expenses associated with anaerobic digestion. Consequently, many methodologies have been proposed (Mohamed Ali *et al.*, 2023) for minimizing the retention time such as mixing, reducing solid content, and pretreatment.

2.6.7 Agitation and Mixing the Substrate

Mixing refers to the motion of fluids and solids for enhancement of the outcome of a process, achieved through the use of an agitation mechanism. The slurry processes employ a digester that cycles the slurry and exposes it to a specific level of shear. Pre-determined outcomes of mixing processes can be achieved without the need for experimental investigations. These outcomes encompass factors such as the power needed for agitation, heat transfer, blending of slurries, suspension of solids, transfer of mass to suspended particles, and various applications involving solid-solid interactions.

The sludge in anaerobic digestion (AD) process tends to settle if the feedstock is not sufficiently agitated. Mixing promotes close interaction between microbes and substrates, facilitating the digestive process (Rojas *et al*., 2010). Finally, by decreasing the particle size of the input will enhance the surface area available for absorption, leading to a higher level of microbial activity. Rotary-stator assemblies are employed for the purpose of pulverizing solid materials and producing consistent emulsions and pastes. The 4BP impeller is the most commonly used impeller for creating dispersions for mass transfer reasons. It offers a good balance between flow generation and shear, which is necessary for effectively producing slurry dispersions.

2.6.8 Substrate Dilution

Some elements, like sulfur and nitrogen, have a lesser impact when combined with water. These elements generate byproducts that inhibit anaerobic digestion, like ammonia and hydrogen sulfide. It was found that minimal biogas is generated by anaerobic digestion in the absence of water. Microorganisms need it to survive and move around. Additionally, it facilitates the substrates' digestibility. The anaerobic breakdown is prevented by high concentrations of end products from high solids digestion. As a result, a little dilution might be beneficial. The production of biogas typically increases with the percentage degradation of waste, and vice versa. The lower water content could result in excessive acid formation, which could interfere with the fermentation process.

The amount of bio-methane produced and the efficiency of the AD are significantly influenced by the total solids content and substrate concentration of the bio-digester(Masinde et al., 2020). Anaerobic digesters are classified into three categories based on their solids content. The groups consist of high solid digesters with a waste-to-water ratio of 1:2.5-4.5, medium solid digesters with a ratio of 1:5-7, and low solid digesters with a ratio of 1:10. The digester's solution is more compact in a dry anaerobic system, which also provides high loading rates. Because of the high loading rate and compacted byproducts, the dry approach is capable of generating a greater quantity of biogas compared to a wet procedure, increasing the level of material digestion.

Bacterial activity was hampered by excessively dry digestion (above 40%), Bacteria are only able to utilize organic substances that are in a dissolved state within water. Dry anaerobic digestion (AD) is a superior method compared to wet AD because of its reduced water usage, lower reactor capacity, and higher volumetric methane yield for the same material loading rate. Nong et al., (2022); Prapinagsorn *et al.,* (2018); Van Tran *et al.,* (2022), explored the co-digestion of grass and cow dung for the production of biogas. When cow dung and grass are mixed without water under anaerobic conditions, a little amount of biogas is generated.

Opoku Jnr (2011) investigated the production of biogas from kitchen waste generated on the KNUST campus and found that high biogas generation was achieved with 1:2 ratios. In order to operate the reactor, various water dilutions ranging from 8 to 20 liters were used. In contrast to the 8-liter dilution, which generated the smallest quantity of biogas (0.65-1.36 L/day), the 20 L dilution produced the highest amount (8.91-3.15 L/day). The degradation was least noticeable in the 8L dilution and most noticeable in the 20L dilution. The experiment demonstrated that the dilution increased as material degradation increased, improving biogas yield.

2.6.9 Pre-Treatment

Pretreatment of substrates can enhance the biogas generation and increase the volatile solids and solubility of substrates, so rendering them more amenable to enzymatic activity. These organisms play a vital role in degrading lignocellulosic materials due to their high levels of cellulose or lignin. Pretreatment has the ability to chemically, thermally, physically, or biologically break down these stubborn polymers. Introducing pretreatment can either increase the rate at which biogas is produced or decrease the time it takes for the process to begin. However, it is important to weigh the higher expense against the resulting benefits in efficiency (Carrere *et al.,* 2016; Gu *et al.,* 2015).

Lignocellulose is a tough substance with an intricate, sturdy, and inflexible composition that can withstand mechanical pressure and resist enzymatic degradation. It is not soluble in water. The inability of water molecules to penetrate the lignocellulosic fiber arises from a variety of factors, including limited surface area available for interaction, the existence of lignin and the crystalline arrangement of cellulose. Lignin provides protection and reinforcement to the fibers, effectively blocking the activity of enzymes (Giacobbe *et al.,* 2020). Moreover, the crystalline structure of cellulose reduces the enzyme accessibility to its surface. Pre-treatment of feedstock is occasionally necessary to enhance the methane production during the anaerobic digestion process.

Pre-treatment breaks down the intricate organic structure into less complicated molecules that are more easily broken down by microorganisms (Camargo *et al*., 2023; Chandra *et al.,* 2012; Kaur & Phutela, 2016). To improve the economic sustainability of anaerobic digestion (AD) systems, centralized systems have been recommended to treat many types of wastes from a variety of sources. Furthermore, studies have actually demonstrated that the biogas production is related to the type of interactions in the various waste streams hinder the digestibility of the waste during anaerobic digestion.

This has prompted extensive study aimed at demonstrating the most effective mix of waste streams to get maximum biogas yield, commonly referred to as co-digestion. Research has determined that the combination of sewage sludge, organic fraction of municipal solid waste (OFMSW), agricultural crops, lignocellulosic waste, and algal biomass produces the highest amount of bio-methane with the best quality. Nevertheless, pig or cow manure, when fermented together with biogas crops, is the prevailing and widely utilized fundamental substrate in agriculture. In addition to the co-digestion of raw materials, research has demonstrated that specific pretreatment methods can enhance the efficacy of the anaerobic digestion process, boost the production of biogas, and offer new options for locally sourced substrates. Pretreatment of raw materials for anaerobic digestion (AD) can enhance biogas production efficiency and decrease the number of volatile solids.(Bharathiraja *et al.,* 2018).

2.6.10 Total Solid Content

Total solids content refers to the total quantity of solids that remains once all moisture has evaporated. Within the process of anaerobic digestion, there exist three primary classifications.:

i. Processes involving low or moist solids with a total solids' concentration of less than 10%.,
ii. Medium or semi-dry solids have a moisture content ranging from 10% to 20%.
iii. High or dry solids systems typically have a solids content ranging from 20% to 40%.

The performance of the digestive process and biogas generation can be influenced by the total solid content and the initial concentration of substrate in the biodigester. Dry anaerobic processes are more condensed and provide elevated loading rates. The treatment process is more straightforward and cost-effective. Enhancing compactness and increasing the loading rate positively impact the degree of stabilization. Dry methods yield a greater quantity of biogas in comparison to wet processes. Bacterial activity will be limited under situations with a moisture level of more than 40%. Microorganisms exclusively rely on organic compounds that are solubilized in water. Nevertheless, arid climates offer greater advantages compared to humid conditions. Dry digester techniques are typically employed for vegetable waste or municipal solid waste, rather than manures.

2.6.11 Types of Pretreatments

The methods of pretreatment include physical, chemical, and biological.

2.6.11.1 Physical pretreatment

Physical methods of pretreatment include mechanical and thermal. For physical pretreatment, extensive research has been conducted on the breakdown of lignocellulosic biomass for energy production. The study aimed to investigate the impact of crushed and uncrushed lignocellulosic biomass on the generation of biogas (Raud *et al.,* 2020), he researched mechanical pretreatment using mills, which reduces the size of the substrate pieces or compresses them to cause breakage in the cellular structure. He found out that this increased enzymatic attack, which is particularly important for lignocellulosic substrates, also reduces viscosity in the digester, hence reducing the problem of floating layers. The substrate's particle size directly affects the digestive process because it directly indicates the surface area available for hydrolysis by enzymes, especially with plant fibers. It was found that methane yield and fiber breakdown improved when the particle size in the feed was reduced from 100 mm to 2 mm (Bharathiraja *et al.,* 2018).

During pure thermal pre-treatment, the substrate is exposed to elevated temperatures (usually ranging from 125 to 190 °C) and pressure for a duration of up to one hour. Pressure cookers, autoclaves, or microwave heaters can be used to do this experiment in the laboratory. Prior to thermal treatment, dry substrates require more water. Heat and water cause the hydrogen bonding present in crystalline cellulose and lignocellulose complexes to break, resulting in the expansion of biomass *(*Saragih *et al.,* 2019).

2.6.11.2 Chemical pretreatment

Various chemicals, including acids and bases of differing strengths, have been utilized to investigate their effects on biogas production under various conditions. Alkali treatments, such as lime, promote the expansion of lignocellulosic materials and partially dissolve lignin. In contrast, acid pretreatment does not directly target lignin but is thought to function by breaking down hemicellulose and disrupting the chemical bonds between lignin and hemicellulose (Gu et al., 2015; Taufikurahman & Delimanto, 2020; Thomas et al., 2018; Van Vlierberghe et al., 2022).

Oxidative pretreatment using hydrogen peroxide or ozone has a similar effect on lignocellulose as alkaline pretreatment, as it can also degrade lignin. Recent research by Song, Yang et al. (2012) examined the effects of hydrogen peroxide and ammonium pretreatment on biogas production from rice straw. The findings revealed that this treatment more than doubled the biogas output. The pretreatment was conducted at ambient temperature over an extended period of 7 days, using chemical concentrations of up to 4% w/w. However, a notable drawback of this method is that the introduction of additional oxygen into the system increases the carbon dioxide fraction in the biogas produced.

2.6.11.3 Biological pretreatment

Research has explored the use of fungal pretreatment as a means to eliminate phenolic toxins from wastewater prior to anaerobic digestion (Taufikurahman & Delimanto, 2020; Wan & Li, 2012). Additionally, it has been employed for the purpose of detoxifying coffee cherry husks in preparation for anaerobic digestion. Research has been undertaken as well on the fungal pretreatment of garbage in order to enhance the production of biogas (Ge *et al.,* 2015; Nair et al., 2018; Uzun *et al*., 2023) . The impact of fungal pretreatment on biogas yields remains uncertain. While white-rot fungus can remove lignin from substrates, they also eliminate some of the organic matter that could potentially contribute to biogas production. On the other hand, enzymes directly added to the biogas reactors do not have a notable effect and are rapidly degraded after being introduced.

2.6.11.4 Liquid Hot Water.

The application of liquid hot water treatment efficiently dissolves hemicellulose and lignin, hence minimizing the likelihood of producing inhibitors like furfural and enhancing the accessibility of enzymes. Nevertheless, this procedure necessitates elevated temperatures and is solely efficient inside a specific temperature range (Bharathiraja *et al*., 2018).

2.6.11.5 Thermal/Thermochemical Pretreatment

Preheating the substrate prior to anaerobic digestion has demonstrated enhanced methane production and decreased volatile solids. Research has additionally demonstrated that preheating a substrate that has undergone treatment with chemical additions (thermochemical) yields superior outcomes (Bharathiraja *et al.*, 2018). Kasinath et al. (2021) showed that subjecting chicken dung to pretreatment with sodium hydroxide heated to a specific temperature of 100°C enhanced both the production of biomethane and the ability of the raw material to be broken down by biological processes.

2.7 Conceptual Framework

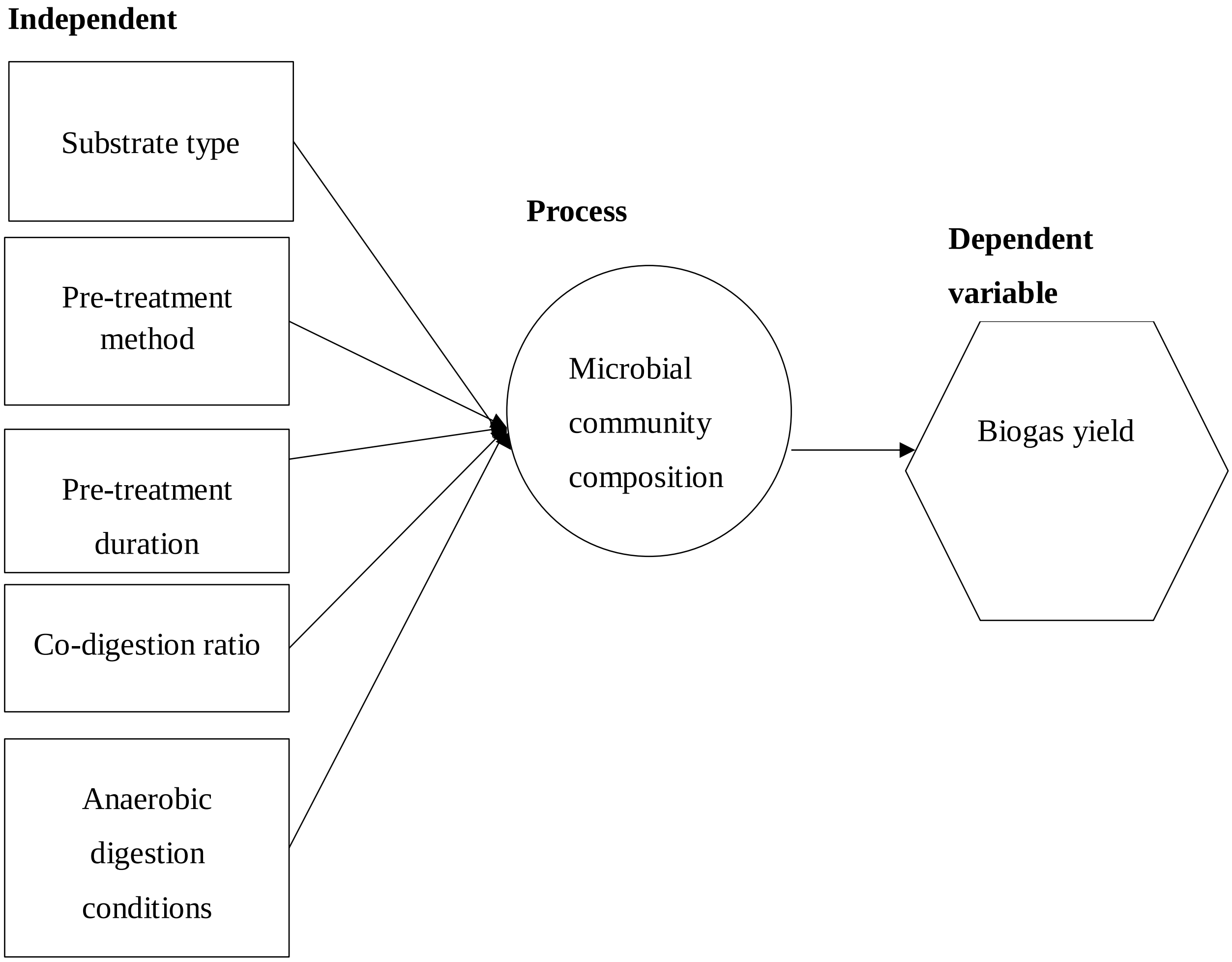


Figure 0-2: Conceptual Framework

The figure illustrates the conceptual framework of the research. The independent variables were: Type of Substrate-the mixture of cow dung and maize stalk residues (including different ratios); Pre-treatment Method-chemical pre-treatment techniques applied to maize stalk residues; Duration of Pre-treatment-the length of time maize residues is subjected to pre-treatment before being mixed with cow dung; Co-digestion Ratio-the specific ratio of cow dung to maize stalk residues (e.g., 70:30, 50:50, 30:70); Anaerobic Digestion Conditions-factors such as temperature and pH levels maintained in the anaerobic digesters. The dependent variable was Biogas yield (the total volume of biogas produced, the quality of biogas and the rate at which biogas is produced over a defined period). The moderating variable of the study was Microbial Community Composition.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Maize residues and cow dung were utilized as co-substrates for production of biogas. Maize residue samples were collected from Mosoriot area in Nandi County. The selection of the residue was based on its abundant production in the region. and most often, they are dumped or flared resulting in a widespread fire hazard and environmental pollution.

Sodium carbonate (Na_2CO_3), conical flasks(250ml), beakers (ranging from 250-1000ml), measuring cylinder, electronic weighing balance with an accuracy of 0.01g, aluminum foil, masking tape, distill water, crucible tongs, Water bath, gas sampling bag, multi-gas detector, laboratory oven, pH meter, rubber hose, cocks, non-return valve, syringe, silicon sealant and oven were used during the experiment.

Seeding involves initiating a freshly commissioned biogas plant by introducing pre-digested materials from an already operational plant. Moreover, substances like animal dung or municipal wastewater are frequently employed to initiate a newly established biogas facility, with the aim of minimizing the time required for the plant to become fully operational. The purpose of this approach is to introduce inoculum into the system (Bharathiraja *et al.,* 2018).

Microbial samples were extracted from the slurry of active biogas digesters during the anaerobic decomposition of cow manure. at a private farm in Kesses village. Cow dung was used as the main co-substrate for biogas production (Lahbab *et al.,* 2021).

Pretreatment of maize stalk and total solids determination was conducted at the Chemistry Lab at Masinde Muliro University of Science and Technology.

Table 0-1: Summary of equipment used in this study

Equipment $materials	Purpose	Source
Crucible	For holding samples	MMUST, Chemistry lab (SPD) Kakamega
Conical flasks	Acts as digester to carry out co-digestion	MMUST, Chemistry lab (SPD) Kakamega
Water bath	To keep constant the temperature required	Chemical and processing lab Moi university (Kesses)
Gas sampling bag	For the collection of gas	Market
Multi-gas detector	For analyzing the quantity of the gas	Chemistry and process lab Moi university Kesses
Electronic weighing machine	For measuring substrates' weight	Moi and MMUST
Laboratory Oven	TS determination	MMUST, Chemistry lab (SPD)Kakamega
pH meter	For measuring the pH for the	Chemical and processing lab (Moi University) Kesses
Silicon sealant	To prevent leakages to the digester	Market
Non-return valve	Prevent back flow of biogas to the digester	Market
Syringe	For collection of samples and enhance passage of water in the water collector	Market

Rubber hose	For conveyance of gas from the digester to the gas collector and water from the gas collector to the water collector	market
Cocks	For closing the conical flasks	Market

3.2 Pretreatment of Maize Stalk Residues.

3.2.1 Physical pretreatment

Maize residue samples were collected from Mosoriot area in Nandi County. The maize stalks were air-dried thoroughly to reduce their moisture content. To achieve this, thinly scatter the maize and expose it to sunlight. Proper drying ensured better storage and prevented the growth of mold or fungi.

The size of maize particles was reduced to increase the surface area available for microbial action. This was achieved by milling the maize stalk into smaller pieces of 1-3 cm using a chaff cutter according to (Zhao et al., 2013). They were then further pounded using a grinding mill to reduce the particle size to increase the surface area as shown in **Error! Reference source not found.** and packed in a dry polythene bag and stored at room temperature ready for chemical pretreatment.

Figure 0-1: Physical pretreatment of maize stalk.

3.2.2 Chemical pretreatment

MS was pretreated using Na_2CO_3 and its effect on TS was studied. The MS was soaked in Na_2CO_3 solution with different percentage concentration and duration based on the design of experiment (DOE) in **Error! Reference source not found.**. The DOE had four replicates with center points.

Table 0-2: Design of experiment for maize stalk residue pretreatment

Run	Na_2CO_3 Concentration (%)	Duration (Days)
1	7.00	8.00.
2	3.00	4.00
3	7.00	4.00
4	5.00	6.00
5	5.00	3.17
6	2.17	6.00
7	5.00	6.00
8	5.00	6.00
9	7.82	6.00
10	5.00	8.82
11	3.00	8.00
12	5.00	6.00
Untreated MS	-	-

The maize residue was prepared based on the design of the experiment where different samples were pretreated at different Na_2CO_3 concentrations and varied duration. Twelve pretreatments were conducted in the Chemistry lab (SPD) at Masinde Muliro University of Science and Technology.

Na_2CO_3 solution was prepared by dissolving the required quantity of sodium carbonate with distilled water. To achieve a 7% concentration of sodium carbonate solution, 14 grams of sodium carbonate solids were dissolved in 200ml distilled water and stirred to achieve homogeneity. Sodium carbonate was used to pretreat milled maize stalks according to (Jin et al., 2013) .Ten grams of reduced and dried maize stalk residue were soaked in 200ml Na_2CO_3 solution of different concentrations in 250ml conical flasks and stirred until it reached a homogeneous state and stored at room temperature for the designated number of days as per the design of the experiment. This was repeated for all sodium carbonate concentrations for varied durations. Following the specified duration, the chemical solution was poured out prior to total solid determination for each sample.

Figure 0-2 Masses of Na_2CO_3 and maize residues while taking weight

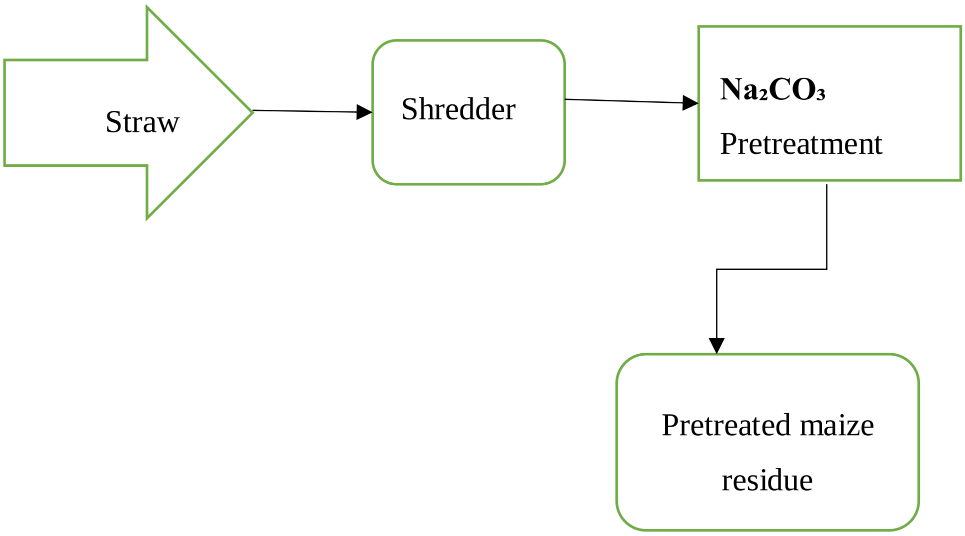


Figure 0-3:Pretreatment of maize stalks.

3.3 Proximate Analysis

The proximate analysis was performed in this experiment on Cow dung and maize residues based on several standards to determine the total suspended solids.

3.3.1 Determination of Total solids for maize stalk residue

The process of determining the moisture content of samples included multiple phases. To begin, the weight of the empty crucible with its cover was measured using a mechanical balance, and its weight was obtained. Then, 10g of each pretreated maize stalk residue sample was put inside the empty dried crucible, weighed, and its weight was obtained. The crucible without cover containing pretreated maize residue was labeled as per the sodium carbonate concentration and duration to be taken in the oven. Then the pretreated maize residue in the crucible was put in an oven set at a temperature of 105°C and maintained for 24 hours. After that, the crucible containing the sample was removed from the oven, and the cover was promptly placed on top. The weight of the sample was measured upon reaching the room temperature. The following equation was used to obtain the total solids:

$$\boldsymbol{T = \left(\frac{C-A}{B-A}\right)100} \qquad 0\text{-}1$$

where:

TS = Total solids

A = mass of dry beaker

B = mass of dry beaker and pretreated maize residue

C= mass of dry beaker and oven maize residue (at 105°C to constant weight)

3.3.2 Determination of Total solids for cow dung.

Determining the total solids in cow dung is an important aspect of analyzing its composition and understanding its potential uses. Total solids refer to the non-volatile components present in a sample, including organic and inorganic matter. The determination of total solids provides valuable information about the moisture level and overall quality of cow dung.

The technique that was used to determine total solids for cow dung was the gravimetric method, which involved measuring the sample weight prior and after drying to obtain the percentage of total solids. A sequential procedure used for determining the total solids content is as outline below:

A sample of fresh cow dung was taken from different areas, from different cows at Masinde Muliro University of Science and Technology dairy farm. The sample was well-mixed to obtain a homogeneous mixture. Care was taken to avoid contamination from foreign materials. A crucible that was devoid of any contents, free from dirt or impurities, and completely free from moisture was measured using an electrical weighing balance. The weight was precisely recorded.

A total of 20 grams of the cow dung sample was carefully put into the crucible. The weight of the added sample was recorded. The sample was placed in a crucible and heated in an oven at 105°C for 24 hours. This process removed moisture from the sample. The crucible was taken out of the oven once it had dried, covered with a lid, and left to cool to room temperature to prevent moisture absorption from the atmosphere. Once cooled, the crucible was weighed with the dried sample using the same electrical weighing balance as before. The weight was accurately recorded. Seven sets of experiments were conducted to mitigate variances. The total solids content was determined by applying the equation 3-1. Subsequently, a mean value of the total solids was recorded.

Table 0-3:Total solids of cow dung sample

Sample No.	1	2	3	4	5	6	7	Average
Total solids (%)	14.206	14.20	12.13	13.81	12.75	13.56	12.98	13.38

From these samples (Table 0-3), the average TS for cow dung was 13.38%.

3.4 Experimental Set up and Procedure

3.4.1 Laboratory method

Bench-scale experiments were conducted to create anaerobic digesters, in which biogas was generated by the decomposition of organic matter. The tests were conducted in sealed serum conical flasks with a capacity of 250 ml, and a working volume ranging from 190 to 250 ml. Every conical flask was hermetically sealed with a rubber stopper that had two openings. The initial output was connected to a gas pipe with an internal diameter of 6mm and was submerged below the surface of the solution to extract samples without introducing any air into the digester. The second exit was positioned higher than the solution's surface to facilitate the collecting of gas. These tubes were fitted with non-return valves to ensure that no gas flowed back to the digester. Each digester was connected to another conical flasks and bottles which had also two holes drilled at the cap with two 0.6cm pipe, one receiving gas from the digester. All the digesters were placed in a hot water bath, where the water temperatures were kept constant at 35˚C for the entire experimental period as recommended.

Sodium carbonate pre-treatment was conducted on maize stalk with sodium carbonate of 7% concentration for 4 days as per the pretreatment outcome in **Error! Reference source not found.** (which had the highest total solids), before being added into the digester together with cow dung slurry. The quantification of co-digestion of substrate ratios was based on 100g. After mixing the cow dung with maize stalk residue at the predetermined ratios as per the design of the experiment, all the digesters were diluted with predetermined volume of water according to the DOE respectively.

A 250 mL conical flask with a working volume ranging from 190 mL to 210 mL was utilized for the digestion of substrates in the biogas generation test. In accordance with the experimental design, the substrates were introduced into the reactor at different ratios, as shown in **Error! Reference source not found.**.

The amount of biogas from each digester was measured by the downward displacements of water using a graduated cylinder daily and the volume of water displaced was assumed to be equal to the volume of biogas generated (Nasir et al., 2015).

Gas chromatograph was used to measure the biogas compositions and nitrogen was used as carrier gas. Each run will last around 45 min as recommended by (Çelik & Demirer, 2015). The initial pH of the sample was determined directly prior to sealing and during the entire experimentation. Also, throughout the retention time pH and gas samples were measured every day.

Table 0-4:Substrate ratios for co-digestion.

Run	Water	Cow dung	Maize Stalk
		Ratios	
1	3.0	1.0	1.0
2	1.0	1.5	1.5
3	1.8	1.0	1.0
4	1.7	1.0	1.0
5	2.0	1.0	3.0
6	5.0	1.0	3.0
7	1.8	1.0	1.0
8	1.8	1.8	1.0
9	1.8	1.0	1.0
10	1.8	1.0	1.0
11	1.8	1.0	1.8
12	2.0	3.0	1.0
13	5.0	1.0	1.0
14	1.8	1.0	1.0
15	11.0	1.0	6.3
16	2.0	1.0	1.0
17	5.0	3.0	1.0
18	1.8	1.0	1.0
19	11.0	6.3	1.0
20	1.0	2.1	2.1

An experiment was conducted to assess the effect of altering these operational factors on the production of biogas and to identify the most effective conditions for the digestion of maize stalk and cow manure. The quantification of co-digestion entails utilizing a ratio that was calculated based on 100 grams. Reactors were sealed using silicone sealants. On a daily basis, the amount of biogas produced is quantified for each trial, and batch digestion occurs in the reactor over a period of 21 days. The experiments were carried out at a temperature of 35°C, which is within the range of mesophilic conditions.

The organic components were gently agitated for approximately one minute each day. Mixing is effective in preventing the formation of temperature gradients that can cause stratification, as well as the deposition of sediments, scum, and crust. By doing this, it ensures consistent physical, chemical, and biological conditions within the digester (Ingabire et al., 2023). Agitation is especially beneficial when dealing with raw materials that have a high fiber content. This is because it promotes a strong connection between the substrate and decomposing organisms, which is crucial for species like Clostridium, a typical fermentation agent in anaerobic systems (Gupta N.& Kushwaha, 2011)

Anaerobic digesters were set up according to DOE and results were tabulated as averages. As shown in Figure 0-4, the amount of water that biogas production displaces on a daily basis in milliliters represented the amount of biogas generated.

Table 0-5: Overview of experimental configuration

Operational Parameters	**Value**
The digester volume	250mL
Reaction volume	210-250 mL
Temperature	Mesophilic (35°C)
Retention time	15 days
Mixing	Shaking manually (daily)

3.5 Biogas Production and Methane Content Analysis

The daily measurement of biogas production was carried out and the methane concentration was assessed using gas chromatography (MRC/GC/3962138D Vaiant) model.

3.5.1 Biogas Production

The biogas production was quantified employing the water displacement technique, which measures the volume of water displaced in the water collector by the generated gas in milliliters. This procedure is depicted in Figure 0-4, below.

Figure 0-4:Illustration of anaerobic batch digester of maize stalk and cow dung experimental setup

The quantification of biogas produced was performed employing the liquid displacement technique (Budiyono et al., 2019). Biogas volume produced was measured daily for about 15 days (Ferrer et al., 2010). The cumulative biogas production is calculated according to equation 0-2 below:

$$\textbf{Accumulated Biogas Yield} = \sum_{n=1}^{n} Vn \qquad \textbf{0-2}$$

V(mL) is the volume of biogas produced every day and the variable "n" denotes the number of days of analysis.

The three conical vials are arranged such that the first vial contains the substrate; the middle part holds the water and the last part to collect the water released from the second container after being displaced by gas produced from the first digester(Akunna *et al*., 2007; Chia *et al.*, 2014) as shown in **Error! Reference source not found.**

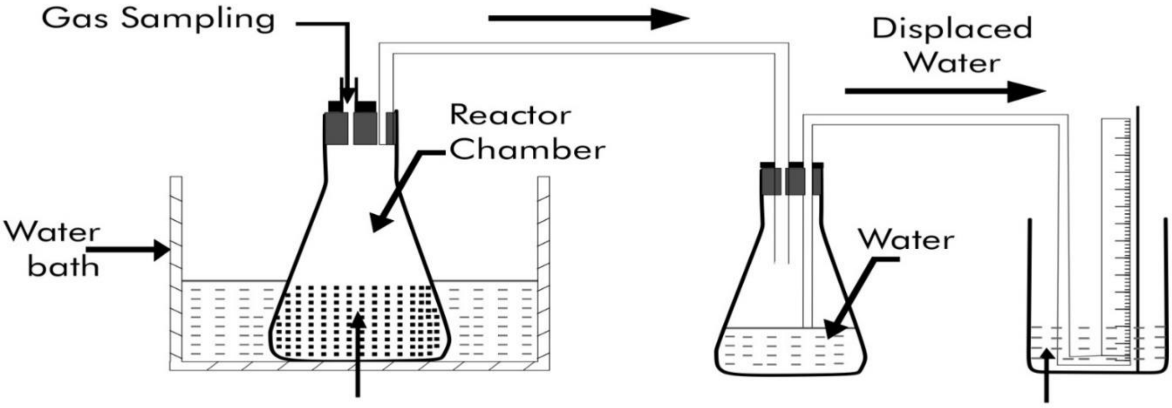


Figure 0-5: Digester Setup(Roy et al. 2019)

3.6 Optimization of Biogas Production

3.6.1 Design of Experiment

A technique referred to as "design of experiment" (DOE) is used to build an experimental model that consumes the fewest resources possible. The Design Expert13 software was utilized to ascertain the optimal quantity of variable inputs and identify the appropriate number of experimental runs for maximizing biogas yield in the co-digestion of maize stalk and cow manure. This software contains CCD, ANOVA, and RSM. To analyze the relationship between the variables and the regression coefficient, analysis of variance (ANOVA) was employed. Variable substrate ratio CD: MS 25-75 and dilution (25-125 ml) were the two factors that controlled the co-digestion of CD and MS. **Error! Reference source not found.**, provided displays the levels of design and the corresponding parameters, for AD. Twenty runs with six center point replications were performed in accordance with the experimental design. For each digestion, anaerobic digestions were set up in duplicate, and the outcomes were presented as means. The experiment's response was measured in milliliters (ml) of biogas yield.

3.6.2 Characterization of Biogas

The biogas sample's composition was retrieved from the digester experiment runs using a syringe. The study was conducted using an Agilent gas chromatography instrument (MRC/GC/3962138D rVaiant) equipped with an ionization detector (FID), as reported by (Chamarthi *et al.,* 2013). The pH of the substrates was measured directly using a Hanna pH meter.

3.6.3 Biogas composition

The composition of the biogas was analyzed using gas chromatography. During the preparation of the calibration gas in the laboratory, samples were collected from the optimal run labeled as run #1. Figure 0-10 displays the chromatogram of the calibration gas, with a volume of 2 µl. The chromatogram displays a characteristic peak corresponding to methane. The methane gas had a maximum retention period of 1.546 minutes when the column temperature was set to 150°C and the input and detector chamber temperatures were set to 200°C and 250°C, respectively. Graphs depicting the total amount of biogas produced were created using various ratios of maize residue to cow dung.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of Chemical Pretreatment and Pretreatment Duration

The pretreatment that gave the highest TS was the one pretreated with 7% Na_2CO_3 concentration for four days, which gave a TS value of 15.15%, indicating an increase of 7.26% TS as shown in **Error! Reference source not found.**. It was established that the optimal conditions were 7% NaCO3 (w/w) for four days, and 15.1515% TS was achieved and was subsequently used to carry out co-digestion with the core substrate, which was cow dung. alkali pretreatment hydrolyzed most organic materials and eased the anaerobic digestion process.

Table 0-1 : **% TS for the pretreated and untreated MS**

Run	**Na_2CO_3 Concentration %**	**Duration of Pretreatment Days**	**Total Solids %**
1	7	8	9.54
2	3	4	11.48
3	7	4	15.15
4	5	6	7.79
5	5	3.2	12.71
6	2.17	6	6.08
7	5	6	8.02
8	5	6	8.06
9	7.82	6	10.43
10	5	8.8	8.37
11	3	8	11.61
12	5	6	8.01
Untreated MS	-	-	7.89

Table 0-2: **ANOVA for Quadratic Model**

Source	Sum of Squares	df	Mean Square	F-value	P-value	Significance
Model	82.07	5	16.41	6.29	0.0223	significant
A- Na_2CO_3 Concentration	7.54	1	7.54	2.89	0.1402	
B-Duration of Pre-treatment	16.87	1	16.87	6.46	0.0440	
AB	8.18	1	8.18	3.13	0.1271	
A^2	13.78	1	13.78	5.28	0.0613	
B^2	43.51	1	43.51	16.67	0.0065	
Residual	15.67	6	2.61			
Lack of Fit	15.67	3	5.22			
Pure Error	0.0000	3	0.0000			
Correctional Total	97.73	11				

The Model F-value of 6.29 presented in Table 4.2 indicates that the model demonstrates statistical significance. The probability of obtaining an F-value of this magnitude purely by chance is a mere 2.23%. P-values below 0.0500 suggest that the model terms hold statistical significance. In this scenario, B and B^2 represent important components of the model. Values exceeding 0.1000 suggest that the model terms lack significance. In the presence of numerous inconsequential model terms, excluding those necessary for maintaining hierarchy, the process of model reduction could enhance the efficacy of your model.

Table 0-3: **Fit Statistics**

Std. Dev.	1.62	**R^2**	0.8397
Mean	9.32	**Adjusted R^2**	0.7062
C.V. %	17.34	**Predicted R^2**	-0.1398
		Adeq Precision	6.9388

A detrimental The predicted R^2 suggests that the overall mean could serve as a more effective predictor of your response than the existing model. In certain instances, a model of a higher order may yield superior predictions. Adequate Precision assesses the relationship between the signal and the noise present in the data. A ratio exceeding 4 is preferable. The ratio of 6.939 suggests a satisfactory signal quality. This framework serves as a tool for traversing the design landscape. The ultimate expression articulated through encoded variables:

$$\mathbf{R= +6.60 + 0.09707\boldsymbol{A} - 1.45\boldsymbol{B} - 1.43\boldsymbol{AB} + 1.47\boldsymbol{A}^2 + 2.61\boldsymbol{B}^2} \qquad \textbf{0-1}$$

The equation with coded factors can facilitate predictions on the response at specified levels of each factor. Default coding assigns +1 to high levels of the components and -1 to low levels. The encoded equation is essential in determining the comparative influence of the factors through an analysis of the factor coefficients. Conclusive equation expressed in terms of actual variables:

$$\mathbf{R1= +30.44414 - 1.03838\boldsymbol{A} - 6.76111\boldsymbol{B} - 0.357500\boldsymbol{AB} + 0.366875\boldsymbol{A}^2 + 0.651875\boldsymbol{B}^2} \qquad \textbf{0-2}$$

The equation expressed in actual factors can facilitate predictions on the response at specified levels of each factor. The levels must be delineated in the original units for each factor. This equation is unsuitable for assessing the relative influence of each factor, as the coefficients are adjusted to fit the units of each factor, and the intercept is not positioned at the center of the design space. Figure 3.1 illustrates the correlation between anticipated total solids and experimental readings. The correlation between the predicted and actual total solids is strong (R2 = 0.8397), indicating that the model is both appropriate and significant, and can effectively replicate the experimental data within the explored range. Figure 4.2 illustrates the graph of externally studentized residuals plotted against the run number. The random distribution of residuals around the line indicates the adequacy of the reduced cubic model. Figure 4.3 displays the normal probability curve of the externally studentized residuals. The data points consistently align along a linear trend line (Figure 4 3), indicating a lack of significant dispersion and that the residuals are normally distributed.

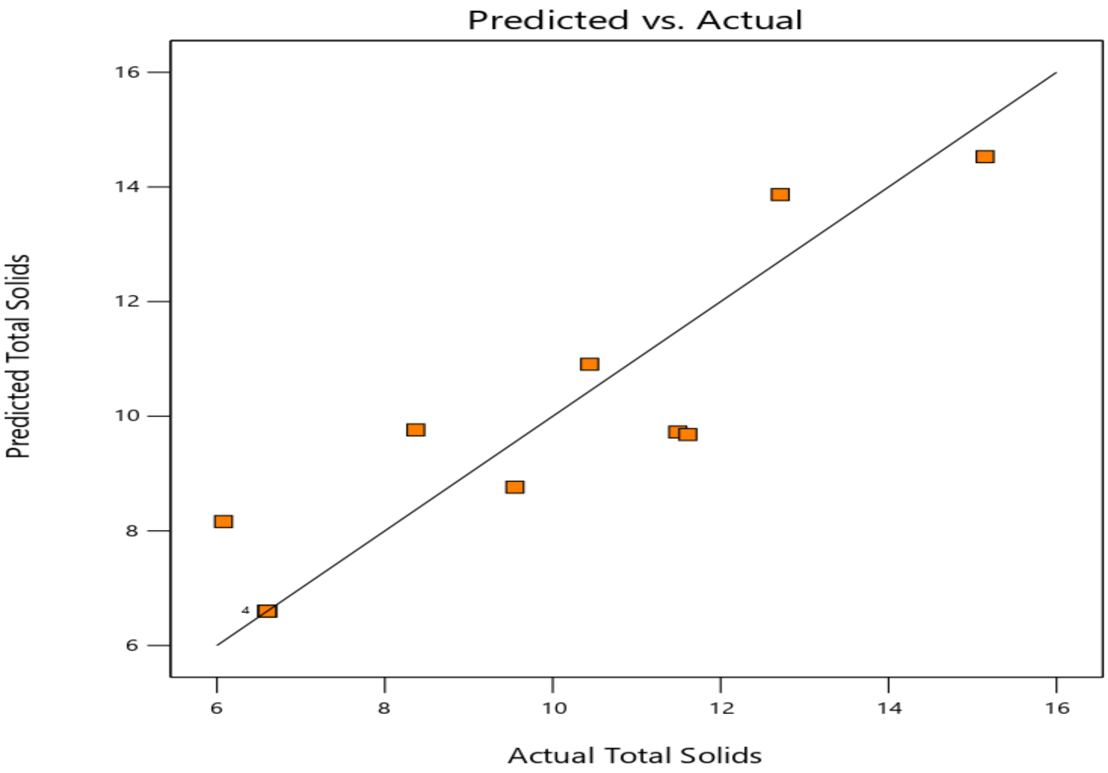


Figure 0-1: Plot of predicted response vs. actual Total solids

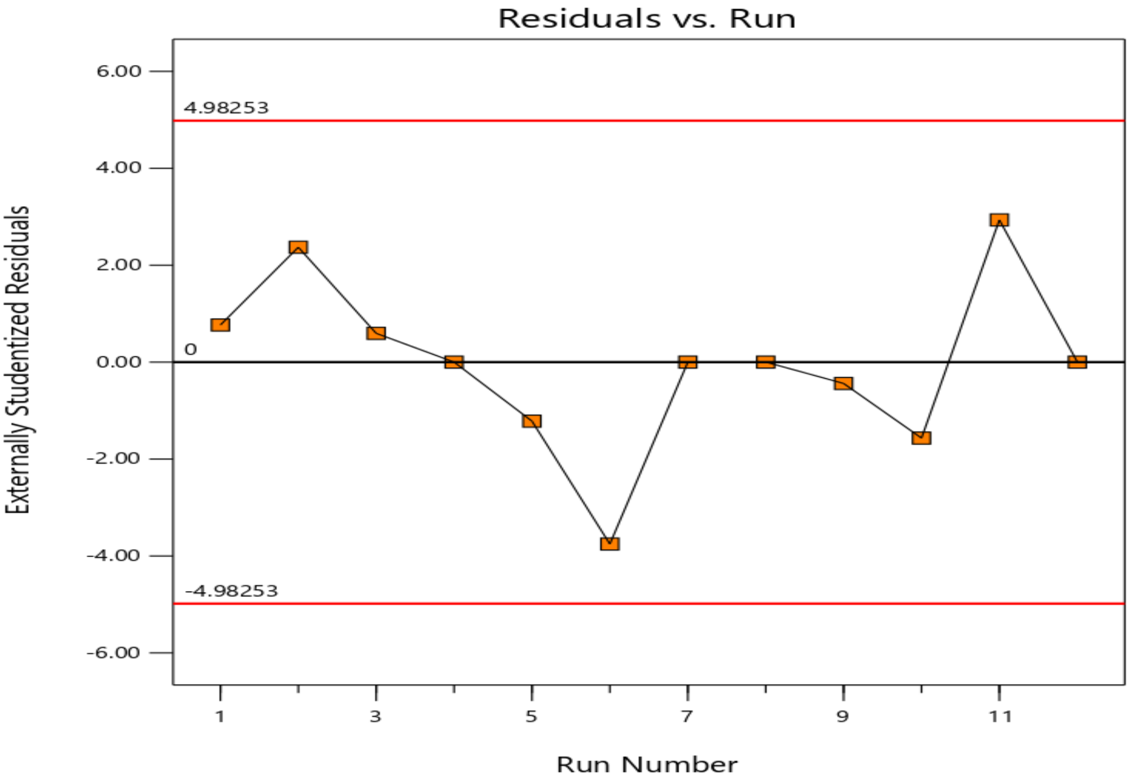


Figure 0-2: The plot of externally studentized residuals against the run number

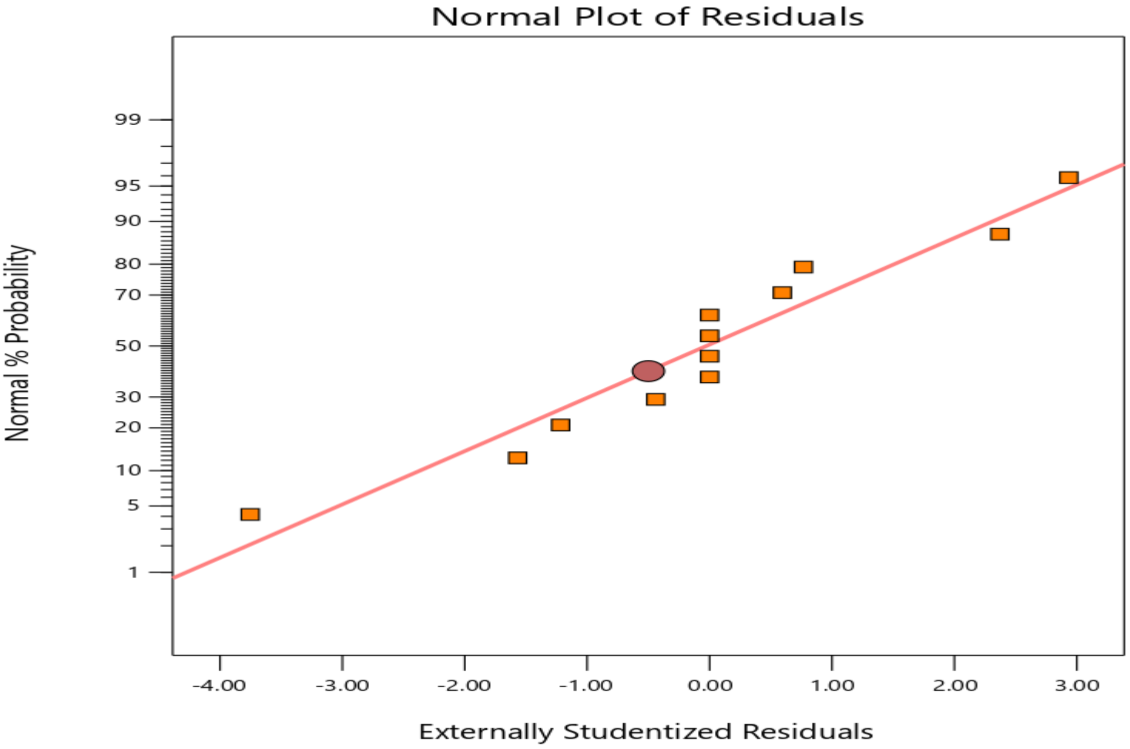


Figure 0-3: Normal probability plot of the externally studentized residuals

4.2 The Effect of Chemicals as A Pre-Treating Medium on Biogas Yield.

The study has investigated various parameters to optimize the sodium carbonate pretreatment process, including concentration and duration. With the rising Na_2CO_3 concentration, a notable loss in weight was noted, and the sample pretreated became brittle and lighter in color which was the same with the observation made by (Kaur & Phutela, 2016).

Results from **Error! Reference source not found.** indicate a significant increase in TS contents of CS with respect to Na_2CO_3, concentration and duration. The obtained TS were as tabulated in **Error! Reference source not found..** From **Error! Reference source not found.** and Figure 0-, this work studied the effect of sodium carbonate concentration and the treatment duration. After treatment, the total solids for all the samples were determined. The outcomes demonstrated that the addition of sodium carbonate (7% w/w for 4 days) obtained the highest total solid of 15.15% which was higher than that of un-pretreated maize stalk, 7.89% (control experiment). The pretreatment that gave the highest total solids (TS) was the one pretreated with 7% Na_2CO_3 concentration for 4 days which gave a TS value of 15.15%, indicating an increase of 7.26% TS and was subsequently used to carry out co-digestion with the core substrate which was the cow dung.

From study findings, it has been observed that optimized concentrations of sodium carbonate and duration of treatment can lead to more significant delignification, increased total solids and improved enzymatic digestibility. However, it is crucial to find the correct balance when it comes to pretreatment conditions because overtreatment can also lead to cellulose degradation and decrease in total solids as witnessed in **Error! Reference source not found.**,Run #9.

The optimal conditions were determined as 7% Na_2CO_3 (w/w) and 4 days, 15.15% TS was achieved. The methane production rate in each digester with pre-treated maize residue reached the maximum between days 9-12 but the peak value was attained on day 12 which indicated that increased methane yield and decreased retention time, alkali pre-treatment hydrolyzed most organic materials and eased anaerobic digestion process. In comparison between co-digestion of maize residue and inoculants with and without pre-treatment, it was found that the digester containing co-digestion of cow dung and pre-treated maize stalk has more production of gas at maximum as compared with the digester containing co-digestion of cow dung and maize stalk without pretreatment.

Table 0-4: Design of Experiments for Chemical Pretreatment of Maize Stalk

Run	Na_2CO_3 Concentration	Duration of Pretreatment	Total Solids
	%	Days	%
1	7.00	8.00	9.55
2	3.00	4.00	11.49
3	7.00	4.00	15.15
4	5.00	6.00	7.80
5	5.00	3.17	12.71
6	2.17	6.00	6.08
7	5.00	6.00	8.03
8	5.00	6.00	8.06
9	7.83	6.00	10.44
10	5.00	8.83	8.37
11	3.00	8.00	11.61
12	5.00	6.00	8.02

4.3 Production of Biogas from the Co-digestion of Cow dung and Maize stalk

The 15-days AD results of cow dung co-digested with maize stalk at mesophilic temperature (35^{o}C) are shown below. According to (Owamah & Izinyon, 2015), the co-digestion of feedstock enhances the production and quality of the anaerobic process by improving the carbon, nitrogen, and nutrient balance in the co-digested material. Results of co-digested cow dung under optimal conditions with maize stalk increase production and quality. Methane production was low due to the low digestibility of the single substrate and accumulation of the inhibitory substances (e.g. lignin, lipids) as the single substrate is unlikely to have buffering and is likely to lack nutritional content (Amin *et al.*, 2017). When co-digested, Cow dung offers various advantages, including enhanced C/N ratio and higher biogas outputs and quality (Iweka *et al.*, 2021).

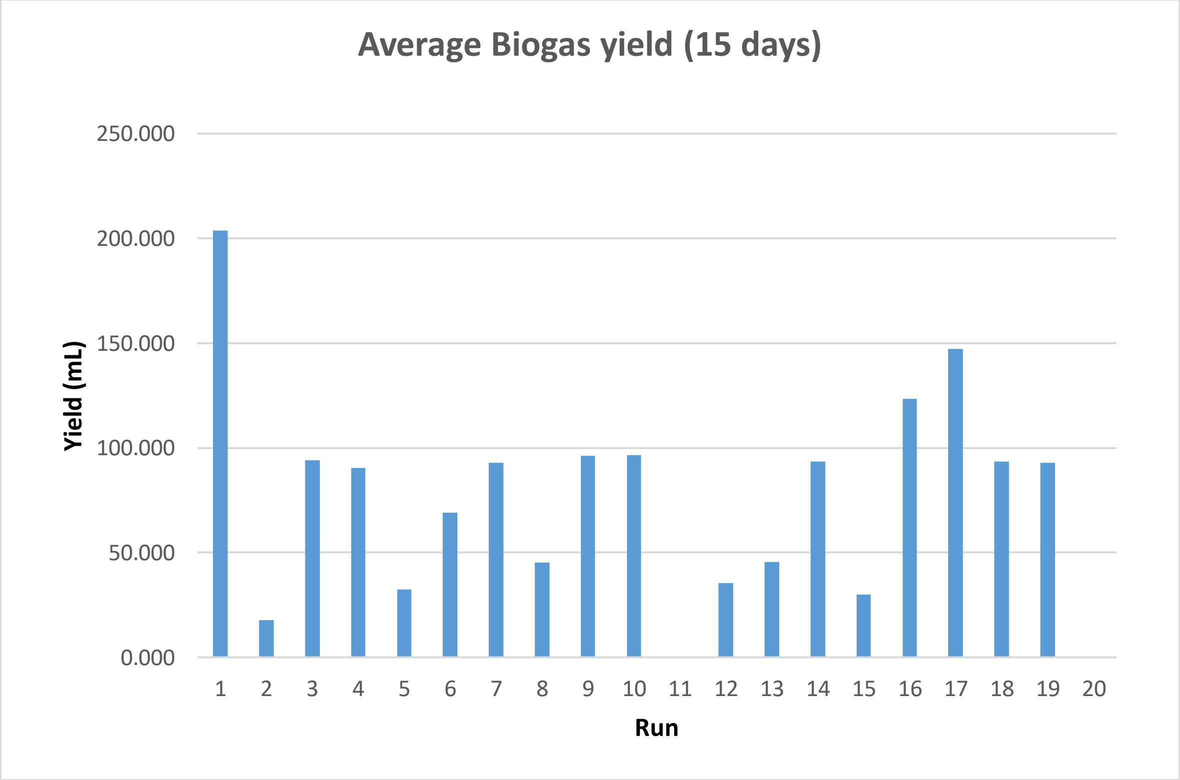


Figure 0-4: Average daily Biogas Yield per Run

In order to assess the influence of substrate ratio, cow dung, maize stalk co-digestion, and dilution on the biogas generation, the daily mean biogas volume and daily biogas output were recorded. Baseline reactors were used to test the variation in substrate ratio for cow dung, maize stalks, and dilution as outlined in Figure 0-. Daily measurements were made for the production of biogas. After 6-7 days of retention, biogas was generated, as demonstrated in the figure below. A total of 20 bio-digesters, with six replicates for each of the center points, were utilized, while two bio-digesters were allocated for the control group. (cow dung and maize stalks). The results of the study were averaged after triplication.

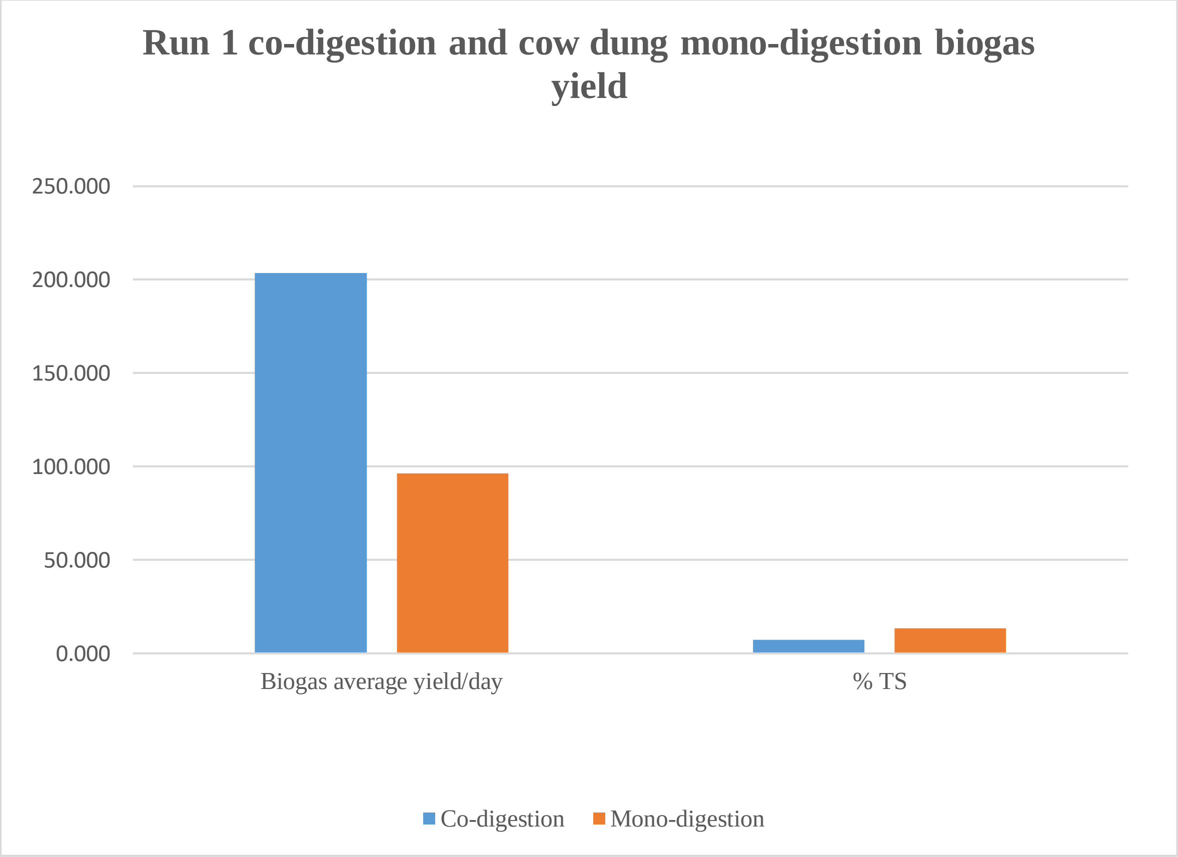


Figure 0-5: Run 1 co-digestion and cow dung mono-digestion

Run 1 was the one that produced the most biogas yield of 203.64ml with substrate ratios of: 3:1:1 W:CD:MS at a pH of 7.2 and a temperature of 35^0C. Runs 17 and 16 followed with 147.27 and 123.55 mL of biogas respectively, and they all worked in the same conditions. The findings indicated that, three things; pretreatment, substrate ratio, and co-digestion had a big impact on biogas production. This is similar to other studies done by (Jasinska *et al*., 2022). The outcomes were analyzed using a design expert to show the optimal run 1, which is shown in Figure 4.5.

Table 0-5: Average biogas yield and % TS.

Run	Ratios			Average Biogas yield (15 days)	Average TS (%)
	Water	cow dung	Maize stalk		
1	3.00	1.00	1.00	203.64	7.13
2	1.00	1.50	1.50	17.60	7.13
3	1.75	1.00	1.00	94.18	7.13
4	1.67	1.00	1.00	90.30	7.13
5	2.00	1.00	3.00	32.44	7.35
6	5.00	1.00	3.00	68.91	7.35
7	1.75	1.00	1.00	92.82	7.13
8	1.75	1.84	1.00	45.09	7.00
9	1.75	1.00	1.00	96.36	7.13
10	1.75	1.00	1.00	96.45	7.13
11	1.75	1.00	1.84	0.00	7.26
12	2.00	3.00	1.00	35.36	6.91
13	5.00	1.00	1.00	45.40	7.13
14	1.75	1.00	1.00	93.55	7.13
15	11.00	1.00	6.29	29.90	7.45
16	2.00	1.00	1.00	123.55	7.13
17	5.00	3.00	1.00	147.27	6.96
18	1.75	1.00	1.00	93.55	7.13
19	11.00	6.29	1.00	92.82	6.81
20	1.00	2.05	2.05	0.00	7.12

4.3.1 Effects of substrate ratio - Co-digestion

The column chart below illustrates how substrates affect biogas yield as part of the study's further investigation into the relationship between substrate ratio and yield. Given that runs 11 and 20 were the minimum runs that never yielded. This is because the dilution was low, to demonstrate the correlation between the increase in biogas output and the increase in dilution (water content). The results obtained agreed with (Hortence et al., 2023) The lower water content in the AD leads to a decrease in biogas production. The experiment's results showed that the optimal digestion of CD and MS resulted in a significant increase in biogas production of 203.64mL, as compared to the mono-digestion of each substrate, with the highest methane content of 61.94% found in the substrate ratio of, 3:1:1 for W:CD: MS. While cow dung and maize stalks mono-digestion were done at 100% cow dung and 100% maize stalks. The yields for these runs were compared with co-digestion. The yield was 107.454ml and 153.42ml greater than CD and MS mono-digestion, respectively as shown in Figure 4.6.

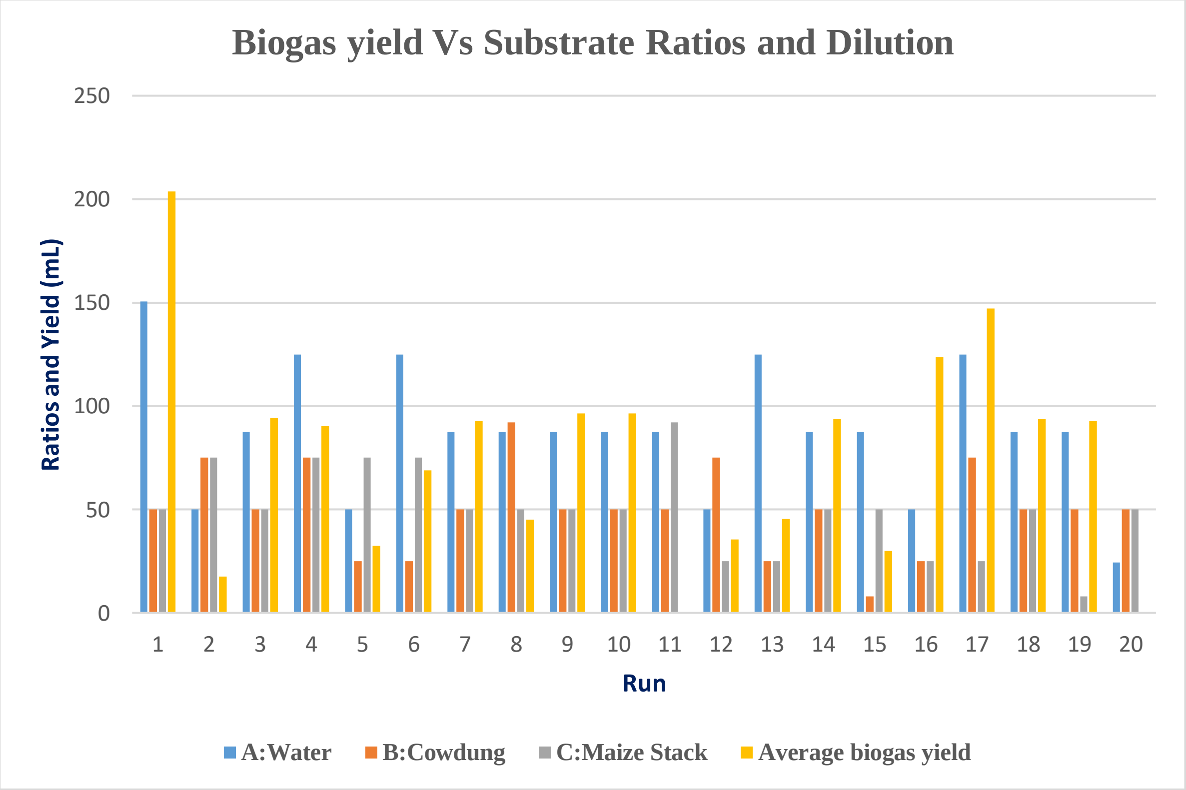


Figure 0-6: The influence of substrate ratios and dilution on the production of biogas

If the amount of cow manure in the reactor is low, only a small amount of biogas may be produced due to low microbial metabolic activity, resulting in low biogas yield. When maize stalks are overloaded, as in the case of runs,11 and 15 in Figure 0-6, organic acid production increases rapidly, leading to acid accumulation, buffer depletion, and pH reduction, inhibiting methanogenic activity, thereby reducing biogas production and can even stop completely as in the case of run 11 in Table 4-5 and Figure 0-.(Induchoodan *et al.*, 2022; Mao *et al*., 2015; R. Singh et al., 2023; Wainaina *et al.,* 2019).

This result is consistent with the results of other authors who studied the ability to produce biogas at different cow dung concentrations in terms of substrate ratio and total solids (Masinde, 2021). The optimum total solid obtained from the co-substrates was 7.13%, which is approximately near 8% TS obtained by (Masinde, 2021) , that gave the highest quantity of Methane. The greater the total solids, the greater the biogas yield and the AD process is more stable (Srisowmeya *et al.,* 2021). For each biodigester containing an equal or slightly higher proportion of cow dung, the biogas production rate is high and fast. Because cow dung and inoculum have the ability to accelerate the digestion of maize stalks, the biogas production efficiency is high, at a span of 6 to 12 days.

This may be due to cow dung containing a lot of biodegradable organic matter. This demonstrates how cow manure and inoculum components play an important role in co-digestion with corn stalks, to accelerate digestion and create biogas on the farm.

The findings of this study additionally indicated that the production of biogas increased with higher levels of dilution, specifically referring to the increase in water content. Runs 2, 5, 8, 12, and 20 demonstrated that anaerobic digestion with low water content led to a reduced biogas yield, which is consistent with the findings of (Jeppu *et al.,* 2022; Wongfaed *et al.,* 2021) Microorganisms rely on it for their life and locomotion. Furthermore, it also facilitates the breakdown of organic materials (Aslanzadeh *et al.,* 2014; Hortence *et al.*, 2023). According to (Poggio *et al.*, 2016; Sageman *et al.*, 2003), the rate of waste decomposition typically rises as dilution increases, and the yield of biogas increases as the decomposition rate increases.

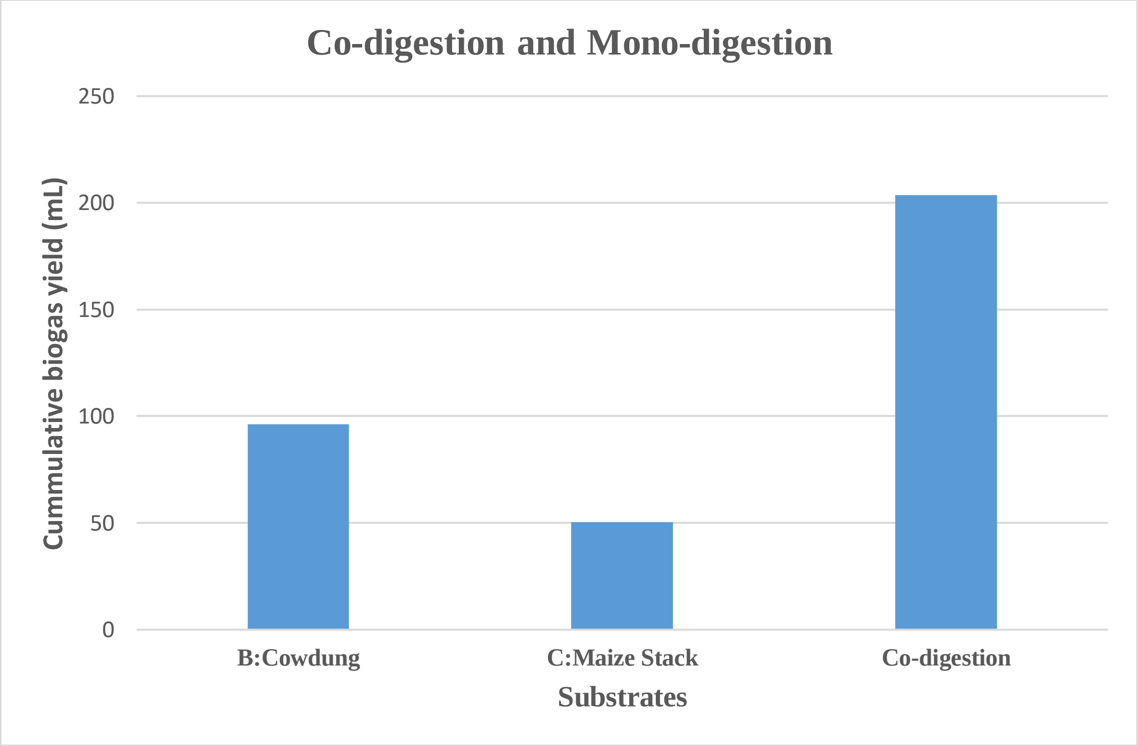


Figure 0-4: The effect of mono-digestion and the optimal co-digestion of cow dung (CD) and maize stalk (MS) on the total biogas output.

4.3.2 Effect of mono-digestion of substrates on biogas yield

As shown in Figure 0-4 above, the cow manure and maize stalk underwent mono-digestion, resulting in the production of 96.182 ml and 50.22 ml, respectively. As illustrated in Figure 0-4, the decrease in average daily biogas production due to mono-digestion from maize stalks is likely due to the Accelerated utilization and exhaustion of biodegradable organic material and the accumulation of toxic compounds due to increased microbial populations, hindering or inhibiting biogas production. fermentation process (Zamanzadeh *et al.*, 2016). However, compared to maize stalk, cow manure contains more biodegradable organic material, which serves as an energy source for microorganisms, leading to a higher average daily biogas yield (Zamanzadeh *et a*l., 2016).

4.3.3 Effect of Dilution on the Production of Biogas

This study took into account the dilution effect, which was evaluated using a ratio as shown in table 4-2. Dilution has been shown to accelerate biogas production. Water will lower some elements' concentrations such as nitrogen and sulfur, leading to the formation of byproducts that interfere with anaerobic digestion products, like hydrogen sulfide and ammonia.

According to Nava-Valente *et al.* (2023) anaerobic digestion without or with reduced water concentrations will result in decreased biogas production. as witnessed from runs 2,5,8,12, and 20. Poggio *et al.,* (2016); Sageman *et al.,* (2003) also made similar observations, the reactor was operated with several water dilutions, ranging from 8 to 20 liters. The 20-liter dilution yielded the highest biogas production, ranging from 0.9 to 3.15 liters per day. In contrast, the 8-liter dilution resulted in the lowest biogas production, ranging from 0.65 to 1.36 liters per day. The findings indicated that higher dilution led to greater material degradation, hence enhancing the production of biogas and methane. yields (Yuan *et al.*, 2014.). The findings demonstrated that higher dilution levels resulted in greater material degradation, hence enhancing the production of biogas and methane (Yuan *et al.,* 2014).

4.3.4 Results for Reduced Cubic Model

Error! Reference source not found. provides the ANOVA results for a comprehensive reduced cubic model obtained by regression analysis using Design expert software. The linear terms A, B, C and the quadratic terms B^2 and C^2 for all components had a substantial impact on the biogas yield due to their p-values being less than 0.05 ($P<0.05$). The biogas yield was strongly affected by the interplay of substrates B, C, and dilution A. The quadratic model for biogas generation was derived using multiple regression analysis and is represented by the equation ($\boldsymbol{Y}=+\mathbf{115.41}+\mathbf{13.43}\boldsymbol{A}+\mathbf{4.12}\boldsymbol{B}-\mathbf{10.45}\boldsymbol{C}+\mathbf{21.65}\boldsymbol{AB}+\mathbf{3.44}\boldsymbol{AC}+\mathbf{1.11}\boldsymbol{BC}-\mathbf{22.39}\boldsymbol{B}^{\mathbf{2}}-\mathbf{7.09}\boldsymbol{C}^{\mathbf{2}}-\mathbf{17.69}\boldsymbol{ABC}$). Final Equation in Terms of Coded Factors

$$\boldsymbol{Y}=+\mathbf{115.41}+\mathbf{13.43}\boldsymbol{A}+\mathbf{4.12}\boldsymbol{B}-\mathbf{10.45}\boldsymbol{C}+\mathbf{21.65}\boldsymbol{AB}+\mathbf{3.44}\boldsymbol{AC}+\mathbf{1.11}\boldsymbol{BC}-\mathbf{22.39}\boldsymbol{B}^{\mathbf{2}}-\mathbf{7.09}\boldsymbol{C}^{\mathbf{2}}-\mathbf{17.69}\boldsymbol{ABC} \qquad \mathbf{0\text{-}3}$$

Where,

Y- Biogas yield (Response1)

A- Water

B- Cow dung

C- Maize stalk

The regression equation is formulated using coded components to produce a precise mathematical link between the response of anaerobic digestion (biogas yield) and the substrate variables given in equation 4.3.

Table 0-6: **ANOVA Results**

Source	Sum of Squares	df	Mean Square	F-value	P-value	Significance
Model	16045.72	9	1782.86	284.39	< 0.0001	significant
A	1443.14	1	1443.14	230.2	< 0.0001	
B	232.16	1	232.16	37.03	0.0005	
C	991.11	1	991.11	158.1	< 0.0001	
AB	3748.93	1	3748.93	598.01	< 0.0001	
AC	94.73	1	94.73	15.11	0.006	
BC	9.86	1	9.86	1.57	0.25	
B^2	6439.87	1	6439.87	1027.25	< 0.0001	
C^2	393.75	1	393.75	62.81	< 0.0001	
ABC	2502.48	1	2502.48	399.18	< 0.0001	
Residual	43.88	7	6.27			

Lack of Fit	25.89	2	12.95	3.6	0.1076	not significant
Pure Error	17.99	5	3.6			
Cor Total	16089.6	16				

The Model F-value of 284.39 presented in Table 4 4 suggests that the model demonstrates statistical significance. The likelihood of an F-value of this magnitude arising purely from random variation is merely 0.01%. P-values that fall below 0.0500 indicate that the terms within the model possess statistical significance. The principal model terms pertinent to this scenario include A, B, C, AB, AC, B^2, C^2, and ABC. Values surpassing 0.1000 indicate that the model terms lack statistical significance. Should your model contain a plethora of extraneous terms, aside from those essential for establishing hierarchy, the process of model reduction may significantly improve its efficacy. The F-value of 3.60 for the Lack of Fit suggests that it does not hold statistical significance when contrasted with the pure error. The likelihood of encountering a Lack of Fit F-value of this magnitude arising solely from random variation is 10.76%. An insignificant lack of fit is preferable, as it indicates that the model effectively captures the underlying process in question.

The quadratic equation can be utilized to derive an accurate estimation for biogas production due to the elevated value of R^2. Chanathaworn concluded that the adjusted R^2 value of 0.9938 demonstrated the suitability of the response surface model in accurately predicting outcomes in the biogas investigation. The R^2 value of 0.8841 indicated a high level of agreement between the anticipated and observed values. The CV of 2.67 indicates a high level of reliability and precision in the experimental outcomes. The reliability of experimental data diminishes as the coefficient of variation (CV) increases. The experimental biogas production results closely matched the projected outcomes, as depicted in Figure 0-5 below. All the data points lie precisely on or in close proximity to the line of fitness. This provides more evidence for the strong R2 value achieved by this model.

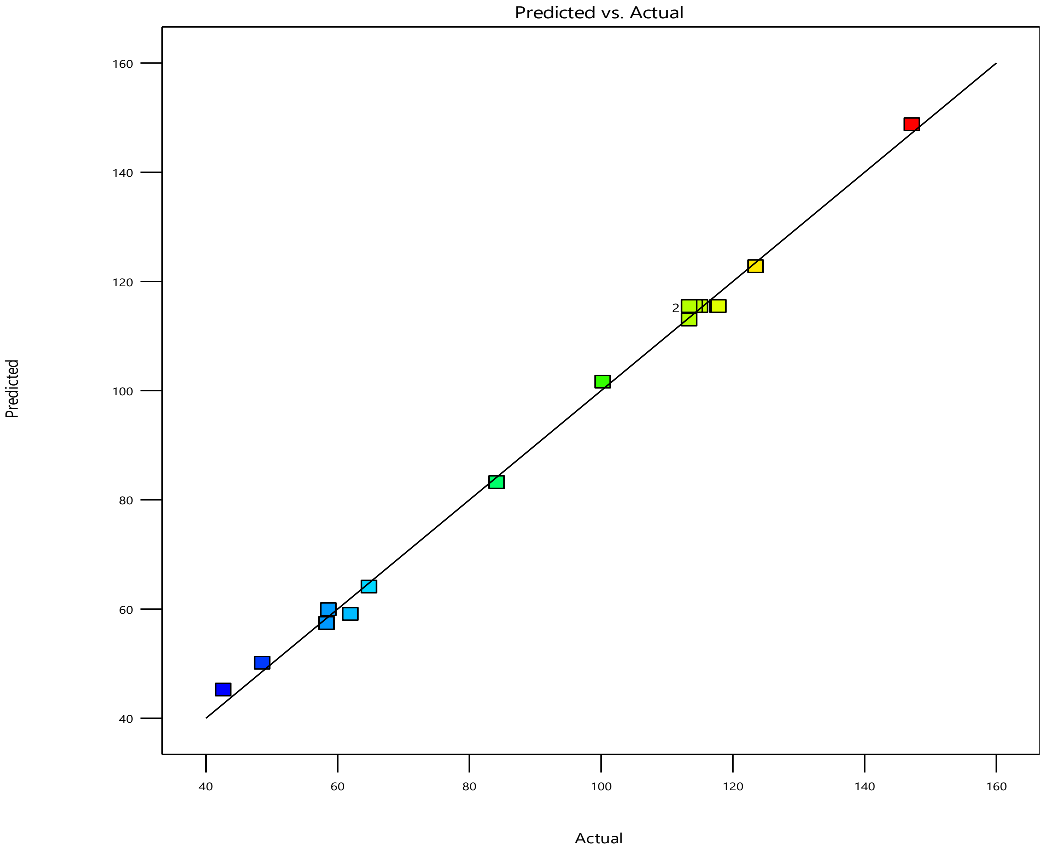


Figure 0-5: Plot of predicted response vs. actual value from response surface

4.3.5 Interactions between Maize Stalks, Cow Dung, and Water

In Figure 0-6,the red line represents the relationship between biogas yield and water content, while keeping the amount of maize stalk constant at a low level of 50g. Conversely, the black line illustrates the relationship between biogas production and water quantity, while keeping the quantity of maize stalks at a high level. There is a noticeable disparity in the quantity of maize stalks between the low (50mL) and high (125 mL) water levels. This is due to the fact that the LSD bars do not intersect in either scenario.

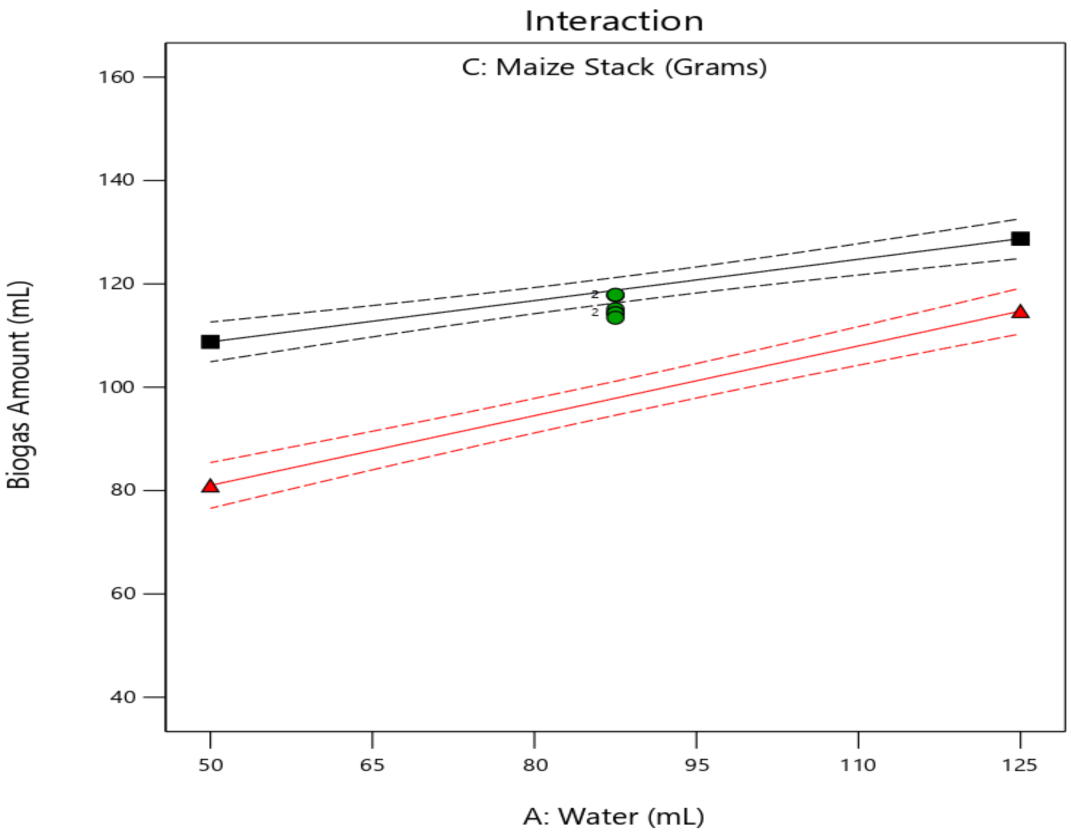


Figure 0-6: Plot of interaction between water and maize stalk while keeping cow dung at mid-level

Figure 0-7 shows the interaction graph between the amounts of cow manure (factor B) and water (factor A), with the amount of maize stalk (factor C) kept at mid-levels. When the water level is reduced, a noticeable difference between the two levels of cow dung is seen. The difference between the two levels of cow dung is also notable when the water level is set high. From Figure 0-6 and Figure 0-7,the results proved that the increased dilution leads to increased material degradation, consequently improving biogas and methane yield

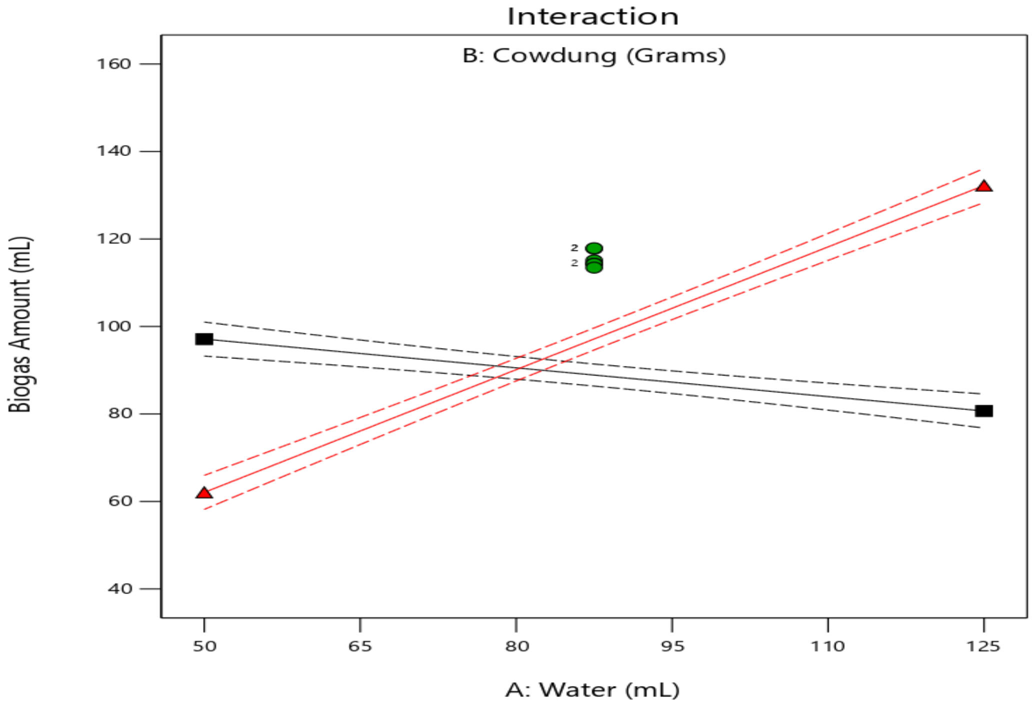


Figure 0-7: **The interaction graph between the amounts of cow manure (factor B) and water (factor A), with the amount of maize stalk (factor C) kept at mid-levels**

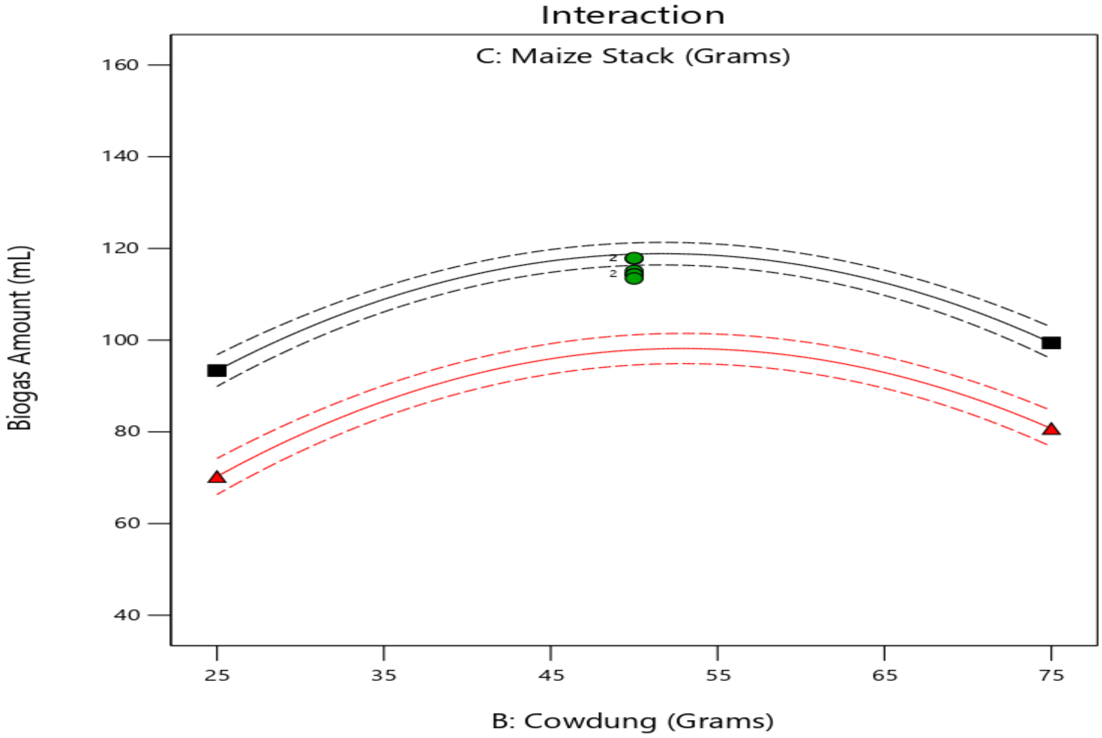


Figure 0-8: Plot of interaction between water and maize stalk while keeping cow dung at mid-level

Maintaining water at mid-levels ensures proper moisture content for microbial growth and metabolic processes. The interaction of maize stalks, cow dung, and water in the biogas digester is highly interconnected. Maize stalks provide the primary substrate for microbial activity by releasing sugars through hydrolysis. Cow dung adds a diverse microbial population that enhances the breakdown of complex organic compounds into methane gas. The biogas digester's methane production efficiency is enhanced by the combined action of maize stalks, cow manure, and water. For the microorganisms engaged in anaerobic digestion, the combination of these substrates offers a balanced nutritional profile. Maximum microbial activity is supported by appropriate water levels, which increases biogas output.

4.4 Biogas composition

Gas chromatography was utilized for the analysis of the composition of the biogas. Specimens were gathered from the most favorable analysis, designated as number 1, while standard gases were generated within the laboratory. Figure 0-9 displays the chromatogram of a 2 µl sample of standard gas. The chromatogram exhibits a prominent peak corresponding to methane gas. The methane gas has a maximum retention time of 1,546 minutes when the column temperature is set at 150°C, the inlet chamber temperature is set at 200°C, and the detector chamber temperature is set at 250°C.

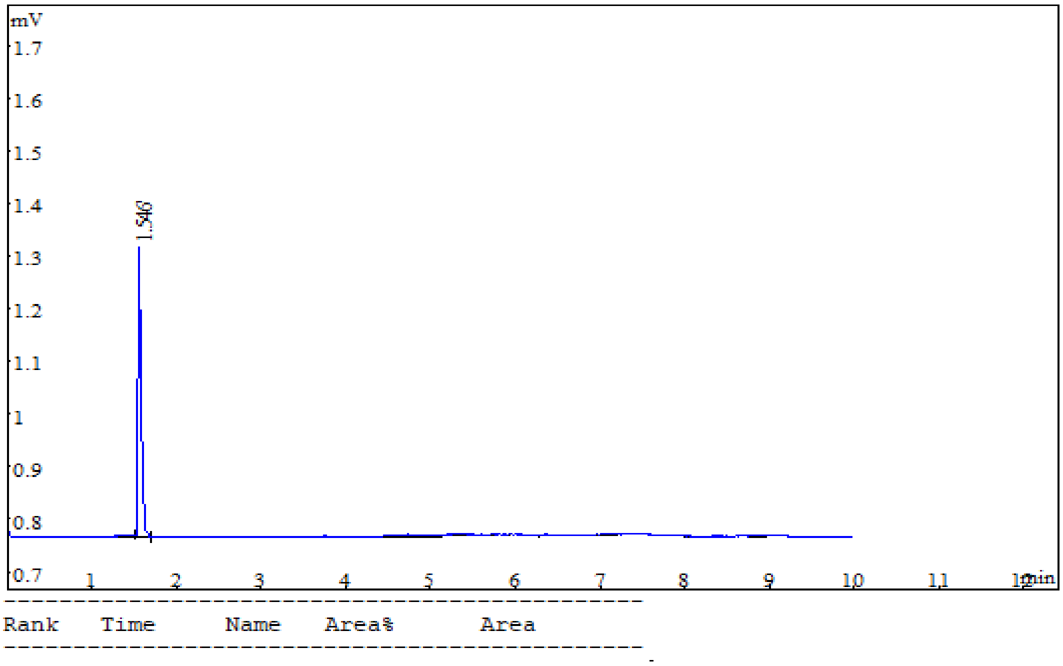


Figure 0-9: Standard gas Chromatogram

The GC was utilized to ascertain the methane content in the samples by introducing an equal volume of reference gas. Gas chromatography was utilized to analyze three samples, and the outcomes are displayed in Figure 0-10,Figure 0-11, and Figure 0-12 below.

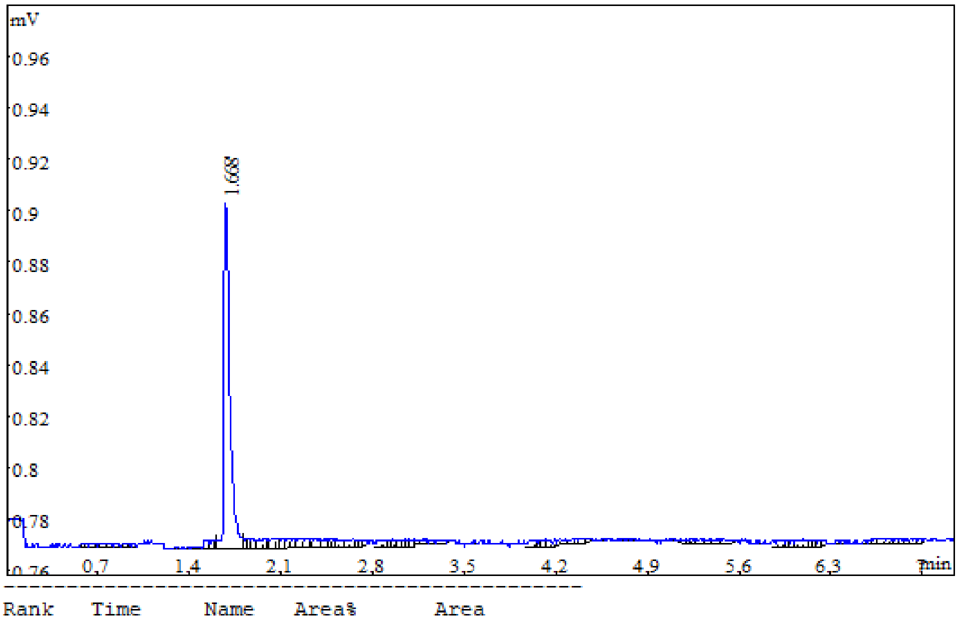
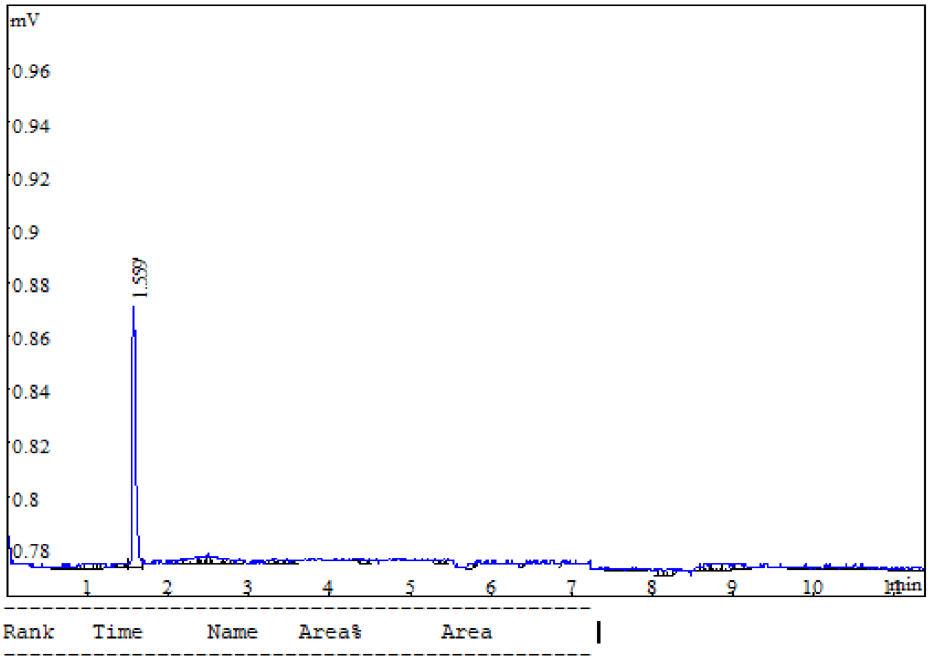


Figure 0-10:sample gas 1



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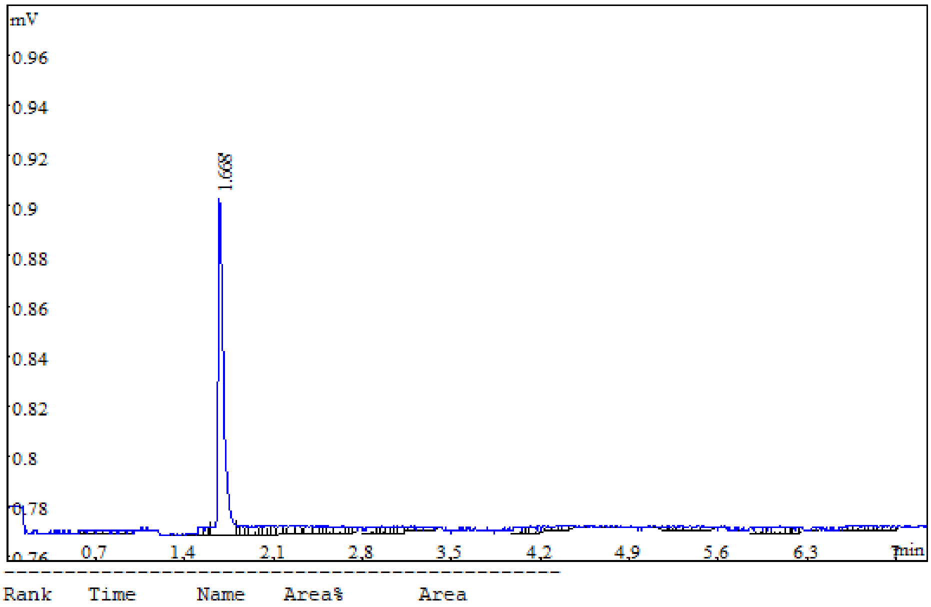
Rank    Time      Name    Area%       Area
-------------------------------------------------
```

Figure 0-11: Sample gas 2



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-------------------------------------------------
Rank    Time      Name    Area%       Area
```

Figure 0-12: Sample gas 3

Table 0-7:Peak area for the standard and sample gases

Gas Samples	Peak Area
Standard	1262
Sample 1	794
Sample 2	672
Sample 3	879

Table 0-8:Percentage of Methane in the sample gas

Gas Sample	Peak Area	% $CH_4 = \frac{peak\ Area}{1262}\ x\ 100\%$
1	794	62.92
2	672	53.25
3	879	69.65
Average		**61.94**

To figure out how stable the AD system is, it's important to look at how much methane CH4 is in the biogas produced. The composition of the substrate combination significantly impacted the production of biogas, with the highest methane yield (61.94%) seen when CW and MS were co-digested. The results are pretty close to (Bote *et al.*, 2020; Katima, 2001).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Effect of Chemicals as a Pre-Treating Medium on Biogas Yield

The use of chemical pre-treatment on maize stalk residues enhances biogas yield by breaking down the complex lignocellulosic structure, increasing the digestibility of the substrate. Studies show that chemical pre-treatment significantly improves the rate of biogas production by facilitating the release of more fermentable sugars. This leads to increased methane content in the biogas and accelerates the digestion process. The CW and MS co-digestion method has a higher hydrolysis rate than the single-material digestion method. It was found that MS improved biogas production from co-digestion of CW and MS, while CW and inoculum were excellent substrates for bacterial incubation used to promote biogas synthesis from the co-digestion of organic materials, (CW and MS).

5.1.2 Impact of Maize Residue Pre-Treatment Duration

The duration of pre-treatment plays a critical role in maximizing biogas yield. An optimal pre-treatment time allows sufficient degradation of lignocellulose in maize stalks, enhancing microbial activity during digestion. However, over-treatment can result in the loss of volatile solids or inhibitory by-products, potentially reducing biogas production. Experimental results suggest that a moderate pre-treatment duration is ideal for improving biogas yield, as it provides enough time for structural breakdown without overexposure. Bio digesters have a retention time of six to fifteen days. Under different sodium carbonate pretreatment concentrations, the maize stalk pretreated by 7% Na_2CO_3 concentration, for 4 days achieved the highest TS value of 15.15% which was 7.26% more than the untreated one.

Maize stalk pretreatment using sodium carbonate showed great potential for improving the efficiency of biomass conversion process. Alkaline pretreatment hydrolyzes most organic matter and facilitates anaerobic digestion. Comparing the co-digestion of corn residue and inoculants with and without pre-treatment, it was discovered that the co-digestion of cow dung and pre-treated maize stalk produced more gas at a minimum level as compared with the mono-digestion of cow dung by 2.12 times. Therefore, sodium carbonate pretreatment was found to be an efficient approach for enhancing the efficiency of maize stalk biodegradation and boosting biogas production.

5.1.3 Optimal Ratio of Cow Dung to Maize Stalk Residue for Biogas Production

The co-digestion ratio of cow dung to maize stalk residue affects biogas yield and digestion efficiency. Based on research findings, an optimal ratio of cow dung to maize stalk residue tends to result in the highest biogas production. Cow dung provides essential microbes and nutrients that enhance the anaerobic digestion process, while maize stalk residue acts as a carbon source. An excess of maize stalk residue without sufficient cow dung may lead to process inhibition due to high C ratio, while too much cow dung could reduce the energy potential of the feedstock.

The potential for co-digestion of different substrates in biogas production through anaerobic digestion might assist advance the utilization of cleaner fuel. This would eradicate environmental contamination coming from the burning of maize stalk. Utilization of cow dung substrate as a co-substrate, to maize stalk has been proven to be an effective method for generating biogas through AD. The produced biogas contains 61.94% methane by volume. The study found that the optimum substrate ratio for cow dung and the optimal substrate ratios for biogas production from maize stalks through co-digestion of cow dung, pre-treated maize stalks, and dilution were 1:1:3, respectively. These ratios, along with the dilution, created favorable conditions for bacterial growth.

5.1.4 Impact of Co-Digestion on the Quantity of Biogas Produced

Co-digestion of cow dung with maize stalk residues results in a synergistic effect, producing more biogas than using either feedstock alone. The combination provides a balanced C ratio, improving microbial growth and digestion efficiency. The synergy enhances the biodegradability of the materials, leading to a more stable digestion process and increased methane production. Compared to mono-digestion, co-digestion improves both the quantity and quality of biogas by leveraging the complementary characteristics of cow dung and maize stalk residues.

The daily average biogas yield produced by the substrate ratio of 1:1:3 (CD:MS: W) respectively, was 203.64mL while that of mono-digestion of cow dung yielded 96.18 mL, indicating that co-digestion produced 2.12 times greater than that of cow dung mono-digestion. The substrates used in this study could facilitate large-scale biogas generation efficiently and sustainably. Co–digestion bioreactors operating with low cow dung concentration vis a vis maize stalks, yielded low biogas compared with the ones with balanced substrate ratios or cow dung being slightly higher. It was deduced that co-digestion improves the efficiency of the digester, resulting in a higher biogas yield. The findings demonstrated that higher dilution levels result in enhanced material breakdown, thereby enhancing biogas and methane production. Therefore, Co-digestion of cow dung and maize stalk residues, when optimized in terms of pretreatment, mixing ratio, and pre-treatment duration, results in a significant enhancement of biogas production.

5.2 Recommendations

5.2.1 Recommendations from Findings

From the study findings, the following recommendations were made

- Trials with different chemical pre-treatments and evaluating the performance of various chemical treatments under different conditions to determine the most cost-effective method that maximizes biogas yield while maintaining sustainability should always be done.
- Regularly measure biogas production during experiments to ensure that pre-treatment time is aligned with optimal gas output.
- Regularly monitor the digestion process: Keep track of pH, temperature, and C ratio to avoid inhibition or inefficiency in the digestion process when adjusting the ratios.
- Promote the use of co-digestion, as it significantly enhances biogas production compared to mono-digestion. The combined use of cow dung and maize stalk residues provides a more balanced feedstock for anaerobic digestion.

5.2.1 Recommendations for further Research

- Additional Research is needed to improve the pretreatment conditions and expand the technique for industrial use.
- Important factors like mixing are not controlled during the different stages of methanogenesis due to the lack of continuous agitation throughout the digestive

process. The study suggests regulating specific parameters to enhance methane generation. Modify the agitation to increase the biogas production.

- Biogas has not been valorized, research is still needed to purify or valorize it to remove CO_2 and improve methane content for direct use for culinary purposes or as a source of energy for vehicles.

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