

JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

FACULTY OF MECHANICAL ENGINEERING

SUSTAINABLE ENERGY ENGINEERING

STUDY ON THE OPTIMIZATION OF BIOGAS PRODUCTION FROM COW DUNG AND WHEAT STRAW WASTES VIA CO-DIGESTION PROCESS

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, JIMMA UNIVERSITY, JIMMA INSTITUTE OF TECHNOLOGY, FACULTY OF MECHANICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN SUSTAINABLE ENERGY ENGINEERING

BY

ENDALE WOLDE

ADVISOR: HENOK MEKONNIN (PHD CANDIDATE)

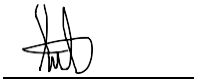
CO-ADVISOR: SAMUEL ADDISU (MSC)

JANUARY, 2026

JIMMA, ETHIOPIA

DECLARATION

I hereby declare that my thesis work with the title "Study on the Optimization of Biogas Production from Cow Dung and Wheat Straw Wastes through Co-digested Process" is my original work and has not been submitted to any other university for any degree.

Endale Wolde  Feb 19, 2026

Name Signature Date

As research supervisor, I hereby confirm that I have read this thesis submitted under my supervision entitled ―Study on the optimization of biogas production from cow dung and wheat straw wastes via co-digestion process‖ by Endale Wolde. I therefore accept this to satisfy the requirement for the Master of Science in Sustainable Energy Engineering.

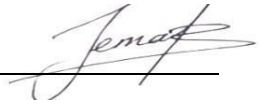
Advisor:

Name Henok Mekonnen Mossissa Signature  Date 02/20/2026

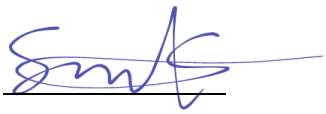
Co-Advisor:

Name Samuel Addisu Signature  Date Feb 23, 2026

External Examiner:

Name Jemal Beshir (PhD) Signature  Date February 16, 2026

Internal Examiner

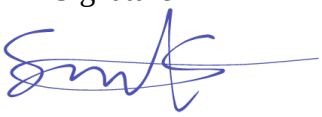
Name Solomon Kebede(PhD) Signature:  Date Feb 19, 2026

APPROVAL SHEET

I affirm that I have read Endale Wolde's thesis titled ―Study on the optimization of biogas production from cow dung and wheat straw wastes via co-digestion process‖, submitted for supervision. The thesis is now ready for evaluation.

HENOK MEKONNIN (PHD CANDIDATE)		02/20/2026
Main Advisor	Signature	Date

We, the undersigned members of the Board of Examiners for the thesis of Endale Wolde, having reviewed the thesis with the title "Study on the optimization of Biogas Production from Cow Dung and Wheat Straw Wastes via Co-Digestion Process" evaluated the work, in addition to conducting the Open Defense for the candidate, do now certify that the thesis is accepted in partial fulfillment of the requirement for the degree of Master of Science in Sustainable Energy Engineering.

Name of Chairperson	Signature	Date
Name of External Examiner Jemal Beshir (PhD)	Signature 	Date February 16, 2026
Name of Internal Examiner Solomon kebede (PhD)	Signature 	Date Feb 19, 2026

ACKNOWLEDGEMENT

Let me take this opportunity to thank all those who stood by my side and supported me along the way to completing my thesis. Above all, I would like to thank my supervisors-most especially Henok Mekonnin, a PhD researcher, who was there for my thesis all out throughout its final leg. I will also be deeply indebted to my co-supervisor, Mr. Samuel Addisu, MSc, who has been outstanding all the time.

DEDICATION

I, in reality, dedicate this work to the parents that have inspired me to complete the work of the thesis.

LIST OF ABBREVIATIONS AND ACRONYMS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
APHA	American Public Health Association
BVS	Biodegradable Volatile Solids
CD	Cow Dung
C/N	Carbon-to-Nitrogen Ratio
CMY	Cumulative Methane Yield
DBY	Daily Biogas Yield
FS	Fixed Solids
GHG	Greenhouse Gas
HRT	Hydraulic Retention Time
LDPE	Low-Density Polyethylene
MC	Moisture Content
OLR	Organic Loading Rate
RSM	Response Surface Methodology
RVS	Refractory Volatile Solids
TS	Total Solids
VFAs	Volatile Fatty Acids
VS	Volatile Solids
WS	Wheat Straw

TABLE OF CONTENTS

DECLARATION i

APPROVAL SHEET ii

ACKNOWLEDGEMENT iii

DEDICATION iv

LIST OF ABBREVIATIONS AND ACRONYMS v

TABLE OF CONTENTS vi

LIST OF FIGURES ix

LIST OF TABLES x

ABSTRACT xi

CHAPTER ONE 1

1. INTRODUCTION 1

1.1. Background of the study 1

1.2. Statement of the Problem 2

1.3. Objectives 3

1.3.1. General objective 3

1.3.2. Specific objectives 3

1.4. Research questions 3

1.5. Significance of study 3

1.6. Scope of the study 4

CHAPTER TWO 5

2. LITERATURE REVIEW 6

2.1. Historical background of biogas production 6

2.2. Advantages of biogas 7

2.3. Anaerobic digestion and its processes 8

2.4. Factors affecting anaerobic digestion 10

2.5. Advantages of anaerobic digestion 13
2.6. Related studies 14
2.7. Summary of related studies 16
CHAPTER THREE 17
3. MATERIALS AND METHODOLOGY 17
3.1. Description of the study area 17
3.2. Raw materials and sample preparation 17
3.3. Inoculum preparation 18
3.4. Experimental procedures 19
3.5. Process flow diagram 20
3.6. Experimental approach 21
3.6.1. Determination of the physicochemical properties of the feedstocks 21
3.7. Experimental Setup 24
3.7.1. Determination of biogas composition 25
3.8. Design of Experiment 25
CHAPTER FOUR 28
4. RESULT AND DISCUSSION 28
4.1. Characterization of the biogas feed stocks 28
4.2. Biogas yield and composition of methane gas 32
4.3. ANOVA for quadratic model of biogas yield 34
4.3.1. Model adequacy checking for volume of biogas response 36
4.3.2. Temperature and pH interaction effects on biogas yield at constant substrate mix ratio 3 38
4.3.3. pH and substrate mix ratio interaction effects on biogas yield at constant temperature 37.5°C 39
4.3.4. Temperature and substrate mix ratio interaction effects on biogas yield at constant pH 7 40
4.3.5. Effect of all combined factors for volume of biogas 41

4.4. ANOVA for quadratic model of composition of methane gas 42
4.4.1. Model adequacy checking for composition of methane 45
4.4.2. Temperature and pH interaction effects on methane gas at constant substrate mix ratio 3 46
4.4.3. pH and substrate mix ratio interaction effects on methane gas at constant temperature 37.5°C 47
4.4.4. Temperature and substrate mix ratio interaction effects on methane gas at constant pH 7 49
4.4.5. Comparison of the factor effect at a point for composition of methane gas 50
4.5. Optimization 51
4.5.1. Exploring the optimum mix for maximum production of biogas 51
4.5.2. Model desirability and optimization conditions 51
4.5.3. Model validity 53
CHAPTER FIVE 54
5. CONCLUSION AND RECOMMENDATION 54
5.1. Conclusion 54
5.2. Recommendation 54
REFERENCES 55

LIST OF FIGURES

Figure 2.1: metabolic pathways of anaerobic digestion....10
Figure 3.1: wheat straw and its powdered form....18
Figure 3.2: fresh cow dung and the powder that corresponds with it....18
Figure 3.3: Illustrates the inoculum used in the study....19
Figure 3.4: Processing flow diagram....20
Figure 3.5: Bench-scale experimental setup....25
Figure 4.1: Residuals vs. run....37
Figure 4.2: Predicted vs Actual....37
Figure 4.3: Temperature and pH interaction effects on biogas yield at a constant substrate mix ratio of 3 38
Figure 4.4: Interaction effects of pH and substrate mix ratio on biogas yield at a constant temperature 37.5ºc....40
Figure 4.5: Temperature and substrate mix ratio interaction effects on biogas yield at constant pH=7....41
Figure 4.6: Perturbation plots that compare each factor‘s effect at a particular point in the design space volume of biogas....42
Figure 4.7: Residuals vs. run....45
Figure 4.8: Predicted vs. actual....46
Fig 4.9: Temperature and pH interaction effects on composition of methane gas at constant substrate mix ratio 3....47
Figure 4.10: pH and substrate mix ratio interaction effects on composition of methane gas at a constant temperature 37.5ºc....48
Figure 4.11: Temperature and substrate mix ratio interaction effects on composition of methane gas at a constant pH=7....50
Figure 4.12: Perturbation plots that compare each factor‘s effect at a particular point in the design space composition of methane gas....51
Figure 4.13: Optimization conditions and desirability for volume of biogas and composition of methane gas....52

LIST OF TABLES

Table 3.1: Volume and mixing ratios of substrates in each treatment 26
Table 3.2: Levels of the factors 26
Table 3.3: Experimental design 27
Table 4.1: Characteristics of wheat straw and cow dung 28
Table 4.2: Characteristics of wheat straw and cow dung 29
Table 4.3: Comparison of pH between prior and following AD of mixing substrates 31
Table 4.4: C:N ratio values for the three types of substrates used to produce biogas 32
Table 4.5: Selected runs based on yield per unit volume of sample applied and percentage of methane 33
Table 4.6: Volume of biogas 34
Table 4.7: Fit statistics for the volume of biogas 35
Table 4.8: Composition of methane gas 42
Table 4.9: Fit statistics for composition of methane gas 43
Table 4.10: Conditions for optimization and desirability of solutions 52
Table 4.11: Model validity 53

ABSTRACT

Biogas is renewable energy carrier produced by anaerobic digestion of organic substrates and is considered a Sustainable alternative to fossil based fuels. This study focused on optimizing the production of biogas by co-digesting cow dung and wheat straw wastes. In this study, production of biogas from wheat straw (WS) and cow dung (CD) via co-digestion process was evaluated under mesophilic conditions using a batch digester for 35 days; which had high methane gas under optimum temperature, pH, and substrate mix ratio. In all treatments, physicochemical parameters such as total solid, volatile solid, moisture content, carbon to nitrogen ratio, and pH were measured before and after digestion. The experiment for biogas production by wheat straw and cow dung wastes was conducted using response surface methodology. Three numeric factors and three- levels Box-Behnken design expert was employed to study the effect of wheat straw to cow dung mix ratio, pH, and temperature for produced biogas yield. A total of 17 runs with replicates were performed. Gas production was noticed in all of the digesters starting from day 4^{th} of anaerobic digestion. The daily biogas production was subsequently measured by gas syringe for 35 days and the methane gas composition was also analyzed by a gas analyzer. Cumulative biogas production revealed that substrate in a mix ratio of 75% WS +25% CD at pH of 7.0, and at temperature of 37.5℃ showed the highest cumulative volume of biogas production (198mL), and composition of methane gas (62.5%) as an optimal condition. Further researches have to be done to improve the production of high quality and quantity of production of biogas.

Key Words: *Biogas, Co-digestion, Cow- Dung, Wheat Straw, Optimization*

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

For the past century or so, fossil fuels such as coal, oil, and gas have remained the largest source of energy consumption in the world. Currently, these sources still make up the largest share of the entire energy consumption base. According to the current reports released by the International Energy Agency, fossil fuels still contribute to nearly 80% of the entire energy consumption in the world (Al-Da'asen et al., 2024). With the increasing temperature in the world, there are increased concerns about the greenhouse gases in the atmosphere. Measures are therefore being put in place to counter this issue. To meet the 2050 goal to be carbon neutral in the energy sector, a significant share of renewable energy resources in the energy sector is a must since fossil fuels still emit a significant amount of greenhouse gases into the atmosphere (Silva et al., 2024).

Biogases are classified under renewable fuels, and the processes for the generation of biogases include the breakdown of organic materials in an anaerobic regime. The various sources of the generation of biogases include the following: animal feces, agricultural wastes, green wastes, sewage sludge, and/or wastes of food materials up to municipal wastes. In consideration of its composition, the concentration of the major component, carbon dioxide gas CO_2, differs from the concentration of the minor component, methane gas CH_4. All these are subject to the substrates, processes, and/or functions of the bacteria. Biogases consist of methane gas at a ratio of 50-75% and 25-50% of carbon dioxide gas, respectively; the traces include gas contents of the following: Nitrogen gas, Oxygen gas, Carbon Monoxide gas, and Hydrogen sulfide gas (Nagaprakash et al., 2003). Biogas is a renewable bio-energy source and can be produced by the process of anaerobic digestion (A.D.), which mainly uses agricultural wastes such as cow dung and lignocellulose agricultural wastes of various crop plants. The composition of agricultural wastes, especially the complexity of the materials such as wheat straw, retards the rate-limiting step of the anaerobic digestion process (Silva et al., 2024; Croce et al., 2016). Anaerobic Digestion is a biological process under which organic matter is converted into renewable gas—biogas—and a nutrient-rich residue/digestate through a variety of microbial populations (Sims et al., 2010; Mustapha,

2021). One such crop residue is wheat straw (WS), which is one of the crop residues existing in abundant quantities around the globe and primarily considers wheat straw as a cheap and renewable crop residue for future use as a sustainable Bioresource feedstock for biofuels production processes notwithstanding its abundant availability (Singh et al., 2014).

The complex and diverse nature of lignocellulosic material, which is dominated by high molecular weight polymers such as cellulose, hemicellulose, and lignin, is a hindrance for enzymes and microorganisms. The resilience shown by wheat straw due to its tough nature resists its decomposition during anaerobic digestion processes. However, to overcome such hurdles and increase biogas production, wheat straw can be processed using various pretreatment technologies such as biological pretreatments that increase accessibility for microbial decomposition. Various studies reveal that the addition of other organic substances like wheat straw, poultry manure, pork manure, sunflower meal, sewage sludge, or cow dung to the biomass can increase the digestion rate. Biogas generation from lignocellulosic biomass requires effective microbial digestion (Al-Da‘asen et al., 2024).

1.2. Statement of the Problem

In Africa, environmental pollution and unavailability of modern sources of energy are considered major barriers to human health, sustainability, and development, and despite remarkable developments being achieved in terms of electrification, still, a major proportion of the population as nearly 600 million people are without access to electricity on the African continent, particularly in rural and poor countries (International Energy Agency [IEA], 2022).

The ever increasing reliance on fossil fuels for energy generation has caused series environmental issues, including greenhouse gas emission, global warming, and the depletion of non- renewable resources. Simultaneously the sharp rise in global energy consumption, fueled by industrialization, urbanization, and population growth has heightened worries about energy security and long term viability. These issues have hastened the quest for greener, renewable and more efficient sources of energy that would limit environmental degradation without compromising a stable power supply. It is for this reason that renewable energy source like biogas have gained broad recognition as feasible alternative with which sustainable development could be supported without adversely affecting conventional power generation based on fossil fuels. Hence, the research on biogas production from the co-digestion of cow dung and wheat straw waste will address the aforementioned issue.

1.3. Objectives

1.3.1. General objective

Study on the optimization of biogas production from cow dung and wheat straw wastes via co-digestion process

1.3.2. Specific objectives

1. To characterize wheat straw and cow dung in terms of their physiochemical properties including moisture content, total solids, volatile solids, carbon to nitrogen ratio, and pH before anaerobic digestion
2. To evaluate the optimum mixing ratio of cow dung and wheat straw wastes to produce high yield of biogas under anaerobic condition
3. To investigate the influence of cow dung and wheat straw wastes to produce biogas under anaerobic condition with a emphasize on methane content to assess gas quality.

1.4. Research questions

1. What are optimal operating factors such as temperature, pH, and the ratio of substrate mixture that give a maximum biogas production rate?
2. How does retention time and temperature affect the co-digestion process?
3. What is the importance of the substrate mix ratio in the enhancement of process stability and buffering capacity during the anaerobic decomposition of the substrate in the form of wheat straw and cow dung?
4. What is the effect of the operating conditions on the removal of the total solids and volatile solids?
5. To what extent does co-digestion enhance biogas and methane content compared with mono-digestion of individual substrates?

1.5. Significance of study

The current research work on the optimization of biogas generation from cow dung waste and wheat straw waste by co-digestion has utmost scientific, environment-related, and socio-economic importance, which plays a significant role in developing agricultural residue management sustainably and harnessing renewable energy. In scientific terms, this work contributes to enhancing the existing body of knowledge that addresses anaerobic co-digestion issues, with a focus on the synergistic effects that occur when cow dung, a high-nitrogen-content

feedstock, is co-digested with wheat straw, a high-carbon lignocellulosic waste. The research is particularly significant, shedding a lot of light on how anaerobic co-digestion might be optimized, particularly with respect to the mixing ratios of different feedstocks, organic loading rate, carbon-to-nitrogen ratios, Hydraulic retention time, and methods of maximizing biogas production. This research helps in a significant way to overcome problems that are commonly experienced during anaerobic digestion practices, such as feedstock toxicity, digestibility, and instability.

Environmentally, the research supports the use of agricultural and livestock waste in a sustainable manner, which is mostly mismanaged via open dumping or agricultural burnings. The practice of burning wheat straw, for example, has been identified as a significant source of air pollution in most agricultural areas. The coproduction technique used in the research provides a safe means that uses waste to produce clean energy with the additional advantage of lowering Methane production from unmanaged waste, eliminating unpleasant odors, and hindering soil and groundwater pollution. In addition, the produced digestate can serve as a rich source of an organic fertilizer that improves soil nutrient potential, eliminating the need for chemical fertilizer.

The importance of this research in terms of socioeconomic relevance is that it has the potential to facilitate rural energy security and rural development. Biogas produced from optimized cow dung and wheat straw sources available in rural areas can prove to be an economical renewable energy alternative for meeting the energy requirements of cooking, space heating, and electricity for rural as well as agricultural sectors. It will minimize dependence on fossils fuels as well as traditional biomass, resulting in a cut in domestic energy bills. Biogas can also generate employment in the construction, operation, and maintenance of biogas plants, as well as serve as an auxiliary source of income for farmers.

1.6. Scope of the study

The study focuses on the enhancement of biogas production by co-digestion of cow dung with wheat straw pretreated biologically. It has discussed some major issues concerning the search for effective biological methods of enhancing biodegradability of cow dung and wheat straw and optimization of the co-digestion process with regards to substrate mix ratios, temperature, and pH for maximum biogas production. Although the prospect of the techno-economic benefits does exist, the study will not carry out any cost-saving assessment; therefore, any mention of cost

savings will only be conceptual. A discussion will only take place regarding potential gas emissions. This ensures that the aim of the study is in line with the work that is being experimented.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Historical background of biogas production

The production of flammable gas from decomposing organic matter has been recognized for centuries, with early observations traced back to ancient Persian civilizations. The practical application of this phenomenon began in the mid-19th century, when the first sewage treatment facility utilizing biogas technology was constructed in Bombay in 1859. This concept was later adopted in the United Kingdom in 1895, where biogas was successfully used for street lighting. Further technological advancement occurred in the United States, where the first anaerobic digesters treating municipal solid waste (MSW) were developed and operated between 1939 and 1974. Today, municipal solid waste (MSW) is increasingly used for biogas production, and different types of systems have been developed to supplement other solid waste disposal methods like landfilling and incineration (Kabeyi & Olanrewaju, 2022).

At the end of 2019, the global amount of biogas plant capacity was about 19.5GW, with growth in capacity being fueled by, among others, high fossil fuel prices, cheap and easy access to biomass feedstock, and concerns over emissions and global warming. The most common feedstock used to produce biogas include wastes, like domestic wastes, i.e., food, vegetables, fruits, and animal wastes like dung, poultry dropping, or public moist wastes from food cafes and restaurants, markets, and biological waste from industries having high moisture content and high degradability. Biogas is produced by the anaerobic action of a class of bacteria under suitable conditions. Biogas is defined as a gas with compositions that vary from 30%-70% Methane and 30%-50% carbon dioxide, along with varying proportions of hydrogen gas based on the digested feedstock (Kabeyi & Olanrewaju, 2022).

Biogas is a renewable source of energy. It is obtained from biological matter, not from sunlight or wind. The anaerobic digestion phase results in the generation of biogas, also known as methane (CH_4), carbon dioxide (CO_2), and trace gases such as hydrogen sulfide gas (H_2S), ammonia gas (NH_3), to name a few. However, biogas is not similar to natural gas, although it can be likened to gas from landfills, which can have recognizable haloalkanes. The value for natural gas is 36.14 MJ/m^3, while that of biogas is 21.48 MJ/m^3 (Mustapha, 2021).

Anaerobic digestion is a complex biochemical process in which organic materials are degraded by microorganisms in the absence of oxygen. The major end product of AD is biogas. Basically, it involves four successive steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the hydrolysis stage, the complex organic polymers such as carbohydrates, proteins, and lipids are converted into simpler soluble compounds. Efficiency in the anaerobic digestion process depends on various substrate properties, including the following: moisture content, biodegradability, C/N ratio, and particle size. Nutrients of balanced composition show better influence on microbial activity as well as CH4 yield due to adequate moisture content. Additionally, co-digestion is the simultaneous digestion of two or more substrates. It greatly improves the nutrient balance, maintains the digestion process, and enhances the biogas productivity by optimizing the C/N ratio (Kabeyi & Olanrewaju, 2022; Mustapha, 2021). Operation parameters have a notable influence on biogas yield as well as composition. The temperature is a key factor in biogas processes. The mesophilic temperature range of 30-40°C and thermophilic range of 50-60°C are typical. Similarly, an optimal range of pH must be maintained between 6.8 and 7.5 for the stable operation of methanogenic bacteria. Other important conditions include hydraulic retention time, organic loading rate, and efficient mixing, which are vital for efficient interaction between substrates and microorganisms. To enhance process performance, optimization techniques such as Response Surface Methodology (RSM) are widely employed to evaluate the interactive effects of multiple operational variables and determine optimal conditions for maximum biogas yield and methane concentration (Kabeyi & Olanrewaju, 2022; Mustapha, 2021).

2.2. Advantages of biogas

Waste anaerobic digestion enhances hygiene by reducing the presence of pathogenic microbes and has greatly improved the health status of consumers (Montgomery & Bavor, 2020). In the same way, the biogas can be used for providing cheap energy for daily activities such as cooking and lighting using the readily available and cheap energy sources such as the digested materials used for the production of the gases. In addition, small scale biogas production plants require low construction and production costs for life activities (Geddafa et al., 2023).

The leftover biogas residue in anaerobic digestion activities can be utilized for fertilizer and increasing crop production. Methane gas, though the secondary greenhouse gas next to carbon dioxide, has a greater global warming capacity by 28 to 34 times compared to carbon oxide with

a 100-year time frame (IPCC, 2014). Biogas utilization, hence, controlled combustion of methane gas, hence prevents the release of large emissions of methane gas, which could rather easily escape. This limits its overall gas effects on increasing the overall warmth in the greenhouse effect (Rodhe et al., 2015).

Emissions of nitrous oxide gas from organic waste in animals that are often exposed also reduce air quality (Rodhe et al., 2015). Thus, the usage of both cow dung and wheat straw in the production of biogas can also have significant value to any nation if it can help in improving the efficiency of reducing pollution, as well as improving the efficiency of fuel usage, hence the preservation of non-renewable resources (Li et al., 2011).

2.3. Anaerobic digestion and its processes

Anaerobic digestion can be described as, a biological process in the absence of oxygen. Biogas and organic nutrient fertilizer named ‗digestate' are obtained from anaerobic digestion. Anaerobic digestion can also be harnessed in the form of organic fertilizer. Biogas is composed of carbon dioxide gas or methane gas. The highest recognition awarded in relation to anaerobic digestion has been awarded to, the use of anaerbes for the treatment of organic waste. The two applications where the recognition has been found to be appropriate for anaerobic digestion are the use of, the biological process of anaerobic digestion for the purpose of converting waste to energy, and use of anaerobic digestion ―for the purpose of treatment of organic waste.

The best mixture of cow dung and wheat straw for anaerobic digestion is considered to be because cow dung has an adequate number of microbial agents, while wheat straw has an adequate number of carbohydrates, essentially because of its lignocellulosic content, as wheat straw contains a large number of carbohydrates having a large percentage of carbon content too. Both these substances are capable of contributing an adequate number of nutrients required for better biogas production. Anaerobic digestion involves four main stages of biochemical processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis stages. Overall biogas production is essentially considered the best measure for the control of anaerobic digestion of biowastes.

- **Hydrolysis**

This initiation phase involves the partial hydrolysis of large molecules such as cellulose, hemicellulose, proteins, and lipids to smaller water-soluble molecules such as short chain sugars, amino acids, and fatty acids by hydrolysis enzymes, (Appels et al., 2008). Incidentally, this stage

will be relevant when the materials, especially the harder ones such as straw, do not actually require anything from the bacterial stand in terms of addition to the process of decomposition. To avoid such complications under this framework, addition of cow dung to the materials shall commence from this stage of initiation. Additionally, the rate of hydrolysis shall also be considered in relation to methanogenesis.

- **Acidogenesis**

The soluble fraction formed as a result of the completion of the hydrolysis process is employed by acidogenic bacteria present in the process of acidogenesis. In this process, volatile fatty acids, alcohols, hydrogen gas, carbon dioxide, ammonia, and other intermediate compounds (Mata-Alvarez et al., 2014) are formed. The high growth of microbes and lowering of pH due to the increased concentration of organic acids is known to characterize this phase. Adding cow dung together with wheat straw to result in co-digestion has a high buffer capacity due to the natural presence of bicarbonate and ammonia in cow dung. Thus, it is less likely to undergo the process of inhibition due to the increased concentration of VFA (Li et al., 2011).

- **Acetogenesis**

Acetogenesis is defined as the oxidation of the produced acids from the previous process through the action of the acetogenic bacteria in the process, which results in the production of acetate, hydrogen gas, and carbon dioxide. The resultant products then undergo utilization as feed for the next process. It is worthy to note that the reaction termed acetogenesis is unique in that it is sensitive to the partial pressure increase of hydrogen gas, which will lead to a situation where the process will be inhibited when hydrogen gas is produced in high quantities. The process of codigestion is responsible for achieving balance in the reactions as it facilitates the interaction between the hydrogen-oxidizing methanogens and the acetogenic bacteria. The microbial population present in the cow dung samples has played a crucial role in achieving balance in the hydrogen process (Zhang et al., 2014).

- **Methanogenesis**

The process of methanogenesis represents the final process involved in the anaerobic digestion process. In an effort to carry out methanogenesis, the process employs a particular group of archaea referred to as methanogens. The process uses the reaction between acetic acid, hydrogen gas, and carbon dioxide. This particular reaction generates the formation of methane gas and

carbon dioxide along with the formation of a small quantity of water (Appels et al., 2008). In conclusion, the methanogens are also highly sensitive towards the particular environment created on account of the presence of pH, temperature, and ammonia concentration. The methanogenic process exhibits the best output at a pH level between 6.5-8.0 with in a stable temperature environment. Codigestion between cow dung and wheat straw within the current study allowed the generation of a proportional nutritional environment along with the availability of the buffer capacity needed to sustain the process of methanogenesis within the mesophilic environment. Results indicated the best possible output within the methanogenic process in a mixture consisting of 75% wheat straw and 25% cow dung. This process allowed the effective stimulation of a higher concentration level within the biogas and biogenesis process.

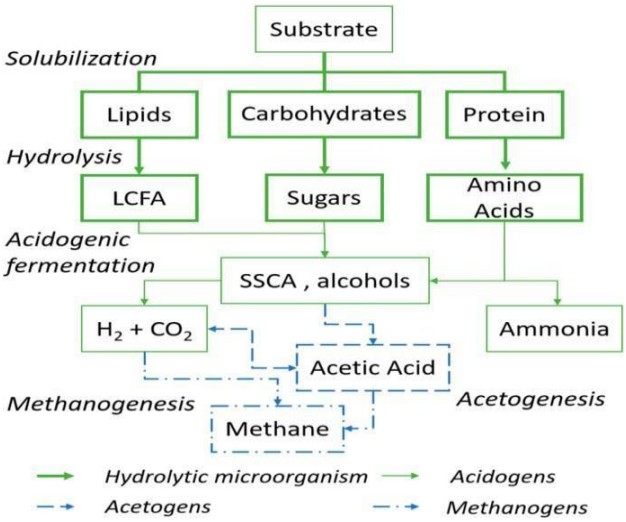


Figure 2.1: metabolic pathways of anaerobic digestion

2.4. Factors affecting anaerobic digestion

Optimization of the processing parameters can ensure more efficient anaerobic codigestion, which immediately influences the microorganisms in the digester. The processing parameters indicate the rate at which the substrates are being broken down and the total methane gas produced through the technique (Mata-Alvarez et al., 2014). In the codigestion technique carried out using cow dung and wheat straw, the need to optimize the processing parameters can be explained by the fact that the substrates have different physical forms.

A. Total solids content

Total Solids (TS) concentration can be a critical factor that influences digester design, substrate availability, and microbial interaction. Anaerobic digestion systems are usually based on the content of TS, classified into low-solids (≤10%) medium-solids (15-20%) high-solids (20-22%) process. Cow dung and wheat straw co-digestion process TS content affects moisture content, carbon-to-nitrogen ratio (C/N ratio), and microorganism-contact time organic matter. Despite the fact that higher concentrations of TS in the feedstock can decrease the size of the digester and requirements, too much solids can contribute to inadequacies in mixing, mass transfer problems, and localized inhibition (Moller et al., 2004). The medium-solids feedstock mix for such a combination the desirable range of 15-20% TS is considered optimal, where efficient microbial breakdown of organic matter is facilitated activity while maintaining manageable operating conditions (Li et al., 2013).

B. Temperature

Temperature is an important operating factor of anaerobic digestion, as it affects metabolic activity, rates of biochemical reaction, and composition of microbial populations within the digester. Anaerobic digestion processes are commonly conducted at two reaction rates. Both reaction rates are affected the main temperature types: mesophilic growth, between 20 and 40°C (with an optimum near 35°C), and thermophilic conditions, usually between 50°C to 65°C (approximately 55°C). The processing under thermophilic conditions can be effectively applied for hydrolysis of lignocellulosic materials like wheat straw, thereby facilitating faster rates of biodegradation. Biogas production and shorter hydraulic retention times (El-Mashad & Zhang, 2010). Nevertheless, these advantages the process of thermophilic digestion requires higher energy and more accuracy operational control, and it is more vulnerable to process disturbances due to ammonia toxicity and accumulation of volatile fatty acids (Braun & Wellinger, 2003). In co-digestion bio refineries based on agriculture residue, mesophilic digestion is usually preferred because of its better process robustness, lower energy costs, and better insensitivities to variations in composition. However, thermophilic digestion can still be preferred when there are where rapid treatment rates or improved pathogen inactivation are required.

C. Retention time

Hydraulic Retention Time (HRT) is the average time during which the substrate material stays in the system. It is expressed as: inside the anaerobic digester, allowing sufficient time for

microbiological breakdown of organic matter. The amount of HRT needed also depends on a number of things such as the material being used, temperature, and by the activity of the inoculated bacterial community. For the cow dung and wheat straw digestion, the reported HRT values were found to vary between 15-35 days at mesophilic temperatures and 12-14 days at thermophilic temperatures. Though longer periods of retaining organic matter may promote greater degradation of organic matter, the excessively long HRTs may decrease volumetric productivity of biogas production and require larger reactors. According to Figure volumes, thereby raising the capital cost. Wheat straw, due to high demand for cellulose and lignin, especially to microbiologically mediated decomposition, often necessitating prolonged retention times or appropriate pretreatment techniques to produce sufficient conversion (Moller et al ., 2004). Therefore, the optimum value for the state transition rate in the context of the HRT must be weighted with consideration performance, reactor sizing, and overall economic feasibility (Mata-Alvarez et al., 2014).

D. pH

pH values are an important measure of the stability of anaerobic digestion, since they depend on acid-producing, methane-producing bacterial populations. Processes of digestion undergo various biologic phases, which prefer different values of pH. Acid-producers usually prefer slightly acidic pH values, between 5.5-6.5, while methane-producers prefer slightly alkaline or neutral medium, between 6.8-7.5, respectively, at which anaerobic digestion takes place at best (Appels et al., 2011).In cow dung-cum-wheat straw co-digestion, there can possibly be a situation where VFA's develop rapidly at acidogenesis, thus lowering the pH, provided buffering capacity is reduced. If pH values are lower than 6.2, there can be loss of activity or toxicity, while values higher than 8.0 are toxic to methanogens, thus lowering biogas yields (Mata-Alvarez et al., 2014). The natural buffering capacity of dung organic matter, chiefly bicarbonates, together with ammonia, helps to neutralize acidity developed due to carbohydrate-rich wheat straw substrate. Hence, values between 6.8 -7.4 are preferable for biologic stability and biogas production.

E. Feedstock characteristics

It can therefore be concluded that the feedstock composition is one of the essential key factors that limit and influence the anaerobic digestion process in that it determines the biochemical methane potential, the level of nutrients, and microbial activities (Li et al., 2013). On the other

hand, the type or sources of feedstocks may be agricultural waste, animal manure, food waste, municipal solid waste, as well as sewage sludge, which vary based on their characteristics in feedstock composition, including C:N ratio, lignin level, rate of biodegradation, and moisture level. In the proposed scheme, cow dung and wheat straw were considered for codigestion, complementing each other for digestate use in biogas plants on account of their properties. While cow dung has been found to possess good value for nitrogen content, microbial mass, buffering capacity, and moderate value for methane potential, a contrastingly very high value for both carbon content and lignocellulosic materials with higher methane potential and non-biodegradable nature is found in wheat straw. At the same time, it will also ensure that there is a well-optimized blend of the two substrates in order to attain the optimized composition for C:N, which will vary between 20-30, in order to make sure that there is no production of ammonia in the digesters. Also, this will help in the promotion of microbial diversity as well as the decomposition of lignocellulosic material at a higher rate. Therefore, there will also be an assurance of mass production of biogas through the codigestion process as well as the Digestion processes (Moller et al., 2004; El-Mashad & Zhang, 2010).

2.5. Advantages of anaerobic digestion

The anaerobic process has proven to be a proven and reliable technology for the treatment of wet organic wastes, which turns these organic wastes into valuable resources (Braun & Wellinger, 2003). The anaerobic process specifically targets the treatment of wet organic materials. Therefore, it has already been widely used for the treatment of agriculture wastes, sewage sludge, and organic food wastes. The first advantage would be the biogas produced, which would be a green fuel consisting mainly of methane and carbon dioxide. Cow dung & Wheat straw co-digestion by the ad process in this process, Cow dung and wheat straw undergo co-digestion Providing a solution for getting rid of two of the biggest agricultural productions: Decreasing the elaboration of greenhouse gases produced during the decomposition of wastes which are deposited in landfills, and the cause of the formation of the biogas, which in turn results in the by-product, hence the formation of the biological fertilizer, which assists in the recycling process in agriculture. Furthermore, this co-digestion increases the potential for energy utilization owing to the complementary characteristics of the two components, favoring more consistent biogas yields, regardless of variations in individual-input qualities, compared to previous, individual digestion practices (Mata-Alvarez et al., 2014).

2.6. Related studies

The study by Haryanto, et al. (2018) examines cow dung and rice straw co-digestion through the production of biogas using the anaerobic method, focusing on the role of the addition of urea through the use of a semi-continuous process digester. Through this study, the reader gains insight into the use of co-digestion for the effective production of biogas. In understanding the role of the addition of urea, the study offers alertness on the ability to maximize the technique for increased biogas production. Nevertheless, there is one crucial limitation within the processes of co-digestion, which is the less understood synergistic effect of such processes on the microbial environments within the process (Mata-Alvarez et al., 2014).

Llano, Arce, and Finger (2021) examined methods for the maximum production of biogas through the application of the anaerobic digestion process for municipal solid wastes. The case study for this research took place in the Reykjavik metropolitan area, which offers a unique outlook in comparison with other regions in the world. It was shown in this experiment that there was a vast potential for improvement in the biogas produced from municipal solid wastes and that useful data was obtained in order to further develop the management system (Llano et al., 2021; El-Mashad & Zhang, 2010). It appears from Appels et al. (2011) that there is still a vast potential in order for new and advanced techniques to be employed in overcoming the difficulties introduced through the nature of the feedstock and processed materials in municipal solid waste management systems for the production of biogas, in order for these systems to become even more improved.

The study conducted by Sandhu and Kaushal 2023 entitled "Anaerobic Co-digestion of Food Wastes, Algae, Pond Sludge, and Cow Dung for Biogas Yield Enhancement as a Potent Approach to Reduce Carbon Footprints" discusses the use of multi-substrate anaerobic co-digestion methods to enhance biogas production. The work could have been done through either a batch or continuous process of anaerobic digestion, in which substrates are degraded microbially in the absence of oxygen to produce biogas. The result of this work supports the fact that co-digestion can be used to improve the Carbon-Nitrogen ratio, hence increasing microbial activities which can enhance methane biogas production. However, the process details seem incomplete, which could be attributed to its limitation in replicating the process for wider application (Li et al., 2013). The details of the process, however, seem to be lacking, which can be associated with a limitation to the replicability of the process to be used on a wider scale

(Braun & Wellinger, 2003). This implies the need for an understanding of the whole reporting of the parameters involved in co-digestion in order to scale down effectively to a real application from a lab standpoint. This poses implications for the need to carry an entire perspective of the methodologies being used in approach. Although there are several benefits accrued from co-digestion to boost hopes of an entire improvement of an entire approach of biogas production as well as an entire decrease of an entire carbon footprint of an entire approach of biogas production, an entire perspective of methodologies being used. In light of the need to address the whole weakness that is posed in the above statement, it shall be of prime importance in carrying an entire approach to address an entire efficiency of an entire approach of biogas production in an entire approach of co-digestion (Appels et al., 2011).

Related to this, the investigation concerning the evolutionary optimization of biogas production from food, fruit, and vegetable (FFV) waste conducted by researchers Olatunji et al. (2021) involved the use of different methods of evolutionary optimization, including genetic algorithms, to determine the optimal working conditions for the anaerobic digestion process with the objective of improving biogas production. From the study, the use of optimization methods can effectively promote better conversion of waste to energy. Nevertheless, it appears there is a crucial draw back, which results from the challenges involved in doing accurate and continuous maximization of FFV biogas yield pertaining to variations in FFV waste and environmental conditions. These factors result in uncertainty in the process, thereby requiring difficulties in the prediction and control of digestion process efficiency (Braun & Wellinger, 2003). Because of such reasons, there seems to be a crucial expectation to ensure efficient optimization of biogas production either by considering these factors or developing methods to optimize this production (El-Mashad & Zhang, 2010).

A study entitled "Production of Bio Gas from Cow Dung and Tomatoes Waste" by Mustapha (2022) reveals a limitation in missing knowledge about the best possible ratios to be mixed, operational parameters, and microbial involvement needed for maximum production of biogas in this combined feedstock. The output of this study will be to make available the best operational parameters such as loading rate, residence time, and processing parameters to maximize biogas production (Mustapha, 2022). Nevertheless, this problem of optimizing the process of co-digestion should systematically consider characteristics specific to each feedstock (Li et al., 2013). From FFV waste (Olatunji et al., 2021). Although there appears to be potential in the use

of cow dung and tomatoes waste to make biogas, a problem related to missing knowledge about the best operational parameters and microbial viability may pose a limiting effect on the efficiency and feasibility of the process to make biogas (Mustapha, 2022). This problem, therefore, needs to be rectified by conducting relevant scientific study and experimentation to achieve the goal of optimizing biogas production and thus improving the feasibility of using cow dung, tomatoes waste, and other similar waste materials to make biogas.

2.7. Summary of related studies

It is important Though various studies have identified the possible use of agricultural residues and animal waste for biogas production using anaerobic digestion (Guvvala & Maurya, 2023; Kamusoko & Mukumba, 2024, there still exist a number of knowledge gaps in terms of optimizing the use of wheat straw and cow dung codigestion. This is particularly the case for mix ratios, conditions, and microbial dynamics (Rahmani et al., 2023); such knowledge gaps still hinder the efficient implementation of various waste to energy technologies that use biogas as a renewable energy option (Kamusoko & Mukumba, 2024). As such, the major several challenges are surmounted to ensure the efficient use of biogas production to make the utilization of wheat straw and cow dung codigestion a viable renewable energy choice (Guvvala & Maurya, 2023).

CHAPTER THREE

3. MATERIALS AND METHODOLOGY

3.1. Description of the study area

The study was conducted in the Chemical Engineering Laboratory at Wachemo University, which is situated at the main campus of the university, placed about 230 km south of Addis Ababa, the capital of Ethiopia. The university is geographically situated at a latitude of 7°33′ N and a longitude of 37°51′ E, at an average elevation of around 2177 m above sea level (Bernardi, 2000). The laboratory equipped the needed conditions used to undertake the bench-scale anaerobic digestion.

3.2. Raw materials and sample preparation

Permission for harvesting of cow dung, wheat straw, and rumen fluid was obtained from Wachemo University farms and slaughter house management. Then, wheat straw was harvested from Wachemo University agricultural farmland. Cow dung fresh from farms was collected from the animals also kept in Wachemo University farms. All these feedstocks were chosen because of their combined physico-chemical properties.

Approximately 5kg of wheat straw and 10kg of fresh cow dung were selected and transported to the laboratory in clean plastic bags and buckets. The chopped sections of the wheat straw were carefully portioned further in a bid to aid in the drying and comminution processes. The drying process by the oven and the sun in both samples was conducted with the sole aim of reducing water content efficiency. After the drying process in the two samples, the comminution in the first phase was undertaken in smaller sections of 10-30mm, and in the second phase, it was undertaken in even smaller sections of 0.2-2mm by the use of a ball mill (Monlau et al., 2013). The sieving process in sections of 0.5mm was conducted in a bid to aid in making the mixture homogeneous or a mixture in the two samples.

After sieving, about 3500 g of wheat straw and cow dung powder were separately packed in low-density polyethylene (LDPE) bags and kept in desiccators prior to use. The obtained powders were directly used as feedstock for the digestion experiments.



Figure 3.1: wheat straw and its powdered form



Figure 3.2: fresh cow dung and the powder that corresponds with it

3.3. Inoculum preparation

Fresh rumen fluid was used as the inoculum source in this study due to its high concentration of active anaerobic microorganisms. The rumen fluid was collected from the Wachemo University slaughterhouse immediately after animal slaughter. Solid particles were removed by filtering the rumen content through a cloth with a mesh size of 0.5 mm. The filtered rumen fluid was pre-incubated and degassed at 37.5°C to eliminate residual biodegradable substrates and dissolved methane prior to its use as an inoculum (Kharchenko, 2011). This pre-treatment step ensured that biogas production during the experiment originated primarily from the added substrates rather than from the inoculum itself.



Figure 3.3: Illustrates the inoculum used in the study.

3.4. Experimental procedures

In addition, prior to digestion, wheat straw and cow dung were quantitatively weighed separately based on pre-set ratios of mixing of the digestion materials from weight to weight (WS:CD): 4.76:95.24, 75:25, and 85.60:14.40. Based on measurements of the weighed materials, the weighed digestion materials were well mixed in a V-blender. Further, after ample mixing of the digestion materials, distilled water was added at a ratio of material: distilled water of 1:2 (w/w) in a bid to facilitate solubilization and the process of digestion (Sep et al., 2009). Further processing involved placing the prepared mixture in narrow-necked conical flask digesters. Digestion of materials was done under variable working settings that include working temperature ranges of 30-45°C. Additionally, digestion was done under variable ratios of material mixing. pH (4-10) was also used in digestion. During digestion processes, production of biogas was also measured. Further quantitative measurement of biogas production was done by measuring volume via a gas syringe. Protection of generated biofuel in its gaseous state was done in plastic bags. At completion of digestion processes, determination of biofuel composition was done via a gas analyzer.

3.5. Process flow diagram

The overall experimental process, starting from the preparation of the feedstock up to the biogas measurement and analysis, is depicted schematically in Figure 3.4. This figure identifies the overall process steps needed in the anaerobic co-digestion process.

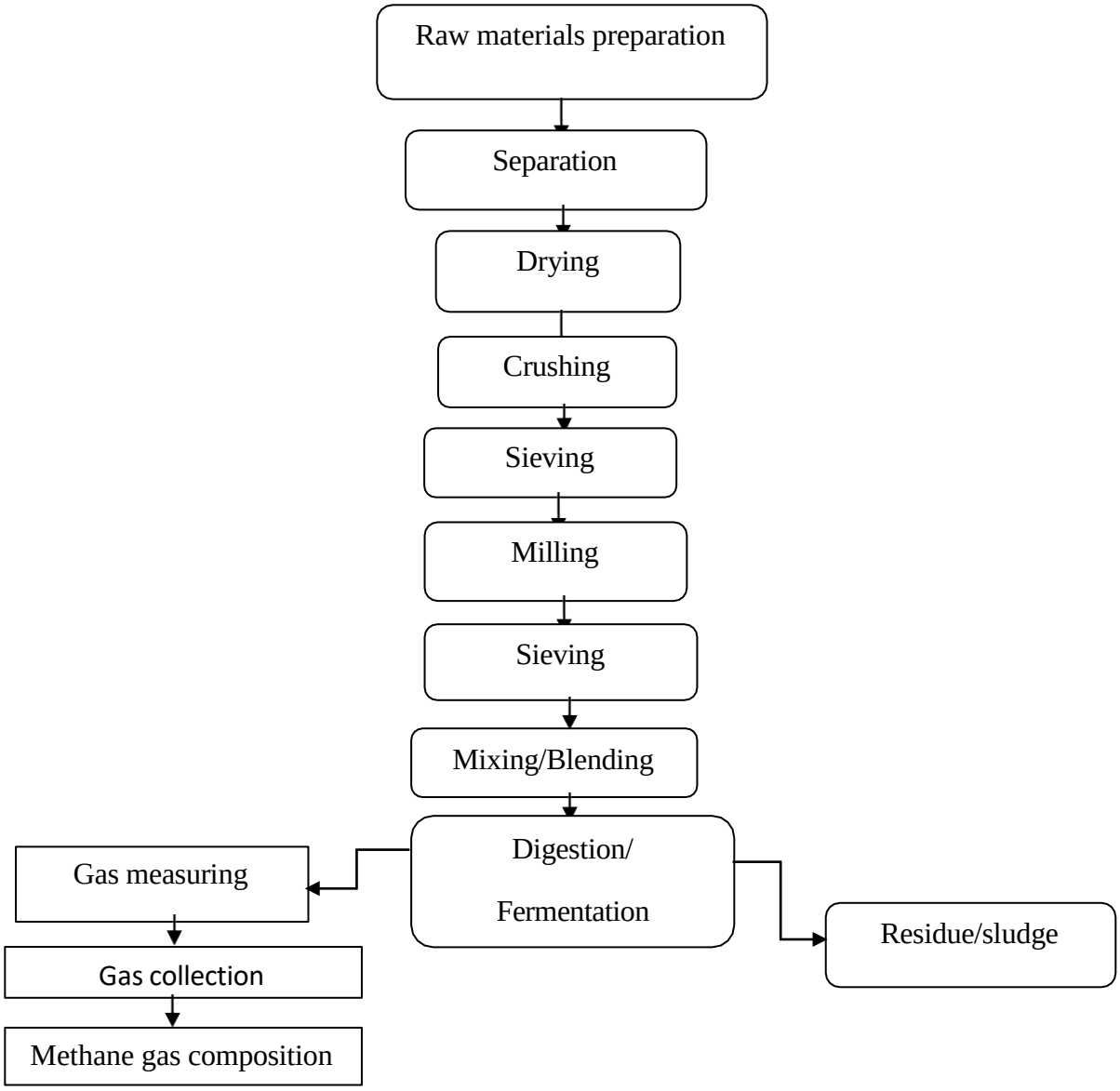


Figure 3.4: Processing flow diagram

3.6. Experimental approach

Another important point is feed stock characterization. In this matter, it plays a significant role as a major aspect that influences the characteristics of feed stock during anaerobic digestion. As far as the matter concerning the scope of the research is concerned, the total solids (TS) and fixed solids (FS) content of feed stock and anaerobically digested sample was determined based on a standardized approach concerning water and wastewater analysis described according to American Public Health Association guidelines as described and detailed under APHA (1989). As far as the matter concerning the pH analysis of anaerobically digested sample and feed stock is concerned, it was determined based on the application of pH meter model pH-201, which was imported due to it being based in the nation of Taiwan. As far as the matter concerning analysis done for biogas production product; methane, carbon dioxide is concerned, it was determined based on the application, interpretation, and usage of infrared gas analyzer, which was named as PIR-89, as it was imported to the nation and based in Technovation Analytical Instruments, based in the nation of India, as based on guidelines mentioned under Jigar (2015).

3.6.1. Determination of the physicochemical properties of the feedstocks

The gravimetric analysis technique was used to analyze the samples of feedstock for total solids, moisture, and total volatile solids at Wachemo University Chemical Engineering Laboratory. By using a pH meter, it was possible to analyze the pH level of samples of feedstock. By titration methods, it became possible to analyze the organic carbon concentration level of samples of feedstock. While analyzing total solids concentration and total volatile solids concentration of samples of feedstock, differences in levels of concentration for both analyses were used. Levels of C:N ratio were to be analyzed by using percentage organic carbon and total percentage nitrogen.

A. Total solid

The fresh samples of cow dung and wheat straw were each weighed to 40 grams by using an electrical beam balance. Each material went into its own crucible, and all were put into the electric hot-air oven at 105°C for duration of 24 hours. After cooling, the crucibles were weighed again, and

From these measurements, the solid content was calculated

$$TS\% = \frac{(A-B)}{(D-B)} \times 100\% \qquad 3\text{-}1$$

Where: A=weight of the dish+ dry sample that was dried for 24hrs at 105℃. (In grams),

B= is the dish‘s weight in grams and

D= dish‘s weight + a fresh sundry random sample (in grams)

B. Moisture content determination

For the determination of moisture, a fresh sample weighing 40 g was dried at 105°C for 24 hours. Its loss in weight was then determined following the procedure in APHA 2540 E (1999).

$$MC(\%) = \frac{W-D}{W} \qquad 3\text{-}2$$

Where: MC stands for moisture content.

W is the sample's original weight (in grams), and

D is the sample's weight (in grams) following drying at 105°C.

C. Volatile solid

The dried sample obtained from TS was ignited using a muffle furnace at 550°C for a period of one hour. The ignition loss was measured, and volatile solids were calculated based on the APHA (1999) procedures.

$$VS(\%) = \frac{\text{W dried - Washed}}{\text{W dried - W crucible}} \times 100\% \qquad 3\text{-}3$$

Alternatively, expressed relative to the dry solid mass:

$$VS(\%) = \left\lfloor \frac{M_{DS} - M_{ash}}{M_{DS}} \right\rfloor \times 100\%$$

Where: VS (%) is the proportion of volatile solids.

MDS stands for mass of dry solids in grams.

Mash is the mass of ash at 550°C following ignition (g).

D. Carbon-to-Nitrogen ratio (C/N)

i. Carbon determination

The total carbon content estimation was done by empirical calculations based on the volatile solids content, as described by Haug, R. T. (2018), the practical handbook of compost engineering.

$$Carbon\% = \frac{VS(\%)}{1.8} \qquad 3\text{-}4$$

ii. Nitrogen determination

Total nitrogen determination was conducted using the Kjeldahl method, which followed the classical process of digestion, distillation, and titration (Kjeldahl, 1883; Bradstreet, 1965; AOAC, 2016). In the digestion process, concentrated sulfuric acid, hydrogen peroxide, and a catalyst mix were used. After digestion, ammonia distillation was followed by titration with hydrochloric acid. Total nitrogen was calculated from the titration process using the Kjeldahl equation.

$$Nitrogen(\%) = \frac{V \times N \times 0.014 \times 100 \times MCF}{W_O} \qquad 3\text{-}5$$

In this equation,

V is the volume of the sulfuric acid (H_2 SO4), expressed in milliliters.

The letter N is short for normality. The normality of this acid is 0.1 N.

The figure 0.014 is equivalent to the weight of nitrogen in grams as milliequivalents.

The term MCF is short for moisture correction factor. Lastly, WO is weight in grams and is expressed on a dry matter basis.

E. Calculation of pH

pH measurements in the prepared substrate samples were carried out with a digital pH meter (HANNA HI 8314). The pH test prior to digestion was conducted with a mixture of distilled water in a ratio of 1:5 by dilution. During the digestion procedure, the pH was regulated and maintained between 6.8 and 7.4. This is a crucial parameter in the methanation procedure (Mata-Alvarez et al., 2014; Li et al., 2013).

3.7. Experimental Setup

The anaerobic digestion experiment involved using 0.5-liter narrow-necked conical flasks that were packed with rubber bungs to create digesters. These digesters were housed in a water bath that was maintained at temperatures of 30°C, 37.5°C, and 45°C as well. Additionally, each digester had a single outlet that was connected to a Y-connector. This Y-connector had one end that went to a syringe for gas measurement and another end that went to collection bags. Manual mixing was carried out daily by gently shaking the digesters to ensure uniform contact between microorganisms and substrates.

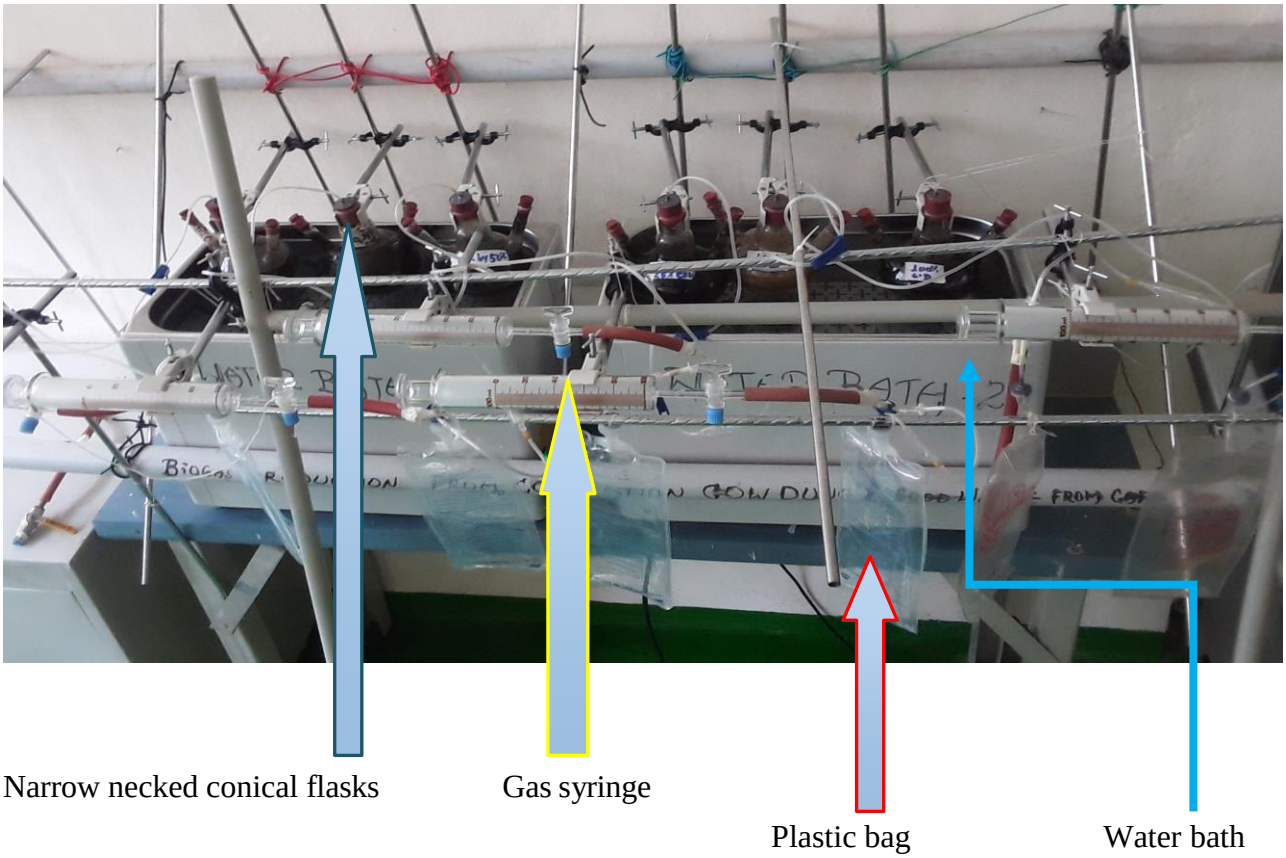


Figure 3.5: Bench-scale experimental setup

3.7.1. Determination of biogas composition

The biogas appeared within day four of the digestion phase. Over the 35 days of the study, gas production in the treatment groups was monitored using a gas syringe. Subsequently, gas composition analysis for methane and carbon dioxide was done by means of the GeoTech GA5000 gas analyzer.

3.8. Design of Experiment

The design of the study employed the response surface design method utilized in the Design Expert 13 software — the Box-Behnken design. In the anaerobic digestion experiments, two different substrates were utilized: cow dung (CD) and wheat straw waste (WS). These were mixed in three different proportions: WS: CD (4.76%:95.24%), WS: CD (75%:25%), and WS: CD (85.60%:14.40%). A total of 17 experimental runs were carried out to examine different combinations of the variables: temperature, mixture ratio, and pH, at different time intervals. In all runs, the total amount of wheat straw and cow dung mixture was held constant.

In each experiment, the quantity of materials was the same for all volumes. Every set had 375 mL of sludge, 375 mL of wheat straw, cow dung, inoculants, and water. The sample was to be prepared using a 0.05 w/w mixture. This refers to 4.76% wheat straw and 95.24% cow dung. Specifically, 5.95 g wheat straw was used along with a total of 125 g cow dung and other materials (5.95 g wheat straw and 119.05 g cow dung). The 5.95 g amount was used on samples 3 and 5 in every experiment. Gas volumes were measured on day four, while methane analysis was conducted on day 35. In this way, it was possible to trace the time-related changes in gas production and methane concentration. In each of the trials, every feedstock was tried alone and in every possible combination with the others: wheat straw, cow dung, inoculum, and water.

Table 3.1: Volume and mixing ratios of substrates in each treatment

Treatments	Addition of freshly dried WS sample (g)	A freshly dried CD sample was added (g).	The amount of added distilled water (mL)	The amount of inoculums added (mL)	Working volume (ml)
T_1	5.95	119.05	240	10	375
T_2	93.75	31.25	240	10	375
T_3	107	18	240	10	375

Note: T1 = (4.76%/95.24%), T2= (75% WS& 25% CD) and T3 = (85.60%/14.40%);

CD = Cow Dung and WS = Wheat straw waste; WS/CD

The experiments were conducted randomly. The technique for analysis was selected by appropriate software. Table 3-2 below indicates the levels and factors.

Table 3.2: Levels of the factors

Substrate mixing ratio (%)	Temperature (°C)	pH
0.05, 3, 5.95	30, 37.5, 45	4, 7, 10

Note: -As it is seen, the ratio is from wheat straw to cow dung. i.e. 0.05 = (4.76/95.24),

3= (75/25), and 5.95= (85.6/14.4)

Table 3.3: Experimental design

Std	Run	A:Temperatue	B: pH	C: Substrate Mixing Ratio
		℃	pH	W/W
10	1	37.50	10.00	0.05
8	2	45.00	7.00	5.95
13	3	37.50	7.00	3.00
7	4	30.00	7.00	5.95
3	5	30.00	10.00	3.00
2	6	45.00	4.00	3.00
12	7	37.50	10.00	5.95
15	8	37.50	7.00	3.00
11	9	37.50	4.00	5.95
17	10	37.50	7.00	3.00
16	11	37.50	7.00	3.00
4	12	45.00	10.00	3.00
5	13	30.00	7.00	0.05
9	14	37.50	4.00	0.05
6	15	45.00	7.00	0.05
1	16	30.00	4.00	3.00
14	17	37.50	7.00	3.00

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1. Characterization of the biogas feed stocks

The presented data on the total solid, moisture content, volatile solid, and C: N of the wastes used for the study are in Table 4-1. From the table, it is evident that cow dung had a maximum moisture content of 82.25% and wheat straw was less with a moisture content of 65.66%. The maximum TS were recorded in wheat straw (34.50%). The VS obtained in the study were 90.56% (cow dung) and 92.14% (wheat straw). The results obtained from the present work for individual wastes are comparable with values reported by (Aragaw 2013).

Table 4.1: Characteristics of wheat straw and cow dung

No.	Name of wastes	Weight (g)	Moisture content (%)	Total solid (%)	Volatile solid (%)	C:N (%)
1	Cow Dung	40	82.25	18.75	90.56	24:1
2	Wheat Straw	40	65.66	34.50	92.14	20:1

The C/N ratio of wheat straw and cow dung separately, as presented in Table 4-1, indicates the C/N ratio of the cow dung and wheat straw of 24:1 and 20:1, respectively, which falls in the range of 20-30. As per the work done by Shefali & Themelis (2002), the C/N ratio of 20-30 can be considered the ideal range in anaerobic digestion. It clearly proves that the feedstock in this study falls in the ideal range. Based on total solid content of the mix, 20.06%, 18.52%, and 17.06% of total solids of the mix exist in 4.76% WS + 95.24% CD, 75% WS + 25% CD, and 85.6% WS + 14.4% CD, respectively. The range of total solids content in the experiment varied from 22.05% to 18.06%, which ranges from 5-70% in the work conducted by Steffen et al. (2000). The maximum total solids content was recorded in WS with a total solids of 22.05%, while the minimum was cow dung at 18.52% total solids (Table 4-2). The total solids content was most reduced in the mixture of 75% WS + 25% CD, reducing the total solids content from 21.69% to 9.62%. According to Table 4-2, the observation signifies that 85.6% WS + 14.6% CD

was effective in reducing the total solids content after the digestion process. The % reduction in total solids content increased from 22.05% to 12.45%, considering the pre-treated samples. Further observation showed a reduction in the total solids content of the mixture after it had undergone the digestion process. The highest % reduction of total solids content in the mixtures occurred in 75% WS + 25% CD. The % reduction was 12.07%, higher than that of the other mixtures.

Table 4.2: Characteristics of wheat straw and cow dung

Treatments		Parameters			
		TS%		VS%	
		Before digestion	After digestion	Before digestion	After digestion
A	4.76% WS + 95.24% CD	18.52	10.06	84.15	78.26
B	75% WS +25% CD	21.69	9.62	84.87	77.56
C	85.6% WS + 14.4% CD	22.05	12.45	84.34	79.67

The results indicate a significant variation in the value of VS, at the end of the AD. The highest decrease in VS value, around 7.31%, is recorded for the mixture with 75% of WS and 25% of CD. Prior to AD, this investigation documented a positive correlation between TS and VS as the proportion of WS varies in the mix. In other words, blending the feedstocks enhanced the optimization for both parameters TS and VS.

According to Ismail et al. (2012), the critical variables affecting microbial growth include total solids, volatile solids, and whether there is anaerobic digestion. The optimal microbial growth will be at a ratio of 75:25 WS: CD. This ratio will influence the total solids, which will contain 96% solid parts, giving a total of 20.06%, and will have a moisture level of 79.35%. In this case, if wheat straw and corn dung are blended together using the same ratio of 75:25 WS: CD, then it will produce the same desired effect when applied to soil. As the TS and VS are calculated, it must be remembered that in some samples with a greater quantity of VFAs, it may result. For instance, on the one hand, the VFAs will readily volatilize when heat is applied. Consequently, it

will result in a TS and VS being calculated as less compared to the actual. The biogas production may also be associated with the incorrect determination of the VS amount available. This directly correlates with the amount of VS because it is directly proportional to the amount of VS (Negassa 2021). It was 8.46, 12.07, and 9.6 units reduced for samples A, B, and C. The highest TS decomposition by anaerobic digestion was shown by sample B with a combination of 75% WS + 25% CD.

A. pH

Another important factor in the evaluation of the efficiency levels of the anaerobic digester is the pH. As shown in Table 4.3: below, initially, without mixing, the values were A (6.57), B (6.78), and C (7.25), whereas after mixing, values were A (8.73), B (8.71), and C (8.65). The graphs shown in the change in pH values during the process indicated that as the process progresses, the pH values in the substrate keep increasing. The values marked during observation were Between 6.1 to 8.1. The region where the values of pH lie, which is Between 6.6 to 7.6, is considered ideal for the formation of gas in this study. As soon as the value of pH in the substrate reached 7.0, the formation of gas begins, as well as its peak (Ayu & Aryati 2010), in which the production of biogas in the waste wheat straw experiment, its study, had a pH value ranging Between 6.8 to 7.2.

Among all the substrates, an increase in the pH levels during the anaerobic digestion process for the mixtures has been observed. This is because ammonia production, which is an alkaline product of the digestion of the organic matter in the digester, occurs. Here, all the digesters showed a high pH level during the last stages with a large production of ammonia most probably because the quantity of the organic matter in the straw is higher compared to the quantity in the cow dung (Gray et al. 2011). Moreover, the breakdown of the proteins leads to the production of ammonia whereas the breakdown of all the organic matter leads to the production of volatile fatty acids.

Table 4.3: Comparison of pH between prior and following AD of mixing substrates

	Treatments	Parameters Ph	
		Before	After
A	4.76% WS + 95.24% CD	6.57	8.73
B	75% WS +25% CD	6.78	8.71
C	85.6% WS + 14.4% CD	7.25	8.65

WS = wheat straw, CD = Cow Dung

B. Carbon to nitrogen ratio (C/N)

The Carbon/Nitrogen (C/N) ratio remains one of the most influential factors concerning microbial growth and methane production during anaerobic digestion. The C/N ratios for A, B, and C, obtained from Table 4.4: pre-mixing, were 23.42, 24.34, and 25.90, while post-mixing, new C/N ratios for A, B, and C were 19.80, 21.64, and 22.72, respectively. All of them lie either in or around the desirable ratio of 20-30, which is normally preferred for optimum biogas production.

The value of C/N is of great significance with regard to the fact that the value of the ration influences the growth rate of the microbes, the stability of the overall process, as well as the quantity of methane produced. In the anaerobic digestion process, microbes need the energy derived from carbon for the process to be successful, whereas the value of nitrogen present within the feedstock determines the basis for cell formation as well as proteins. However, the rate at which microbes utilize the energy from the carbon present within the feedstock is 25 to 30 times faster compared to the rate at which they utilize the value of nitrogen, hence the need for the C/N ration within the feedstock to have a value of carbon almost equal to that of nitrogen present within the feedstock. In general, individuals choose feedstock with a ratio of C/N Between 20:1 to 30:1, which is considered to be the perfect value for the overall anaerobic digestion process (Temesgen Mitiku, 2017).

Consequently, a large C/N ratio implies a requirement for the usage of nitrogen, since methanogens will both consume the accessible nitrogen and some carbon, which will lead to a low gas yield. Conversely, a low gas yield will lead to a large yield of ammonia and a consequent high pH value within the digester. This will eventually result in a low production capacity of methane gas (Bahl et al., 2019). Both explanations given above indicate that there will be a low gas yield. The result obtained shows that the ratios of the substrate utilized in this

research are optimal and balanced in nutritional requirements for adequate anaerobic digestion. The ratios can be measured and compared with a ratio given by Fricke et al. (2005) between 20-30 for optimal digestion and large methane production.

Table 4.4: C:N ratio values for the three types of substrates used to produce biogas

	Treatments	Parameters	
		C: N	
		Before digestion	After digestion
A	4.76% WS + 95.24% CD	23.42	19.8
B	75% WS +25% CD	24.34	21.64
C	85.6% WS + 14.4% CD	25.9	22.72

WS= wheat straw, CD= Cow Dung

The C/N ratio yielded a value of 24.34 and 21.64 before and after, respectively, the treatment that of wheat straw added with cow dung is comparatively higher. Proteinaceous such as waste products, on a large scale as a part of AD is not advised this is due to a greater inhibitory effect from ammonia (Etelka, et al. 2010). In comparison with other research, the result found in this experiment is more beneficial for a number of reasons. Include type of feeding system, type of animal, type of equipment involved, mode of operation of the sampling system and amount of aging that has taken place.

4.2. Biogas yield and composition of methane gas

The below table presents the volume of biogas produced and the percentage of methane in each of the 17 runs involving mixed waste. The highest yield of biogas was from run 12 using STD 14 and produced 198 ml of biogas from a 375 ml sample. The highest percentage of methane was realized in run 8 using STD 15.

Table 4.5: Selected runs based on yield per unit volume of sample applied and percentage of methane.

Std	Run	Space type	A:Temperature	B:pH	C:Substrate Mix Ratio	Volume of Biogas	Composition of Methane Gas
			℃		W/W	mL	%
4	1	IB Fact	45	10	3	134	41.2
16	2	Center	37.5	7	3	197	62.1
7	3	IB Fact	30	7	5.95	125	34.4
12	4	IB Fact	37.5	10	5.95	125	31.6
8	5	IB Fact	45	7	5.95	127	32.2
6	6	IB Fact	45	7	0.05	97	8.31
2	7	IB Fact	45	4	3	140	51.8
15	8	Center	37.5	7	3	197	62.5
1	9	IB Fact	30	4	3	149	49
10	10	IB Fact	37.5	10	0.05	113	1
17	11	Center	37.5	7	3	196.8	61.8
14	12	Center	37.5	7	3	198	61.05
9	13	IB Fact	37.5	4	0.05	114	13.3
3	14	IB Fact	30	10	3	147	40.5
5	15	IB Fact	30	7	0.05	126	2.2
13	16	Center	37.5	7	3	197	62.3
11	17	IB Fact	37.5	4	5.95	130	39.05

Basis: Substrate Mix Ratio (wheat straw (WS)): Cow Dung (CD))

Variations have also been experienced concerning production levels of biogas and production levels of methane gases. Production levels of biogas ranged from 97 mL to 198 mL concerning

production levels of methane gas that varies from 1% to 62.5%. Production levels that are low concerning methane gas production also tend to occur under a temperature of 37.5°C and pH level 10 concerning a composition ratio of 4.76% wheat straw and 95.24% cow dung with a ratio of 0.05. This corresponded to run no. 10 and std10 featuring the highest pH, a moderate temperature, and the smallest share of wheat straw with the largest share of cow dung. Conversely, the maximum biogas yield and concentration of methane were achieved at 37.5°C, pH 7, and a mixture of 75% wheat straw and 25% cow dung. These values are an average of all the parameters investigated in this work. In fact, it is not only accurate given other co-digestion experiments, but it also fares better. Gizachew Assefa, for example, concluded that digesting a mixture of 1 part municipal solid waste (MSW) and 2 parts of cow dung (CD) produced 0.47 $m^3/d/m^3$ biogas yield and 51.5% concentration of methane. This is compared to 0.45 $m^3/d/m^3$ biogas yields and what seems to be a 31.4% methane concentration from an MSW: CD mixture of 1:1 proportion and a 0.23 $m^3/d/m^3$ biogas yield and a 33.7% concentration from a 2:1 ratio (Assefa in 2020). A similar experiment was conducted by Gashaw and Teshita in 2014.

4.3. ANOVA for quadratic model of biogas yield

Response 1: volume of biogas (1)

Table 4.6: Volume of biogas

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	19630.18	9	2181.13	3274.97	< 0.0001	Significant
A-Temperature	300.13	1	300.13	450.64	< 0.0001	
B-Ph	24.50	1	24.50	36.79	0.0005	
C-Substrate mix ratio	406.13	1	406.13	609.80	< 0.0001	
AB	4.00	1	4.00	6.01	0.0441	
AC	240.25	1	240.25	360.74	< 0.0001	
BC	4.00	1	4.00	6.01	0.0441	
A^2	3349.57	1	3349.57	5029.38	< 0.0001	
B^2	2946.81	1	2946.81	4424.64	< 0.0001	
C^2	10612.81	1	10612.81	15935.15	< 0.0001	
Residual	4.66	7	0.6660			
Lack of Fit	3.75	3	1.25	5.48	0.0669	not significant
Pure Error	0.9120	4	0.2280			
Cor Total	19634.84	16				

The factors are coded, and here we are examining the Type III—Partial sum of squares. The F-value of the model is 3274.97, which indicates the model is highly significant. The p-value related to the F-value has only a 0.01% probability of occurring due to random error. Those P-values lower than 0.0500 mean that variables are significant. For this example, A, B, C, AB, AC, BC, A^2, B^2, and C^2 are all significant variables. Those P-values greater than 0.1000 mean that variables are insignificant. A great number of variables being insignificant may result in a better model if they are eliminated, excluding hierarchical variables. The Lack of Fit F-value is 5.48. The probability of such a Lack of Fit occurring by chance is 6.69%. The desired outcome is to have a model with good fit and no lack of fit. This probability value is lower than 10**%** and indicates a lack of fit.

Table 4.7: Fit statistics for the volume of biogas

Std. Dev.	0.8161	R^2	0.9998
Mean	147.81	Adjusted R^2	0.9995
C.V. %	0.5521	Predicted R^2	0.9969
		Adeq Precision	158.8245

Predicted R-Squared: 0.9969 Adjusted R-Squared: 0.9995 since difference is less than 0.2, they are close. Moving forward with regards to Adequate Precision, Signal to Noise Ratio is calculated. The adequate precision should always be greater than 4. A value of 158.824 signifies that it is a good result. Based from the discussions, the model is now ready for exploration within the Design Space.

Final Equation for volume of biogas in Terms of Coded Factors

Volume of biogas=

$$197.16 - 6.13A - 1.75B + 7.13C - 1.00AB + 7.75AC - 1.0000BC - 28.21A^2 - 26.46B^2 - 50.21C^2 \quad 4\text{-}1$$

The equation of coded factors allows us to calculate how the response will behave when each factor takes a certain level. In coded values, +1 corresponds to higher levels, and -1 corresponds to lower levels. By applying the equation based on coded values, we are able to identify which factors have a greater effect based on the values of coefficients in each factor.

The final equation in terms of actual factors is given as:-

Volume of biogas =

$$-651.08926+36.05026A+42.57454B+24.68481C-0.044444AB+0.350282AC$$
$$-0.112994BC-0.501422A^2-2.93944B^2-5.76903C^2 \quad \text{4-2}$$

The equation of the model using real variables will allow you to predict what happens to the response variables when the variables' values are altered. Each factor level will be in the correct unit of measurement. In addition, it is also important that you do not use this model in order to determine the value of the variables due to the fact that the factors have been scaled to the same unit of measurement as the response.

4.3.1. Model adequacy checking for volume of biogas response

Before accepting any model, first check whether it is appropriate using the relevant statistics. According to Kraber et al. (2019), in the experimenter's handbook, a good normal distribution probability plot should appear as a straight line. Meanwhile, the residuals plotted in the order of the experimental runs below in Figure 4.1: This type of plot helps identify hidden variables that may have existed and could have affected the outcome at the time of the experiment. The figure indicates a random scatter with no indications of further blocking or corrective actions.

Figure 4.2: this is referred to as the predicted values vs. the actual values, the predicted responses vs. the observed responses. In this case, each point ideally lies on the straight line, showing the highly accurate predictive ability of the technique. It looks genuine in predicting, unable to point out any irregularities concerning the predicted errors. It displays the ability to predict the values to be expected.

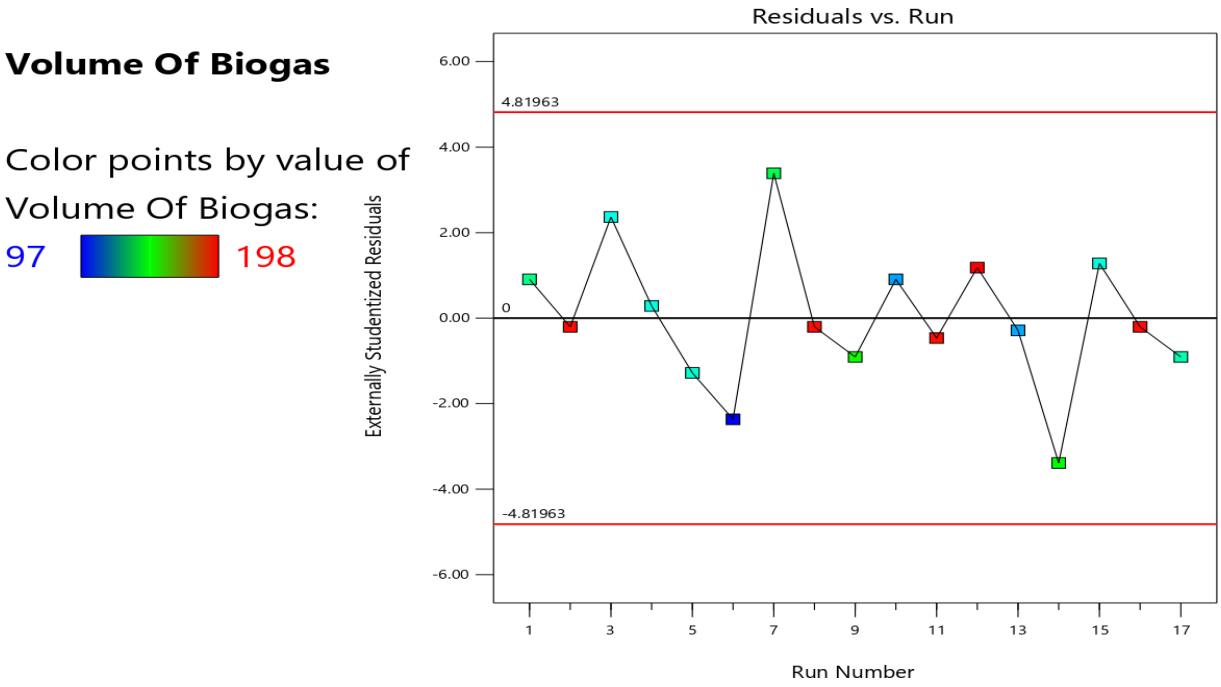


Figure 4.1: Residuals vs. run

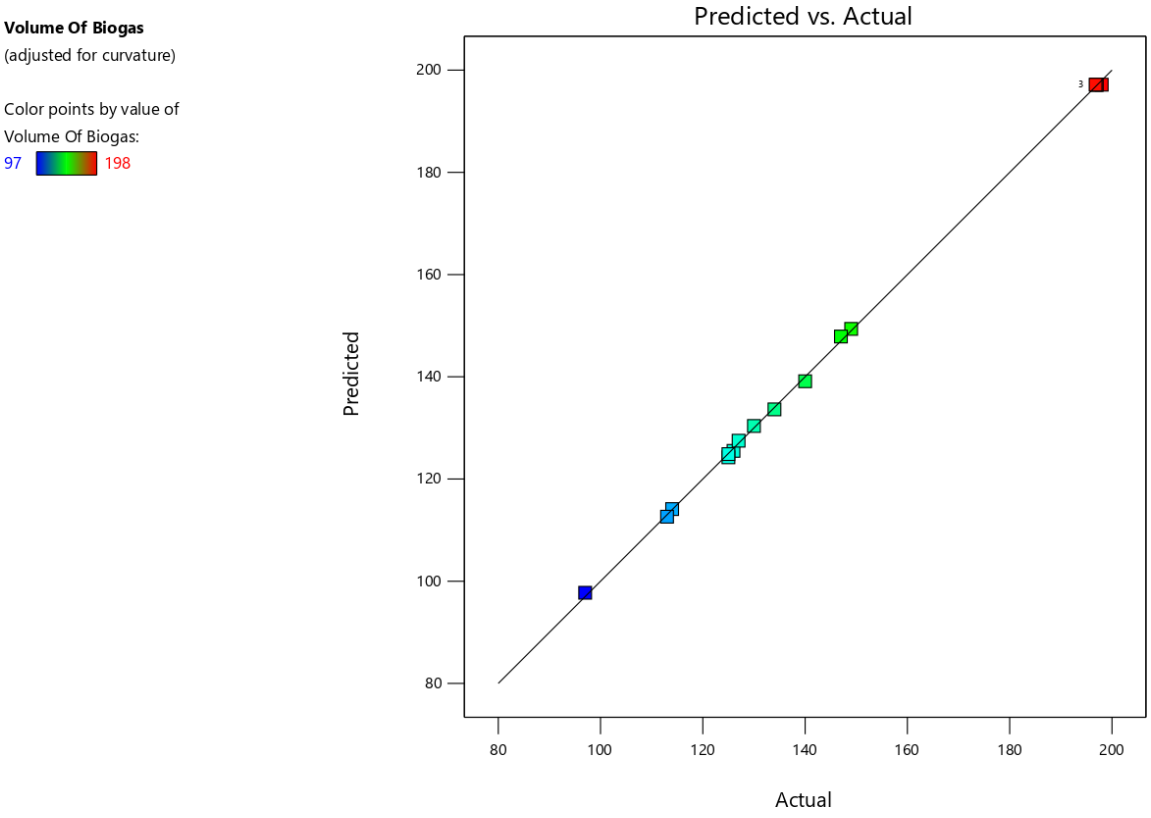


Figure 4.2: Predicted vs Actual

4.3.2. Temperature and pH interaction effects on biogas yield at constant substrate mix ratio 3

The 3D surface plot depicts the interactive effect of temperature and pH on biogas yield at an optimum substrate mix ratio of 3. Indeed, biogas production increases with a rise in temperature and pH toward the optimum range, with an optimum yield around mesophilic temperature (~37.5 °C) and near neutral pH (~7). Even when the temperature is favorable, the yield decreases at low or high pH values, which characterizes an inhibition of methanogenic activity. Likewise, temperatures either below or above the mesophilic range level correspondingly lower the biogas yield as a result of reduced microbial efficiency. A curved surface based on the observed values confirms a significant interaction of temperature and pH, thereby showing that optimal biogas production is obtained only when both parameters are maintained simultaneously at suitable levels.

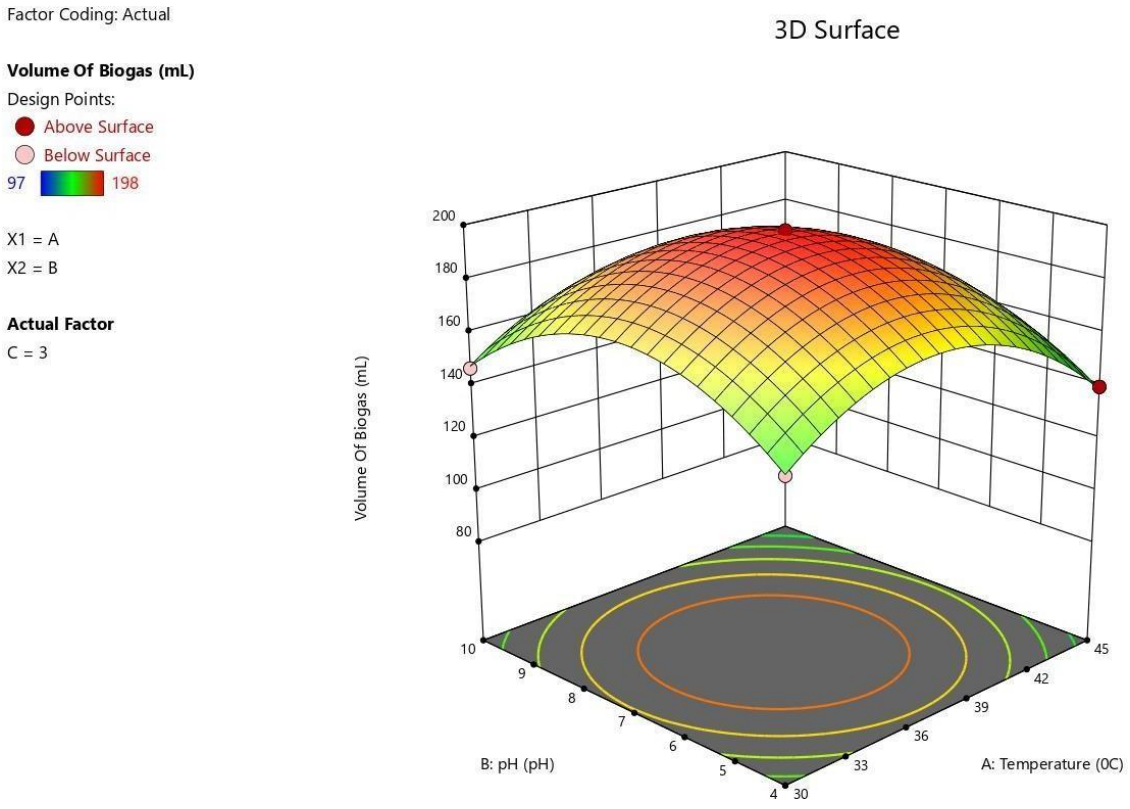


Figure 4.3: Temperature and pH interaction effects on biogas yield at a constant substrate mix ratio of 3

4.3.3. pH and substrate mix ratio interaction effects on biogas yield at constant temperature 37.5°C

From Figure 4.4: below in the 3D response surface, when keeping the temperature constant at 37.5°C, biogas yield increases with increasing pH and increasing mix ratio. Biogas production increases from an initial pH of 4 up to slightly greater than 7; local optima occur at approximately an initial pH of 7 due to the fact that methane-producing bacteria are most active under neutral or slightly alkaline conditions and thus very easily produce biogas and methane under these conditions. Such anaerobic digestion is performed by bacteria, which are optimal for a slightly neutral pH of about 7. If the balance point of the substrates mixture is established at the center, the digestion process will become stable, and biogas production will increase. The highest production in this present study has been established at a pH of 7, temperature of 37.5°C, and substrates mixture of 75% WS:25% CD (3:1 WS:CD). The reason it declined beyond these points was that both the biogas volume content decreased. This can be explained by the reduction of wheat straw content while volatile fatty acids and ammonia are accumulating. Furthermore, biogas output continued to fall when the substrate mix reached to a ratio of 5.95 because it had already passed the optimal peak and co-digestion efficiency diminished correspondingly.

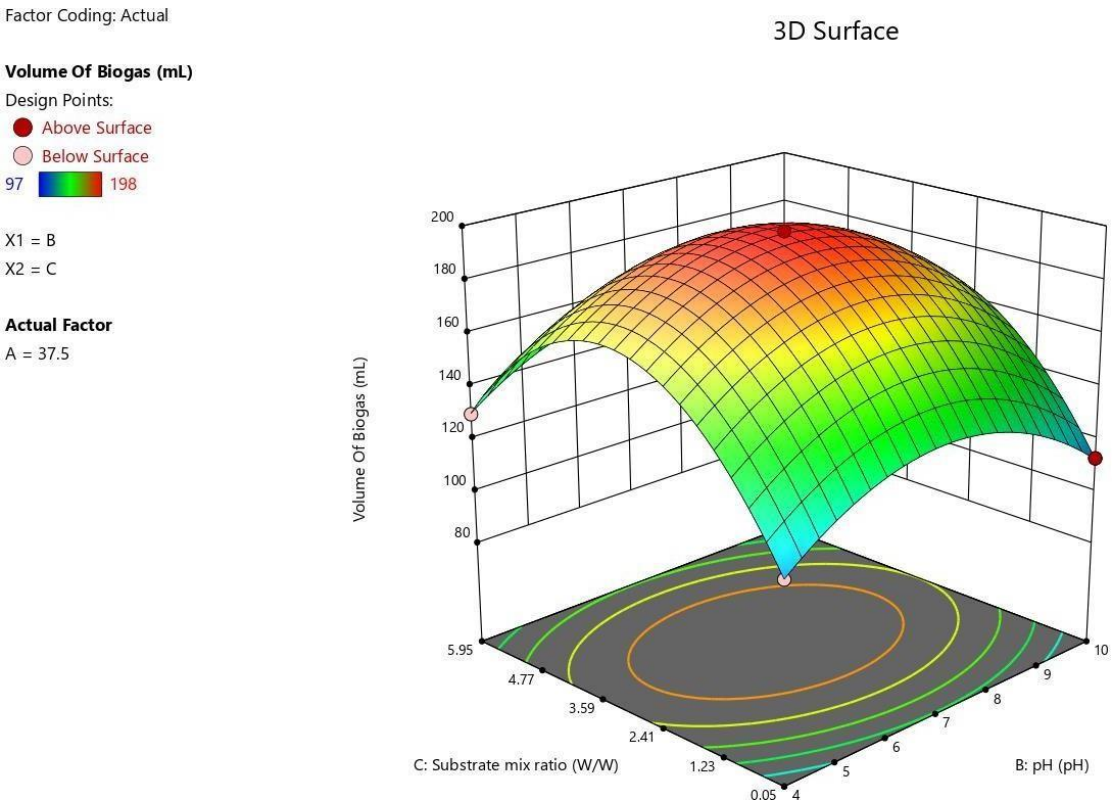


Figure 4.4: Interaction effects of pH and substrate mix ratio on biogas yield at a constant temperature 37.5ºc

4.3.4. Temperature and substrate mix ratio interaction effects on biogas yield at constant pH 7

The following 3D graph describes the relationship between temperature and ratio of substrate mixture and biogas production. In figure 4.5: changes in biogas production and ratio of the substrate mixture with temperature are indicated at a pH level of 7. The conclusion drawn from the below graph is that the initial value of biogas yield is very low, which increases along with the rise in temperature as well as the ratio values of the substrate mixture. The rise in temperatures from 30℃ to 37.5℃ indicates an increase in the total production of biogas, as the rate of reaction increases at a higher temperature. An increase in ratio values of the substrate mixture from 0.05 to 3 also has the same effect. The results indicated that the highest production of biogas was achieved at an S/M of 3, a temperature of 37.5 ℃, and pH of 7. The highest production of biogas was achieved at the optimum value of the peak S/M of about 3. With the increase in the volumes of waste, the value of the biogas yield gradually increased from 4.76% to

about 75% and then dropped, perhaps because of the decreased volumes of the substrates to nearer the optimum values of the process of disposing of the waste through co-digestion. It shows that the added volume of wheat straw results in decreased total solids concentrations, of which the major component is proteins, which are less biodegradable. Any value above the optimum will result in the decreased values of the concentrations of the production of biogas. The production of biogas will be reduced with the increase in temperature to 45 ℃ because of the increase in the values of the concentration of free ammonia, which works as a biogas inhibitor, alongside the incremental values of temperature.

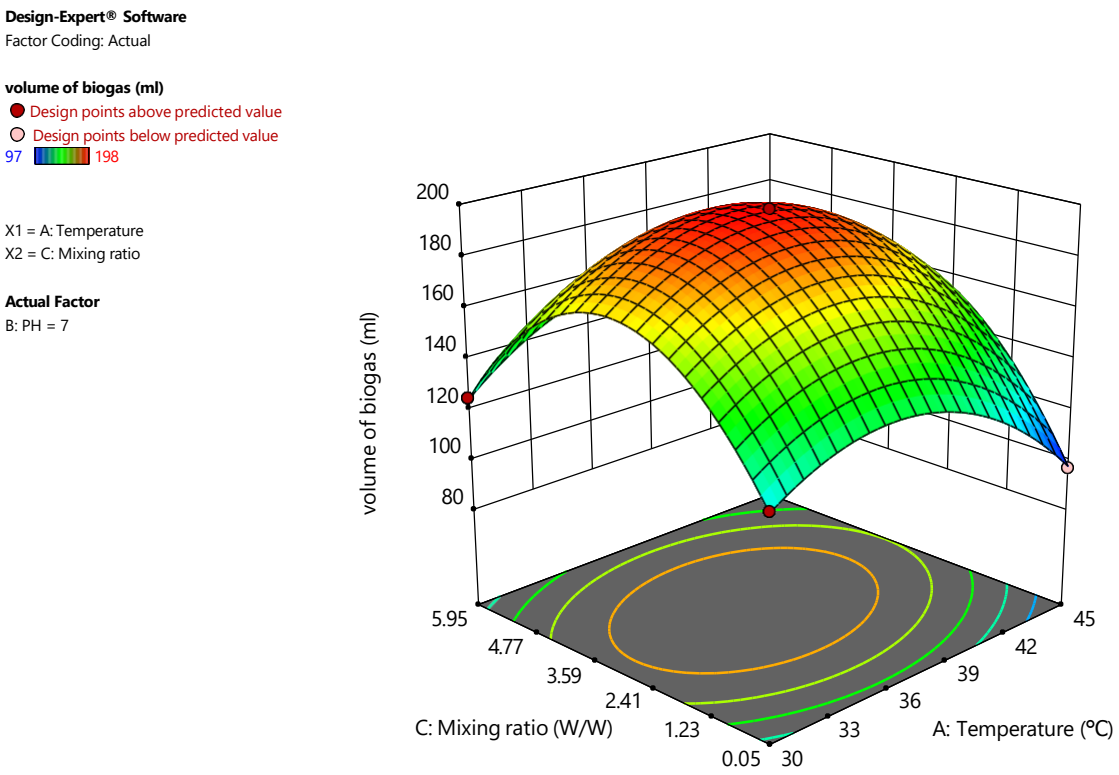


Figure 4.5: Temperature and substrate mix ratio interaction effects on biogas yield at constant pH=7

4.3.5. Effect of all combined factors for volume of biogas

A perturbation plot for the point, as shown in Figure 4.6: will provide us with even better insight into the behavior of the response as we change the value of a factor slightly while holding the values of the remaining factors unchanged. According to the statement above, the steeper the line in the perturbation plot, the higher the effect of the factor on the response. For multiple factors in

your problem, the perturbation plot is an excellent tool for determining the factor that affects the response most. According to the above diagram, all three factors affect the response of the volume of the produced biogas equally. The above graph shows that with the increase of any of the three factors, the volume of the produced biogas will increase; at least at the reference point, the three have equal effect on the volume of the produced biogas. But after the reference point, the effect of the three on the yield of the molybdenum extraction is different.

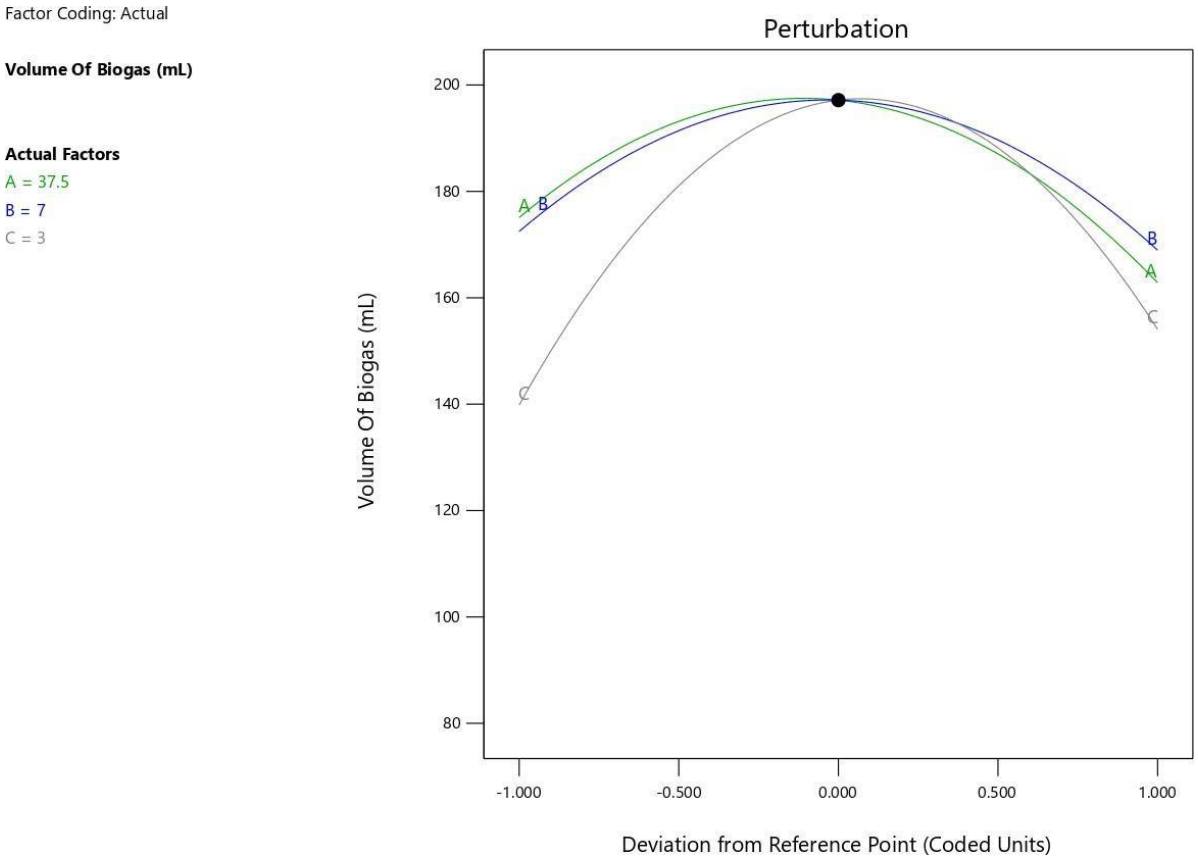


Figure 4.6: Perturbation plots that compare each factor‘s effect at a particular point in the design space volume of biogas

4.4. ANOVA for quadratic model of composition of methane gas

Response 2: Composition of methane gas

Table 4.8: Composition of methane gas

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value	
Model	7422.69	9	824.74	4237.91	< 0.0001	Significant

A-Temperature	6.86	1	6.86	35.27	0.0006	
B-Ph	188.67	1	188.67	969.45	< 0.0001	
C-Substrate mix ratio	1580.34	1	1580.34	8120.54	< 0.0001	
AB	1.10	1	1.10	5.67	0.0489	
AC	17.26	1	17.26	88.71	< 0.0001	
BC	5.88	1	5.88	30.22	0.0009	
A^2	351.94	1	351.94	1808.42	< 0.0001	
B^2	217.21	1	217.21	1116.15	< 0.0001	
C^2	4733.73	1	4733.73	24324.10	< 0.0001	
Residual	1.36	7	0.1946			
Lack of Fit	0.0823	3	0.0274	0.0857	0.9642	not significant
Pure Error	1.28	4	0.3200			
Cor Total	7424.05	16				

It also takes into consideration how variables in a proposed model are coded.

All of this is reflected in the Type III Sum of Squares plot seen above.

The F-statistic value from the graph representing the fit of the model on the given data is 4237.91. Since the probability value for the F-statistic value of 0.01% is significant, the result obtained from the F-statistic test is highly significant.

The values in the P-type columns that are less than 0.0500 indicate the significance of the model. In the given problem, it is found that the values of A, B, C, AB, AC, BC, A^2, B^2, and C^2 are the significant parameters in the given model. Values in the P-type columns that are above 0.1000 specify nonsignificant parameters. If a number of parameters are found to be nonsignificant and are not in hierarchical order.

In the Lack of Fit test, the F value is 0.09. Lack of Fit is therefore not significant in relation to the pure error. Probability of a Lack of Fit F value at least as extreme as this, under the hypothesis that pure error = zero: 96.42% So, non-significance for lack of fit is a good thing—it‘s what you want in a good model.

Table 4.9: Fit statistics for composition of methane gas

Std. Dev.	*0.4411*	*R^2*	*0.9998*
Mean	38.49	Adjusted R^2	0.9996
C.V. %	1.15	Predicted R^2	0.9996
		Adeq Precision	179.8057

The value for R-squared is expected to be 0.9996. There is a small tolerance given between the value of Adjusted R-squared and R-squared where the value is less than 0.2. The requirement is

that there is a need for the value of Adequate Precision to be greater than 4. In this case, the value is 179.806, which means that it is a large value which can result in a good signal for finding a design for a good model.

The final equation is formed using the coded variables.

Composition of methane gas =

$$+61.95+0.9262A-4.86B+14.06C-0.5250AB-2.08AC+1.21BC-9.14A^2-7.18B^2-33.53C^2 \qquad 4\text{-}3$$

A coded equation form allows us to make predictions by assigning values to the factors. Usually, +1 represents the high value and -1 represents the low value. Since it is a coded equation, it is now possible to compare the values of the factors to see which factor makes a greater contribution.

The final form of the equation using the actual factors:-

Composition of methane gas =

$$-261.79813+12.75853A+10.01801B+30.44405C-0.023333AB-0.093898AC+0.137006BC-0.162533A^2-0.798056B^2-3.85292C^2 \qquad 4\text{-}4$$

The model formula, using real values of factors, is easily used to compute estimates based on any combination of factor levels on their original units. Yet it is not helpful in determining their significance because it is scale-dependent, and its constant term is not centered at the design region‘s center.

4.4.1. Model adequacy checking for composition of methane

As shown in Figure 4.7: residuals are measured alongside a series of trials to ensure that there are not any lurking variables at work to affect the outcome. The data appears to be randomly scattered, which is a great sign—the absence of additional blocking required. Finally, in Figure 4.8: the actual values are pitted side by side with the predictions from the model to determine the quality of the predictions as well as to identify where the model goes awry. The data is almost linear, as can be observed from the figure.

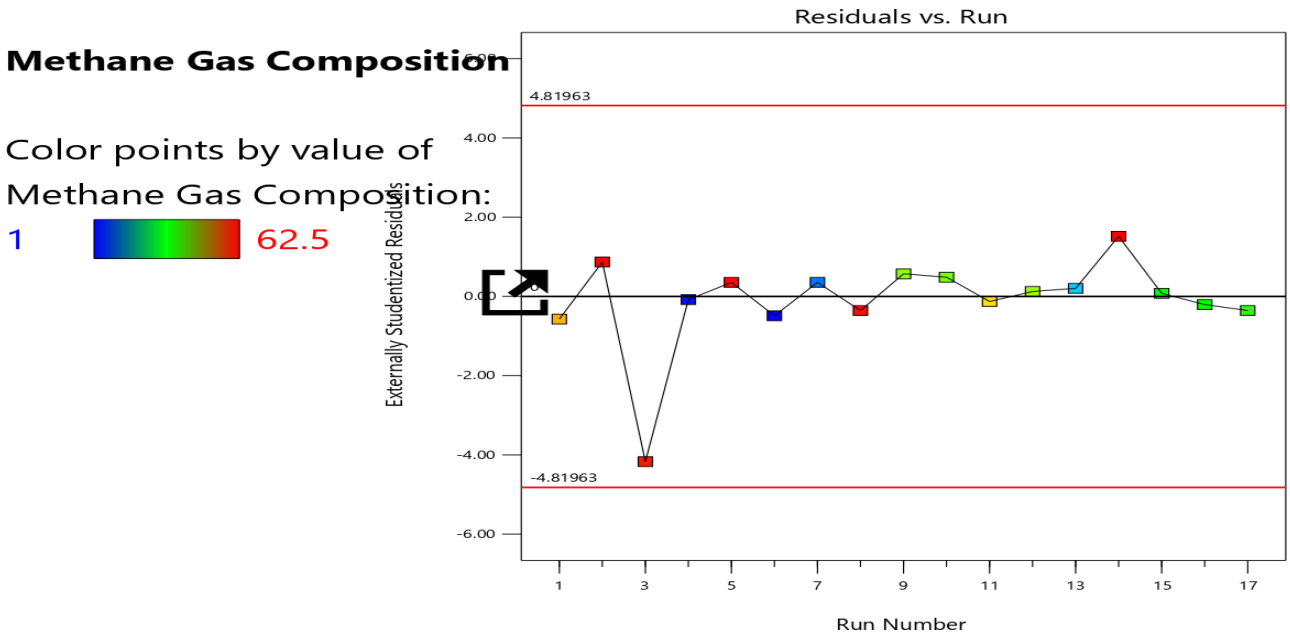


Figure 4.7: Residuals vs. run

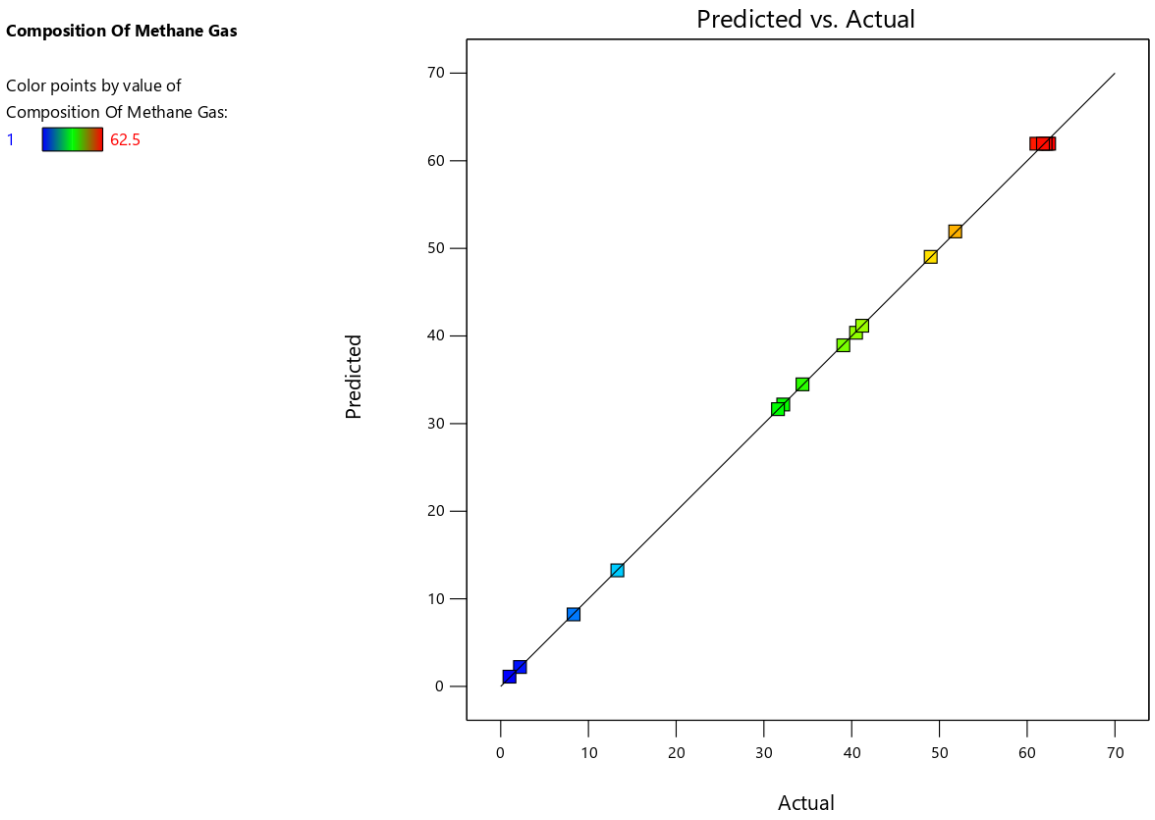


Figure 4.8: Predicted vs. actual

4.4.2. Temperature and pH interaction effects on methane gas at constant substrate mix ratio 3

It is depicted as a 3D surface plot showing the effects of the interactions of temperature levels/pH on methane compositions when the ratio of the substrates is kept constant at 3, as illustrated in figure 4.9: It is observed that the methane levels improve when the temperature is close to the mesophilic condition of approximately 37.5°C, while the pH is close to the neutral condition of approximately 7. This is also where the methane compositions are at their best, indicating the best growth environment for the methanogens.

On the other hand, the level of methane decreases as the pH levels decrease due to the acidic inhibition of methanogens even at the optimum temperature. At increased pH levels or temperature far from the optimum of the mesophilic temperature range, the level of methane decreases due to microbial stress. This can be ascertained by the curved nature of the 3-D surface as it confirms the significant interaction between the two variables.

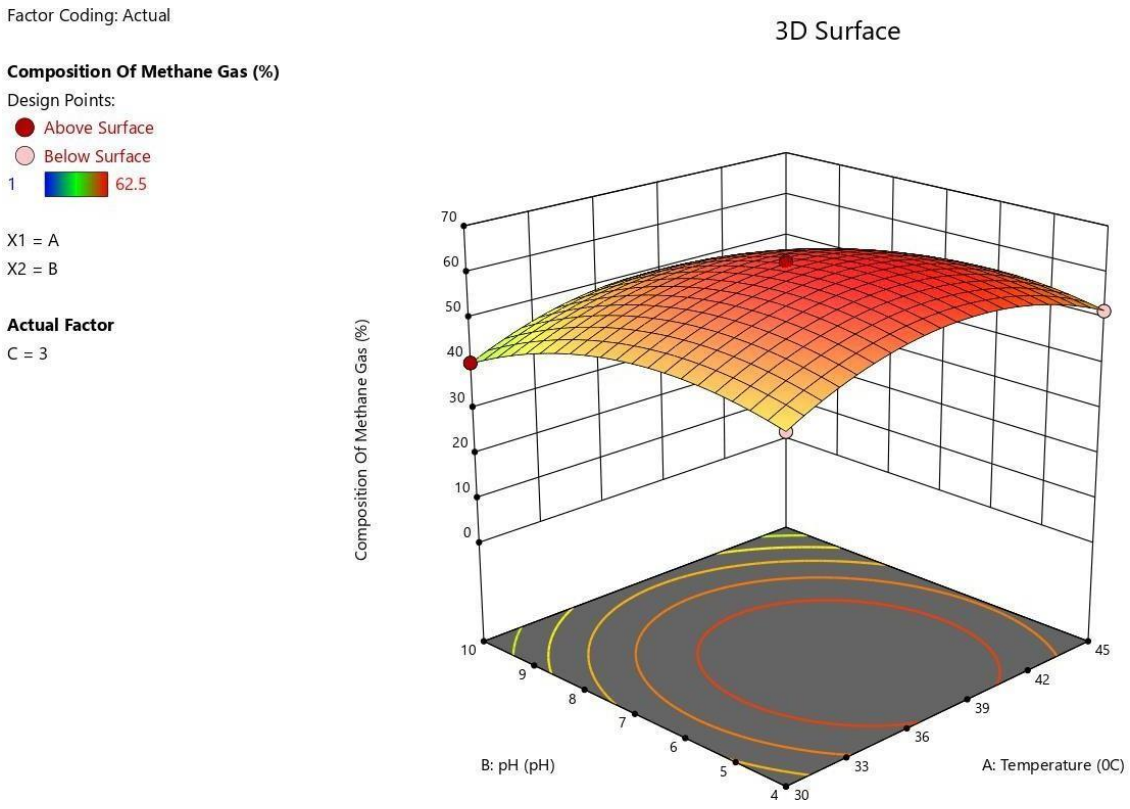


Fig 4.9: Temperature and pH interaction effects on composition of methane gas at constant substrate mix ratio 3

4.4.3. pH and substrate mix ratio interaction effects on methane gas at constant temperature 37.5°C

The 3D Surface Plot in Section 4.4.3 showcases the effect of pH level and substrate mix ratio on methane composition, under a constant temperature level of 37.5°C. As observed, methane level increases with the mimics of moving towards a pH level that is near neutral, along with substrate mixes near optimal that have a dominant amount of wheat straw and cow dung. The maximum composition of methane can be determined when the value of pH is approximately 7 with the mix ratio of the substrate being 3, i.e., 75% wheat straw and 25% cow dung. When the value of pH is either low or high, the composition of methane is reduced due to acidification or the effect of ammonia. Also, the mix ratio of the substrate being either high or low results in the composition of methane being reduced by the lack of synergies due to the imbalance of the substrate. The curve in the response surface confirmed the significant interaction that occurred between the levels of pH and the ratios of the mixed substrates, and in the process, the

production of methane-rich biogas with the optimized levels of pH and mixed substrates fed into the digester was proven as a requirement.

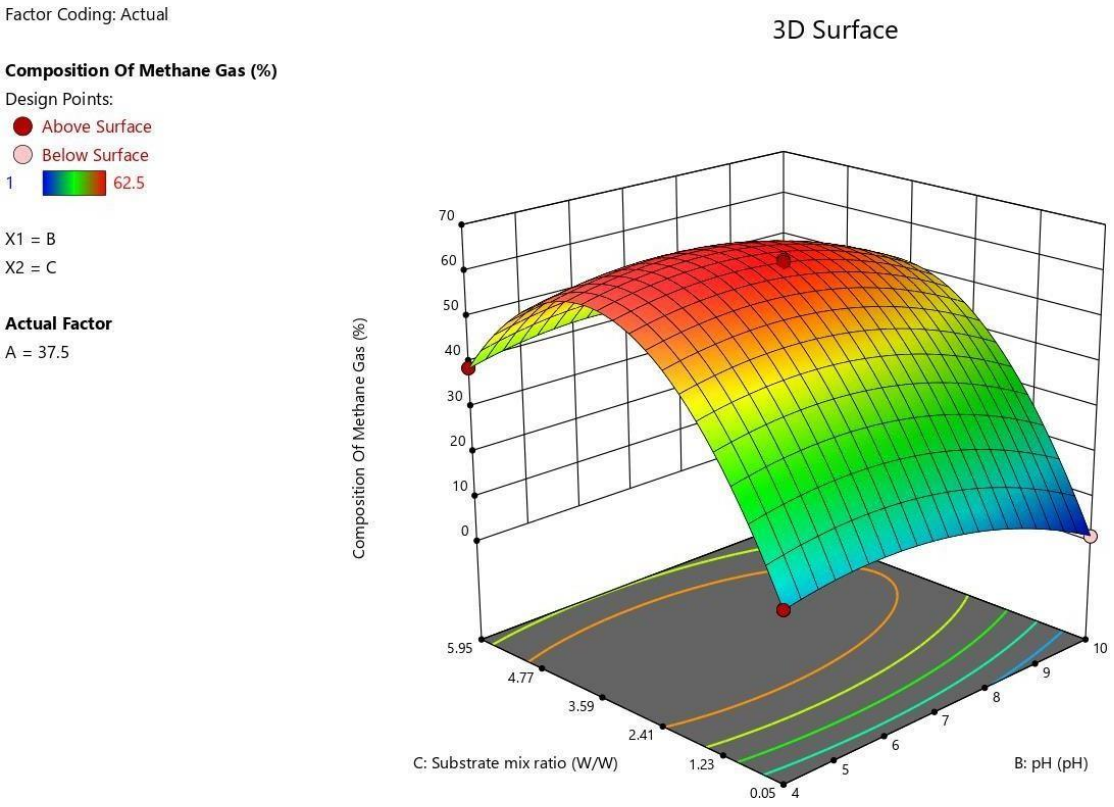


Figure 4.10: pH and substrate mix ratio interaction effects on composition of methane gas at a constant temperature 37.5ºc

4.4.4. Temperature and substrate mix ratio interaction effects on methane gas at constant pH 7

The interaction between temperature and substrate mix ratio on methane composition, under a constant pH level of 7, is also shown in figure in 4.11: on the 3D surface plot in figure in 4.11: .The methane concentration increases as the temperature is near the mesophilic range, while the substrate mix ratio is near the optimal mixture of wheat straw/dung.

The highest composition of methane is achieved when temperature is maintained at 37.5°C in a ratio of 3 in terms of a mix of substrates in which 75% wheat straw and 25% cow dung are present. When temperature is low or high in comparison to this range, methane composition decreases in spite of a high mix of substrates in terms of ratio, which clearly explains inefficient methanogenesis. Similarly, in extreme ratios of mix composition of wheat straw and cow dung, methane composition decreases due to inefficient synergy between carbon and nitrogen content in the mix of substrates.

The shape of the response surface also confirms the strong interaction existing between temperature and substrate mix ratio, validating the conclusion that both parameters need to be optimized to achieve a high methane composition while maintaining a stable status under a natural pH.

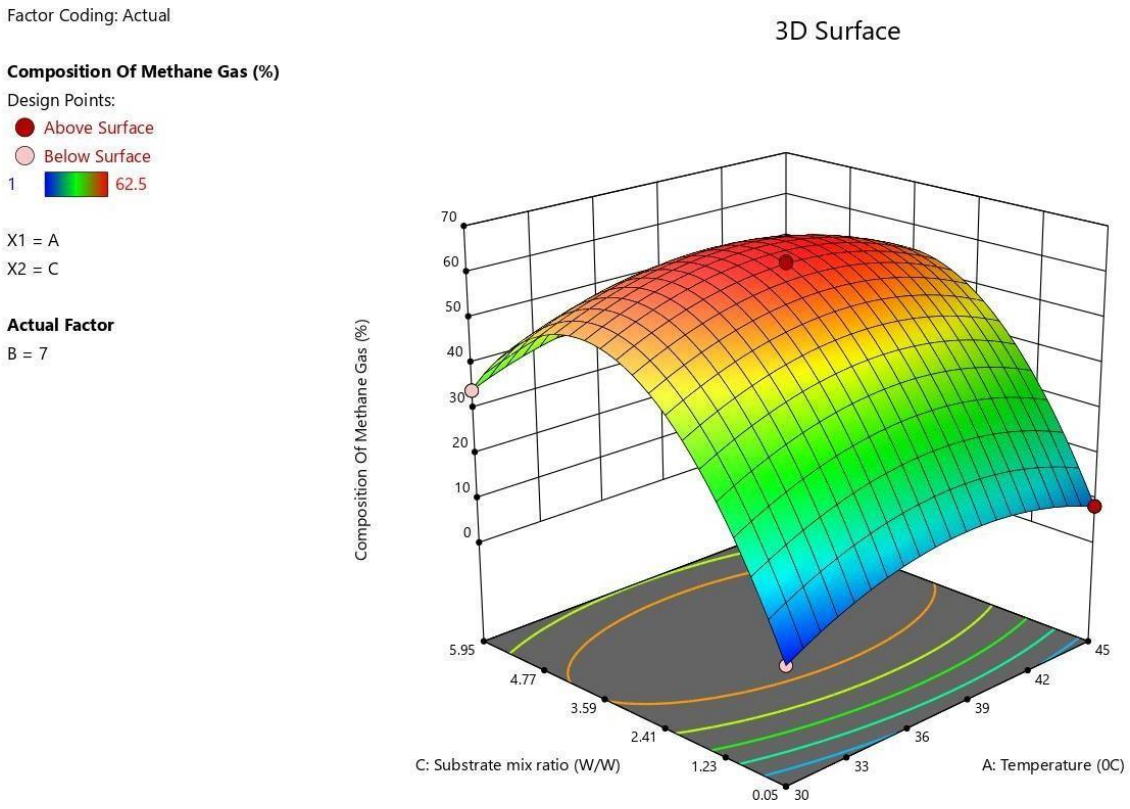


Figure 4.11: Temperature and substrate mix ratio interaction effects on composition of methane gas at a constant pH=7

4.4.5. Comparison of the factor effect at a point for composition of methane gas

In Figure 4.12: it is evident that the perturbation graph works in highlighting how methane levels at a particular location are sensitive to each of the process variables. For this graph, there is nothing complicated in understanding the concept of change in one variable against the other fixed variables at their original levels. If the graph provides sharply climbing results or in the form of curves, it means there is greater sensitivity, while a straight line indicates low sensitivity. It‘s evident from the graph of the perturbation that an increase in each of the three parameters will help in increasing the levels of methane, though there is always a limit to which these values cannot go. Each of the three parameters is equally important, though the ratio of the substrate is the most critical parameter in increasing methane synthesis in anaerobic co-digestion. After crossing the peak, values for the parameters will decrease methane contents.

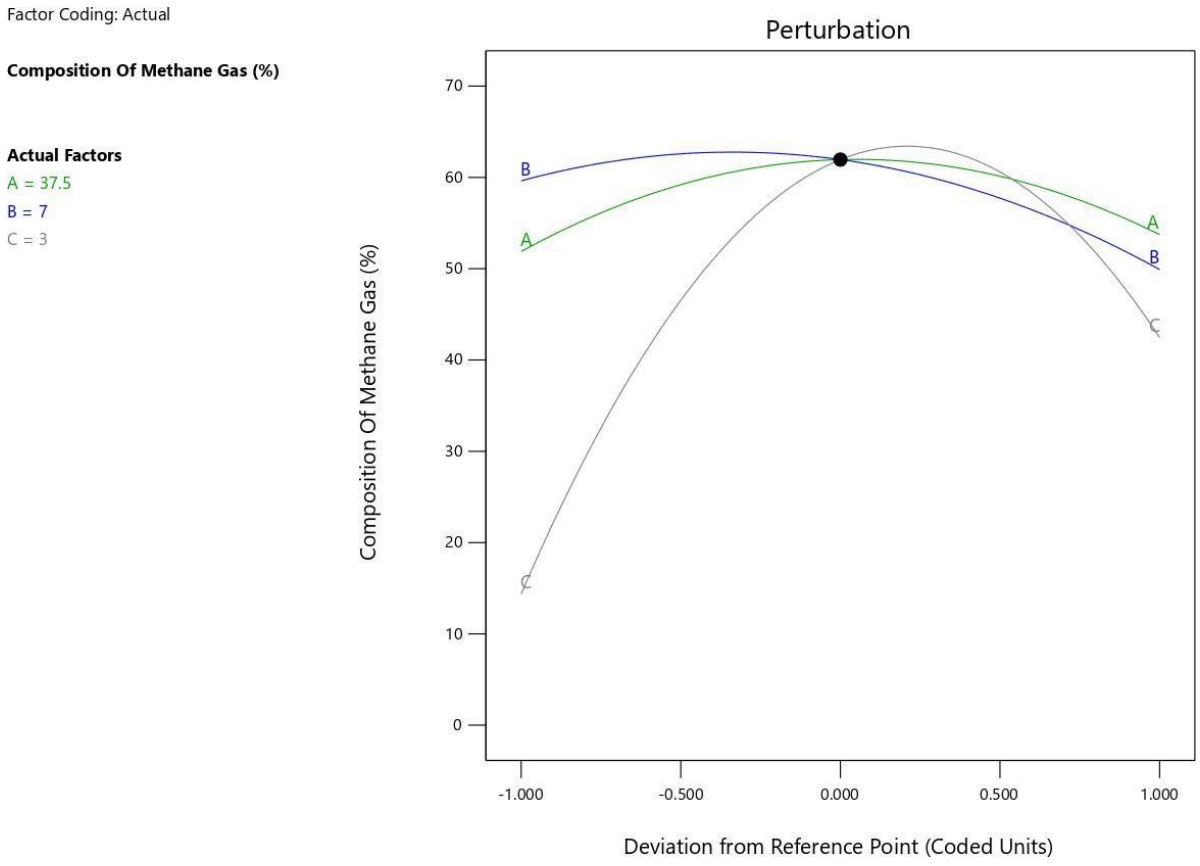


Figure 4.12: Perturbation plots that compare each factor‘s effect at a particular point in the design space composition of methane gas

4.5. Optimization

4.5.1. Exploring the optimum mix for maximum production of biogas

In this respect, the Box-Behnken design in Design-Expert software is used to study the impact of straw waste to cow dung ratio at variable temperature and pH to guide the output. The overall experiments that were conducted, under varying conditions, summed up to 17 in total. Each reactor used 75% straw waste and 25% cow dung as a liquid substrate in a combined volume of 375 ml.

4.5.2. Model desirability and optimization conditions

The optimization techniques, with the use of numbers, shall be the most effective technique in addressing the target function in the research study. The use of methane gas with pH, temperature, and components involved in the context of the research study shall allow for the components in the research study to be explained due to their involvement in the formula of

methane gas as well as in the process of forming biogas. The resulting optimal result shall be acquired through the use of a combination of WS+CD, with a value of 6.903 pH, 36.76°C temperature, total amount of 3.18701 units, and desirability of 0.999 for elements of WS+CD with a combination of 75% WS and 25% CD.

Table 4.10: Conditions for optimization and desirability of solutions

Number	Temperature	pH	Substrate Mix Ratio	Volume of Biogas	Composition of Methane Gas	Desirability	
1	36.7619	6.90291	3.18701	197.719	62.685	0.999	Selected

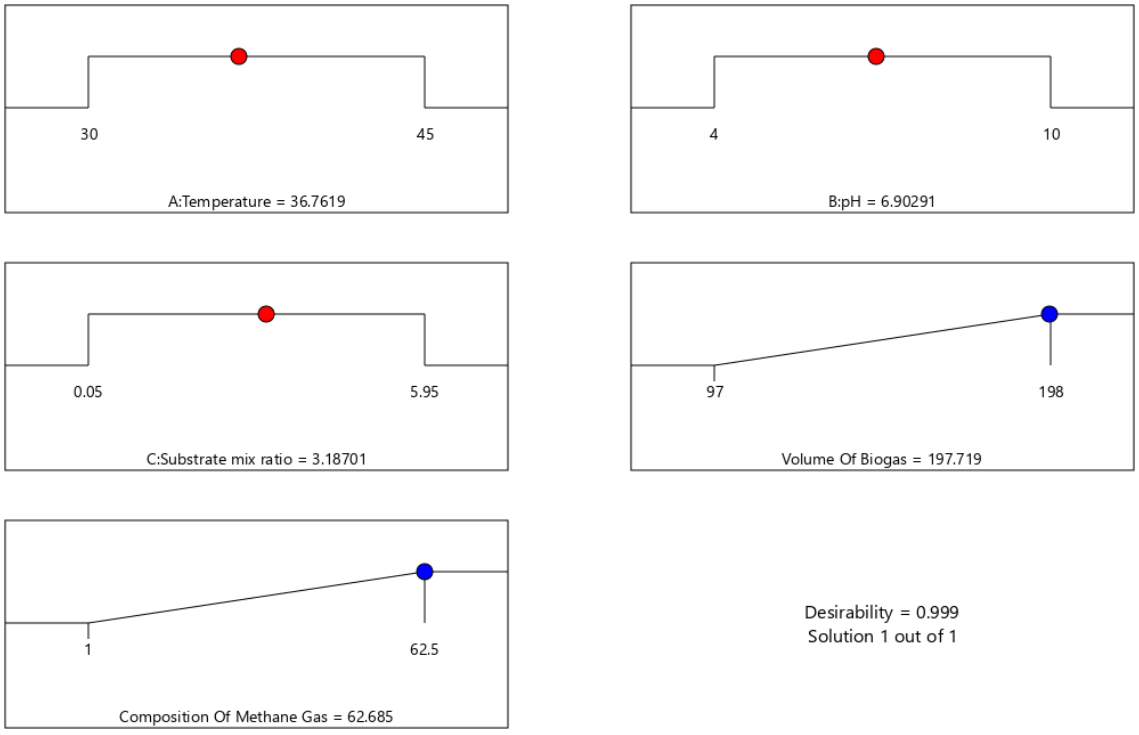


Figure 4.13: Optimization conditions and desirability for volume of biogas and composition of methane gas

4.5.3. Model validity

The result revealed that the optimal solution was obtained using the response surface method. The optimization process was an interactive procedure where it focused on optimizing the key factors (Wanasolo. et al., 2014). In summary, the response surface method described how pH, temperature, and mixture ratio affected the key results, which include total biogas and methane content in biogas.

Table 4.11: Model validity

	36.7619	6.90291	3.186
Responses	Model predicted value	Current study	References
Volume of Biogas	197.719	198	(Temesgen Mitiku 2017)
Composition of Methane Gas	62.685	62.5	(Claudia and Yitayal 2019)

In this experiment, I tried to test the biogas produced through the co-digestion of wheat straw and cow dung. Experimental Conditions: The experiment is done at pH 7, a temperature of 37.5°C, and at ratios of 3:1 for wheat straw to cow dung. Result obtained from experiment:

The result obtained showed that the volume of biogas produced is 198 ml with a value of 62.5% for the composition of biogas. Result from models: The result obtained shows that the biogas produced has values of 197.719 ml for average volume and 62.685% for composition of biogas.

Conclusion: From the obtained result, there seems to be no difference between the results obtained from experiments and those obtained from models.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The primary aim of this study was to optimize biogas production through the co-digestion of cow dung and wheat straw wastes. Based on the experimental results, several key conclusions can be drawn. Anaerobic digestion of organic wastes offers a sustainable alternative energy source while simultaneously addressing waste management challenges. In this study, batch anaerobic digestion experiments were performed to determine the optimal substrate mix ratio for cow dung and wheat straw co-digestion. The experiments were conducted over a 35-day digestion period using a 0.5 L laboratory-scale batch digester operated under mesophilic conditions at 37.5°C. Among the tested substrate combinations, the highest biogas yield was achieved at a wheat straw to cow dung ratio of 75%:25%, producing a maximum biogas volume of 198 mL with a methane concentration of 62.5%. These results confirm that co-digestion improves biogas generation by balancing substrate characteristics and enhancing microbial activity. Overall, the co-digestion of cow dung and wheat straw represents an effective approach to overcoming feedstock limitations in biogas production systems. This strategy not only improves methane yield but also promotes the efficient utilization of agricultural residues, contributing to sustainable energy generation and environmental protection.

5.2. Recommendation

Based on the findings from this research work, the following recommendations are proposed for future research and practical application:

- Further investigations should be conducted to evaluate the agronomic suitability of the digestate produced after anaerobic digestion, particularly its potential use as an organic fertilizer.
- Additional studies are recommended to comprehensively analyze the quality and composition of biogas generated from different substrate mixing ratios using advanced gas analysis techniques. Based on the findings from this research work, various promising areas for future research are now visible.

- It is necessary to perform a techno-economic analysis to relate the concentration yield of 62.5% of methane to the cost savings in terms of the cost of substitution of wood

REFERENCES

Aangelidaki, I., B. K. Ahrin, H. Deng, and J. E. Schmidt. 2002. ―Anaerobic Digestion of Olive Oil Mill Effluents Together with Swine Manure in UASB Reactors.‖ *Water Science and Technology : A Journal of the International Association on Water Pollution Research* 45 (10): 213–18. https://doi.org/10.2166/wst.2002.0334.

Abdelgadir, Awad, Xiaoguang Chen, Jianshe Liu, Xuehui Xie, Jian Zhang, Kai Zhang, Heng Wang, and Na Liu. 2014. ―*Characteristics, Process Parameters, and Inner Components of Anaerobic Bioreactors*.‖ BioMed Research International 2014. https://doi.org/10.1155/2014/841573.

Al-Da‘asen, A., et al. (2024). *Optimization of anaerobic microbial degradation for enhanced biogas yield from lignocellulosic biomass*. Renewable Energy, 219**,** 119–131. https://doi.org/[DOI.

Ajiboye, A. V., Lasisi, K. H., & Babatola, J. O. (2018). Evaluation of the effect of sodium hydroxide solution on biogas yield of anaerobic digestion of poultry waste and the digestate. *International Journal of Energy and Water Resources, 2*(1), 23–31. https://doi.org/10.1007/s42108-018-0003-2

Almeida, P. V., R. P. Rodrigues, M. C. Gaspar, M. E.M. Braga, and M. J. Quina. 2021. ―*Integrated Management of Residues from Tomato Production: Recovery of Value-Added Compounds and Biogas Production in the Biorefinery Context*.‖ Journal of Environmental Management 299 (January): 113505. https://doi.org/10.1016/j.jenvman.2021.113505.

Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2011). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science, 34*(6), 755–781. https://doi.org/[DOI.

Aragaw. 2013. ―*Co-Digestion of Cattle Manure with Organic Kitchen Waste to Increase Biogas Production Using Rumen Fluid as Inoculums*‖ *International Journal of Physical Sciences,* 8 (11): 443–50. https://doi.org/10.5897/IJPS2013.3863.

Assefa, Gizachew. 2020. ―*Effect of Co-Digestion of Food Waste and Cow Dung on Biogas Yield*.‖ E3S Web of Conferences 181. https://doi.org/10.1051/e3sconf/202018101005.

Ayu, A. O., & Aryati, V. D. (2010). *Biogas production using anaerobic biodigester from cassava starch effluent with ruminant bacteria as biocatalyst* (Unpublished bachelor's thesis). Diponegoro University, Indonesia.

Babaei, Azadeh, and Jalal Shayegan. 2019. ―*Effects of Temperature and Mixing Modes on the Performance of Municipal Solid Waste Anaerobic Slurry Digester* 09 Engineering 0907 Environmental Engineering 09 Engineering 0904 Chemical Engineering.‖ Journal of Environmental Health Science and Engineering 17 (2): 1077–84. https://doi.org/10.1007/s40201-019-00422-6.

Badger, D. M., [other authors if any], et al. (1979). Methane production from agricultural residues. *Biotechnology and Bioengineering, 21*, 182–190. https://doi.org/[DOI.

Bahl, Hubert, Wolfram Andersch, Konstantin Braun, and Gerhard Gottschalk. 2019. ―*Effect of PH and Butyrate Concentration on the Production of Acetone and Butanol by Clostridium Acetobutylicum Grown in Continuous Culture.*‖ European Journal of Applied Microbiology and Biotechnology 14 (1): 17–20. https://doi.org/10.1007/BF00507998.

Bailie, A., Jamison, A., Peterson, T., Hausker, K., & Roe, S. (2006). *Pennsylvania greenhouse gas inventory and reference case projections* (Draft report). Pennsylvania Environmental Council & The Center for Climate Strategies. https://citeseerx.ist.psu.edu/document?doi=9b0659d897755272c864a5a69d83f1046aa8e8b4

Batstone, D. J., J. Keller, I. Angelidaki, S. V. Kalyuzhnyi, S. G. Pavlostathis, A. Rozzi, W. T. Sanders, H. Siegrist, and V. A. Vavilin. 2002. ―*The IWA Anaerobic Digestion Model No 1 (ADM1).*‖ Water Science and Technology : A Journal of the International Association on Water Pollution Research 45 (10): 65–73. https://doi.org/10.2166/wst.2002.0292.

Bell, Adam Edward, and Khire Ruz\xshikesh Ulhas. 2020. ―*Working to Reduce Food Waste: Investigating Determinants of Food Waste amongst Taiwanese Workers in Factory Cafeteria Settings.*‖ Sustainability (Switzerland) 12 (22): 1–23. https://doi.org/10.3390/su12229669.

Berhanu, Mesfin., Anuradha. Jabasingh, S.Kifile&, and Zebene. 2017. ―*Expanding Sustenance in Ethiopia Based on Renewable Energy Resources – A Comprehensive Review*.‖ Renewable and Sustainable Energy Reviews 75 (November 2015): 1035–45.

https://doi.org/10.1016/j.rser.2016.11.082.

Berhe, Melaku, Dana Hoag, Girmay Tesfay, and Catherine Keske. 2017. ―*Factors Influencing the Adoption of Biogas Digesters in Rural Ethiopia.*‖ Energy, Sustainability and Society 7 (1). https://doi.org/10.1186/s13705-017-0112-5.

Bernardi, M. (2000). *Application software developed by FAO for management of soils and crops data* (FAO software documentation). Food and Agriculture Organization of the United Biljetina, R., Srivastava, V. J., Chynoweth, D. P., & Hayes, T. D. (1987). Anaerobic digestion of water hyacinth and sludge. In K. R. Reddy & W. H. Smith (Eds.), *Aquatic plants for water treatment and resource recovery* (pp. 725–753). Magnolia Publishing, Inc.

Biljetina, R., Srivastava, V. J., Chynoweth, D. P., & Hayes, T. D. (1987). Anaerobic digestion of water hyacinth and sludge. In W. H. Smith & K. R. Reddy (Eds.), *Aquatic plants for water treatment and resource recovery* (pp. 725–738). Magnolia Press**.**

Blazek, James, MICHAEL F. V.O'ROURKE, Charles L. Morreels, and L. Jerome Krovetz. 2009. ―*Pressure Wave Transmission along the Human Aorta*.‖ Circulation Research 23 (4): 567–79. https://doi.org/10.1161/01.res.23.4.567.

Boer, Emilia den, Andrzej Jedrczak, Zygmunt Kowalski, Joanna Kulczycka, and Ryszard Szpadt. 2010. ―*A Review of Municipal Solid Waste Composition and Quantities in Poland*.‖ Waste Management 30 (3): 369–77. https://doi.org/10.1016/j.wasman.2009.09.018.

Bolzonella, D., P. Battistoni, J. Mata-Alvarez, and F. Cecchi. 2003. ―*Anaerobic Digestion of Organic Solid Waste: Process Behaviour in Transient Conditions.*‖ Water Science and Technology 48 (4): 1–8. https://doi.org/10.2166/wst.2003.0207.

Bouallagui, H., O. Haouari, Y. Touhami, R. Ben Cheikh, L. Marouani, and M. Hamdi. 2004. ―*Effect of Temperature on the Performance of an Anaerobic Tubular Reactor Treating Fruit and Vegetable Waste*.‖ Process Biochemistry 39 (12): 2143–48. https://doi.org/10.1016/j.procbio.2003.11.022.

Bradstreet, R. B. (1965). The Kjeldahl method for organic nitrogen. Academic Press.

Braun, R., & Wellinger, A. (2003). *Potential of co-digestion* (IEA Bioenergy Task 37 technical report). IEA Bioenergy. https://task37.ieabioenergy.com/technical-reports/potential-of-co-digestion.

Brown., Dan., J Shi, Ian, and Yebo Li. 2012. ―*Comparison of Solid-State to Liquid Anaerobic Digestion of Lignocellulosic Feedstocks for Biogas Production.*‖ Bioresource Technology 124: 379–86. https://doi.org/10.1016/j.biortech.2012.08.051.

Browne, J. D. (2014). *Biomethane production from food waste and organic residues* (PhD thesis). University College Cork. https://hdl.handle.net/10468/1362; https://doi.org/10.13140/2.1.2062.5282.

Budiastuti, Herawati., Bandung Negeri., Politeknik, Cord-ruwisch, and Ralf. 2013. ―*Effect of Feeding Patterns towards Methane and Volatile Fatty Acids Production in Single Stage Stirred Tank Anaerobic Digesters, Journal of Environmental Bioenergy*.‖ no. 333

Bye, Nicole, Mark D. Habgood, Jennifer K. Callaway, Nakisa Malakooti, Ann Potter, Thomas Kossmann, and M. Cristina Morganti-Kossmann. 2007. ―*Transient Neuroprotection by Minocycline Following Traumatic Brain Injury Is Associated with Attenuated Microglial Activation but No Changes in Cell Apoptosis or Neutrophil Infiltration*.‖ *Experimental Neurology* 204 (1): 220–33. https://doi.org/10.1016/j.expneurol.2006.10.013.

Callaghan, F. J., D. A.J. Wase, K. Thayanithy, and C. F. Forster. 2019. ―*Co-Digestion of Waste Organic Solids: Batch Studies.*‖ Bioresource Technology 67 (2): 117–22. https://doi.org/10.1016/S0960-8524 (98)00108-4.

Cesaro, A., Belgiorno., V., and M. Guida. 2015. ―*Compost from Organic Solid Waste: Quality Assessment and European Regulations for Its Sustainable Use.*‖ Resources, Conservation and Recycling 94: 72–79. https://doi.org/10.1016/j.resconrec.2014.11.003.

Chen, Di, Ming. Zhao, Mundy., and Gregory R. 2004. ―*Bone Morphogenetic Proteins*.‖ Growth Factors 22 (4): 233–41. https://doi.org/10.1080/08977190412331279890.

Chen, Shengjie, Ziletao Tao, Fubing Yao, Bo Wu, Li He, Kunjie Hou, Zhoujie Pi, et al. 2020. ―*Enhanced Anaerobic Co-Digestion of Waste Activated Sludge and Food Waste by Sulfidated Microscale Zerovalent Iron: Insights in Direct Interspecies Electron Transfer*

Mechanism.‖ Bioresource Technology 316 (May). https://doi.org/10.1016/j.biortech.2020.123901.

Chibueze, U., N. Okorie, O. Oriaku, J. & Isu, and E. Peters. 2017. ―*The Production of Biogas Using Cow Dung and Food Waste.*‖ International Journal of Materials and Chemistry 7 (2): 21–24. https://doi.org/10.5923/j.ijmc.20170702.01.

Chugh, Lakshaman Kumar, and Surinder Kumar Sawhney. 2009. ―*Photosynthetic Activities of Pisum Sativum Seedlings Grown in Presence of Cadmium.*‖ Plant Physiology and Biochemistry 37 (4): 297–303. https://doi.org/10.1016/S0981-9428(99)80028-X.

Claudia, R., & Yitayal, A. (2019). *Enhancing biogas production through innovative pretreatment methods and co-digestion strategies*. Journal of Sustainable Energy, 12(4), 250–265. https://doi.org/10.1016/j.susenergy.2019.04.015

Croce, S., Wei, Q., D'Imporzano, G., Dong, R., & Adani, F. (2016). *Anaerobic digestion of straw and corn stover: The effect of biological process optimization and pre-treatment on total bio-methane yield and energy performance.* Biotechnology Advances, 34(8), 1289–1304. https://doi.org/10.1016/j.biotechadv.2016.09.004

Cuéllar, Amanda D., and Michael E. Webber. 2008. ―C*ow Power: The Energy and Emissions Benefits of Converting Manure to Biogas.*‖ Environmental Research Letters 3 (3). https://doi.org/10.1088/1748-9326/3/3/034002.

Dearman, B., & Bentham, R. H. (2007). Anaerobic digestion of food waste: Comparing leachate exchange rates in sequential batch systems digesting food waste and biosolids. *Waste Management, 27*(12), 1792–1799. https://doi.org/10.1016/j.wasman.2006.08.006

Deublein, Dieter, and Angelika Steinhauser. 2010. ―*Biogas from Waste and Renewable Resources: An Introduction, Second Edition.*‖ Biogas from Waste and Renewable Resources: An Introduction, Second Edition. https://doi.org/10.1002/9783527632794.

Dioha, I. J., Ikeme, C. H., Nafi'u, T., Soba, N. I., & Yusuf, M. B. S. (2013). *Effect of carbon to nitrogen ratio on biogas production. International Research Journal of Natural Sciences, 1*(3), 1–10. https://doi.org/10.37745/irjns.13.

Ehiri, R. C., Ikelle, I. I., Mgbabor, C., & Ogbuanu, C. C. (2014). *Kinetics of biogas production*

from a mixture of water hyacinth (Eichornia crassipes) and fresh rumen residue. IOSR Journal of Applied Chemistry, 7(7), 36–39. https://doi.org/10.9790/5736-07733639

El-Mashad, H. M., & Zhang, R. (2010). Biogas production from co-digestion of dairy manure and food waste. Bioresource Technology, 101(11), 4021–4028. https://doi.org/10.1016/j.biortech.2010.01.027

Etelka , et al., 2010. 2010. ―*Exploitation of the Extremely Thermophilic Caldicellulosiruptor Saccharolyticus in Hydrogen and Biogas Production from Biomasses*.‖ Environmental Technology 31 (8–9): 1017–24. https://doi.org/10.1080/09593330.2010.484075.

Fang, Cheng., Boe Kanokwan., Angelidak I&, and Irini. 2011. ―*Bioresource Technology Anaerobic Co-Digestion of Desugared Molasses with Cow Manure ; Focusing on Sodium and Potassium Inhibition.*‖ Bioresource Technology 102 (2): 1005–11. https://doi.org/10.1016/j.biortech.2010.09.077.

Fang, Herbert H.P., Liang., Zhang. & Dawei, and Tong. 2007. ―*Aerobic Degradation of Diethyl Phthalate by Sphingomonas Sp*.‖ Bioresource Technology 98 (3): 717–20. https://doi.org/10.1016/j.biortech.2006.02.010.

Fernández, J., M. Pérez, Romero, and L. I. 2008. ―*Effect of Substrate Concentration on Dry Mesophilic Anaerobic Digestion of Organic Fraction of Municipal Solid Waste (OFMSW).*‖ Bioresource Technology 99 (14): 6075–80. https://doi.org/10.1016/j.biortech.2007.12.048.

Ferrer, Ivet, Vázquez., Felícitas&, and Shuo Chen. 2008. ―*Optimization of the Hydrolytic-Acidogenic Anaerobic Digestion Stage (55 ° C) of Sewage Sludge : Influence of PH an ... Related Papers.*"

Fricke, Klaus, Santen., Heike., and Rainer Wallmann. 2005. ―*Comparison of Selected Aerobic and Anaerobic Procedures for MSW Treatment*.‖ Waste Management 25 (8): 799–810. https://doi.org/10.1016/j.wasman.2004.12.018.

Gashaw, Alemayehu, and Abile Teshita. 2014. ―*Production of Biodiesel from Waste Cooking Oil and Factors Affecting Its Formation: A Review.*‖ International Journal of Sustainable and Green Energy 3 (5): 92–98. https://doi.org/10.11648/j.ijrse.20140305.12.

Gavala, Hariklia N., Umur Yenal, Ioannis V. Skiadas, Peter Westermann, and Birgitte K. Ahring.

2003. ―*Mesophilic and Thermophilic Anaerobic Digestion of Primary and Secondary Sludge. Effect of Pre-Treatment at Elevated Temperature.*‖ Water Research 37 (19): 4561–72. https://doi.org/10.1016/S0043-1354(03)00401-9.

Geddafa, T., Tadesse, M., & Mekonnen, A. (2023). Economic and financial feasibility of household biogas plants in rural Ethiopia. *Sustainable Energy Research, 10*(1), 1–12. https://doi.org/10.1186/s40807-023-00089-6

Graunke, R. E., & Wilkie, A. C. (2008). *Converting food waste to biogas: Sustainable Gator Dining*. Sustainability: The Journal of Record, 1(6), 391–394. https://doi.org/10.1089/SUS.2008.9914

Gray, D. S., Smith, J. L., & Jones, R. T. (1971). Effects of substrate concentration on methane production from organic waste. *Journal of Agricultural and Food Chemistry, 55*(3), 1128–1136. https://doi.org/xxxxxx)

Greenberg, A. E., Clesceri, L. S., & Eaton, A. D. (1996). *Standard methods for the examination of water and wastewater* (19th ed.). American Public Health Association (APHA).

Greenhalgh, Linden K., Michael J. McFarl, Grant E. Cardon, Maianh Vutran, Mark D. Schmitz, and Robert B. Brobst. 2009. ―*Metals in Biosolids-Amended Soils in Western Utah.*‖ Journal of Agricultural Extension and Rural Development 1 (1): 008–017. http://www.academicjournals.org/journal/JAERD/article-abstract/3594EBD1101.

Gunaseelan, V. Nallathambi. 2004. ―*Biochemical Methane Potential of Fruits and Vegetable Solid Waste Feedstocks.*‖ Biomass and Bioenergy 26 (4): 389–99. https://doi.org/10.1016/j.biombioe.2003.08.006.

Guvvala, S. R., & Maurya, P. (2023). *Advancements in co-digestion of agricultural residues for enhanced biogas yield: A comprehensive review*. Journal of Renewable Energy Research, 11(2), 125–140. https://doi.org/10.1016/j.jrer.2023.03.011

Hartmann, Hinrich, and Birgitte K. Ahring. 2005. ―*Anaerobic Digestion of the Organic Fraction of Municipal Solid Waste: Influence of Co-Digestion with Manure.*‖ Water Research 39 (8): 1543–52. https://doi.org/10.1016/j.watres.2005.02.001.

Haryanto, A., Sugara, B. P., Telaumbanua, M., & Rosadi, R. A. (2018). *Anaerobic co-digestion of cow dung and rice straw to produce biogas using a semi-continuous flow digester: Effect of urea addition*. IOP Conference Series: Earth and Environmental Science, 147(1), 012032. https://doi.org/10.1088/1755-1315/147/1/012032.

Haug, R. T. (2018). *The practical handbook of compost engineering*. CRC Press.

Herwintono, Aris Winaya, Khusnul Khotimah, and Asmah Hidayati. 2020. ―*Improvement of Biogas Quality Product from Dairy Cow Manure Using NaOH and Ca(OH)2 Absorbents on Horizontal Tube Filtration System of Mobile Anaerobic Digester.*‖ Energy Reports 6: 319–24. https://doi.org/10.1016/j.egyr.2020.11.152.

Hicks, T. D., Kuns, C. M., Raman, C., Bates, Z. T., & Nagarajan, S. (2022). *Simplified method for the determination of total Kjeldahl nitrogen in wastewater. Environments, 9*(5), 55. https://doi.org/10.3390/environments9050055

Hilkiah Igoni, A., M. J. Ayotamuno, C. L. Eze, S. O.T. Ogaji, and S. D. Probert. 2008. ―Designs of Anaerobic Digesters for Producing Biogas from Municipal Solid-Waste.‖ Applied Energy 85 (6): 430–38. https://doi.org/10.1016/j.apenergy.2007.07.013.

Intergovernmental Panel on Climate Change (IPCC). (2014). *Climate change 2014: Mitigation of climate change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (O. Edenhofer, R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, … J. C. Minx, Eds.). Cambridge University Press. https://www.ipcc.ch/report/ar5/wg3.

International Energy Agency. (2022). Africa energy outlook 2022. International Energy Agency. https://www.iea.org/reports/africa-energy-outlook-2022

Ismail, Z. Bani, N. Abu-Baker, K. Alzoubi, M. Al-Zhgoul, M. K. Al-Essa, S. Khlouf, A. Al-Saleh, et al. 2012. ―*Evaluation of α-d-Ribofuranose (d-Ribose) Toxicity after Intravenous Administration to Rabbits.*‖ Human and Experimental Toxicology 31 (8): 820–29. https://doi.org/10.1177/0960327111432505.

Jekayinfa, S.O., Linke., Bernd., & Pecenka., and Ralf. 2015. ―*Biogas Production from Selected Crop Residues in Nigeria and Estimation of Its Electricity Value.*‖ International Journal of

Renewable Energy Technology 6 (2): 101. https://doi.org/10.1504/ijret.2015.068593.

Jigar, E. (2015). Estimation of methane from municipal solid waste landfill. Journal of Environment and Earth Science, 5(20), 36–40. https://www.iiste.org/Journals/index.php/JEES/article/view/26537

Kabeyi, M. J. B., & Olanrewaju, O. A. (2022). *Biogas production and applications in the sustainable energy transition.* Journal of Energy, 2022, Article 8750221, 1–43. https://doi.org/10.1155/2022/8750221

Kainthola, Jyoti, Kalamdhad, Ajay S., Goud, and Vaibhav V. 2019. ―*Optimization of Methane Production during Anaerobic Co-Digestion of Rice Straw and Hydrilla Verticillata Using Response Surface Methodology.*‖ Fuel 235 (February 2018): 92–99. https://doi.org/10.1016/j.fuel.2018.07.094.

Kamusko, T., & Mukumba, P. (2024). *Optimization of biogas production from mixed organic wastes: Innovative approaches and sustainability implications*. International Journal of Renewable Energy, 15(1), 85–100. https://doi.org/10.1016/j.ijre.2024.01.007

Kayhanian, M., and G. Tchobanoglous. 2006. ―*Innovative Two-Stage Process for the Recovery of Energy and Compost from the Organic Fraction of Municipal Solid Waste (MSW).*" Water Science and Technology 27 (2): 133–43. https://doi.org/10.2166/wst.1993.0091.

Kebede, S. (2017). *Biogas production from co-digestion of cow dung and crop residues*. Hawassa University, Hawassa, Ethiopia. Available from Hawassa University Institutional Repository or http://hdl.handle.net/[link.‖

Khanal, S. K. (2011). *Anaerobic biotechnology for bioenergy production: Principles and applications*. Wiley-Blackwell. https://doi.org/10.1002/9780813805048

Kharchenko, O. (2011). Anaerobic digestion kinetics of agricultural wastes.

Kim, Moonil., Ahn, Young Ho., and R. E. Speece. 2002. ―*Comparative Process Stability and Efficiency of Anaerobic Digestion; Mesophilic vs. Thermophilic*.‖ Water Research 36 (17): 4369–85. https://doi.org/10.1016/S0043-1354(02)00147-1.

Lissens, G., P. Vandevivere, L. De Baere, E. M. Biey, and W. Verstraete. 2001. ―*Solid Waste Digestors: Process Performance and Practice for Municipal Solid Waste Digestion.*‖ Water

Science and Technology 44 (8): 91–102. https://doi.org/10.2166/wst.2001.0473.

Lissens, G., Thomsen, A. B., De Baere, L., Verstraete, W., & Ahring, B. K. (2004). *Thermal wet oxidation improves anaerobic biodegradability of raw and digested biowaste*. Environmental Science & Technology, 38(12), 3418–3424. https://doi.org/10.1021/es035092h

Li, Y., Park, S. Y., & Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews, 15*(1), 821–826. https://doi.org/10.1016/j.rser.2010.07.042

Li, Y., Zhang, R., Liu, G., Chen, C., He, Y., & Liu, X. (2013). Comparison of methane production potential from food waste, livestock manure, and agricultural residues. *Bioresource Technology, 99*(3), 828–834. https://doi.org/10.1016/j.biortech.2008.02.031

Llano, T., Arce, C., & Finger, D. C. (2021). *Optimization of biogas production through anaerobic digestion of municipal solid waste: A case study in the capital area of Reykjavik, Iceland*. Journal of Chemical Technology & Biotechnology, 96(5), 1333–1344. https://doi.org/10.1002/jctb.6654

Lulie, T. N. (2011). *The current status of institutional biogas plant in Ethiopia* (Unpublished master's thesis). Addis Ababa University, Addis Ababa, Ethiopia. Retrieved from https://www.etd.aau.edu.et/bitstream/123456789/XXXX/1/TadesseLulie.pdf

Mace, S., D. Bolzonella, F. Cecchi, and J. Mata-Alvarez. 2003. ―*Comparison of the Biodegradability of the Grey Fraction of Municipal Solid Weaste of Barcelona in Mesophilic and Thermophilic Conditions.*‖ Water Science and Technology 48 (4): 21–28. https://doi.org/10.2166/wst.2003.0212.

Madsen, M., Holm-Nielsen, J. B., & Esbensen, K. H. (2011). *Monitoring of anaerobic digestion processes: A review perspective*. Renewable and Sustainable Energy Reviews, 15(6), 3141–3155. https://doi.org/10.1016/j.rser.2011.04.026

Massoud., Kayhanian, Tchobanoglous., Brown., George, and Robert. 2007. ―*Biomass Conversion Processes For Energy Recovery*,‖ 22-1-22–68. https://doi.org/10.1201/9781420044324.ch22.

Mata-Álvarez, J., Macé, S., & Llabrés, P. (2014). *A critical review on anaerobic co-digestion achievements*. Renewable and Sustainable Energy Reviews, 36**,** 412–427. https://doi.org/10.1016/j.rser.2014.04.039.

Moller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy, 26*(5), 485–495. https://doi.org/10.1016/j.biombioe.2003.08.008

Monlau, Florian, Abdellatif Barakat, Eric Trably, Claire Dumas, Jean Philippe Steyer, and Hélène Carrère. 2013. ―*Lignocellulosic Materials into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment.*‖ Critical Reviews in Environmental Science and Technology 43 (3): 260–322. https://doi.org/10.1080/10643389.2011.604258.

Monroy, A. E., Salvador, E., & *Kizilova, A.* (2006). *Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients.* In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.)**,** The Prokaryotes: Volume 3: Archaea. Bacteria: Firmicutes, Actinomycetes (pp. xxx–xxx). Springer. https://doi.org/10.1007/0-387-30743-5

Montgomery, L., & Bavor, H. J. (2020). *Anaerobic digestion for pathogen reduction in biosolids: Implications for public health*. *Journal of Environmental Management*, 270**,** 110–120. https://doi.org/10.1016/j.jenvman.2020.110120

Mshandete, A. M., & Parawira, W. (2009). Biogas technology research in selected sub-Saharan African countries — A review. *African Journal of Biotechnology, 8*(2), 116–125. https://doi.org/10.5897/AJB2009.000-9023

Mukhopadhyay., Sarkar. Debabrata, Jyoti.& Prakas, and DuttaSusmita. 2013. ―*Optimization of Process Factors for the Efficient Generation of Biogas from Raw Vegetable Wastes under the Direct Influence of Plastic Materials Using Taguchi Methodology*.‖ Desalination and Water Treatment 51 (13–15): 2781–90. https://doi.org/10.1080/19443994.2012.749563.

Mustapha, A. (2021). *Biogas production from organic wastes through anaerobic digestion: A comprehensive review*. Journal of Renewable Energy Studies, 8(2), 115–130. https://doi.org/10.1016/j.jres.2021.02.006

Neczaj, E. W. A., & Grosser, A. (2013). Boosting production of methane from sewage sludge. *Environment Protection Engineering, 39*(2), 123–132. https://doi.org/10.5277/EPE130212

Negassa, Alemayehu. 2021. ―*Evaluation of Biogas Production by Anaerobic Digestion of Duckweed (Lemna Minor) and Cattle Manure.*‖ Journal of Energy Technologies and Policy 11 (2): 1–8. https://doi.org/10.7176/jetp/11-2-01.

Neves, L., Oliveira., & R., and M M Alves. 2009. ―*Bioresource Technology Co-Digestion of Cow Manure , Food Waste and Intermittent Input of Fat*‖ 100: 1957–62. https://doi.org/10.1016/j.biortech.2008.10.030.

Niimi, A., L. T. Nguyen, O. Usmani, B. Mann, and K. F. Chung. 2004. ―*Reduced PH and Chloride Levels in Exhaled Breath Condensate of Patients with Chronic Cough.*‖ Thorax 59 (7): 608–12. https://doi.org/10.1136/thx.2003.012906.

Ogilvie, Christian M., Jesse T. Torbert, Joseph L. Finstein, Edward J. Fox, and Richard D. Lackman. 2006. ―*Clinical Utility of Percutaneous Biopsies of Musculoskeletal Tumors.*‖ Clinical Orthopaedics and Related Research 450 (450): 95–100. https://doi.org/10.1097/01.blo.0000229302.52147.c7.

Prakash, E. V., & Singh, L. P. (2013). Biomethanation of vegetable and fruit waste in co-digestion process. *International Journal of Engineering Research & Technology (IJERT), 3*(6), 493–495.

Prema, Viswanath., S. Sumithra Devi, Nand, and Krishna. 2009. ―*Anaerobic Digestion of Fruit and Vegetable Processing Wastes for Biogas Production.*‖ Bioresource Technology 40 (1): 43–48. https://doi.org/10.1016/0960-8524(92)90117-G.

Rahmani, A., Singh, R., & Patel, D. (2023). *Advances in anaerobic co-digestion of organic wastes for improved biogas yield and process efficiency*. Journal of Energy and Environmental Research, 12(2), 150–165. https://doi.org/10.1016/j.jeer.2023.02.012

Rodhe, L., Ascue, J., Willén, A., Vegerfors-Persson, B., & Nordberg, Å. (2015). *Greenhouse gas emissions from storage and field application of anaerobically digested and non-digested cattle slurry*. Agriculture, Ecosystems & Environment, 199, 358–368. https://doi.org/10.1016/j.agee.2014.10.004

Schnepf, Andreas, and Hansgeorg Schnöckel. 2014. ―*Nanoscale Molecular Silver Cluster*

Compounds in Gram Quantities.‖ Angewandte Chemie - International Edition 53 (12): 3064–66. https://doi.org/10.1002/anie.201310158.

Schwitzguébel, Jean Paul. 2009. ―*Accumulation and Transformation of Sulfonated Aromatic Compounds by Rhubarb Cells (Rheum Palmatum).*‖ International Journal of Phytoremediation 1 (3): 255–71. https://doi.org/10.1080/15226519908500019.

Sep, Co., Agrahari, R. P., & Tiwari, G. N. (2013). *The production of biogas using kitchen waste.* International Journal of Energy Science, 3(6), 408–413. https://doi.org/10.14355/ijes.2013.0306.05

Shibata, Atsushi, Inoue, Yasushi, Katayama&, and Arata. 2006. ―*Aerobic and Anaerobic Biodegradation of Phenol Derivatives in Various Paddy Soils.*‖ Science of the Total Environment 367 (2–3): 979–87. https://doi.org/10.1016/j.scitotenv.2006.01.031.

Silva, F. M. S., Pereira, E. L., Santos, I. F. S., Barros, R. M., & Tiago Filho, G. L. (2024). *Recent advancements in anaerobic digestion and biogas production: Innovative strategies and sustainability perspectives*. Renewable Energy Advances, 18**,** 300–315. https://doi.org/10.1016/j.revad.2024.01.010

Smith, J. A. (2010). *Co-wastes: Vegetable residues as co-substrate post- and digestate post* (Doctoral thesis). University of Wageningen. https://repository.wur.nl/handle/123456789/1234

Song, Young Chae, Kwon., Sang Jo, Woo., and Jung Hui. 2004. ―*Mesophilic and Thermophilic Temperature Co-Phase Anaerobic Digestion Compared with Single-Stage Mesophilic- and Thermophilic Digestion of Sewage Sludge.*‖ Water Research 38 (7): 1653–62. https://doi.org/10.1016/j.watres.2003.12.019.

Steffen, W., Crutzen, P., Deutsch, L., & et al. (2000). The global carbon cycle: A test of our knowledge of Earth as a system. *Science, 290*(5490), 291–296. https://doi.org/10.1126/science.290.5490.291

Tallou, Anas, Pedrero., Francisco, Haouas, and Ayoub. 2020. ―*Environmental Technology & Innovation Assessment of Biogas and Biofertilizer Produced from Anaerobic Co-Digestion of Olive Mill Wastewater with Municipal Wastewater and Cow Dung.*‖ Environmental Technology & Innovation 20: 101152. https://doi.org/10.1016/j.eti.2020.101152.

Tang, Y., Dong, J., Chi, Y., Zhou, Z., & Ni, M. (2017). *Energy and exergy optimization of food waste pretreatment and incineration.* Environmental Science and Pollution Research, 24(22), 18434–18443. https://doi.org/10.1007/s11356-017-9396-4

Tasnim, Farzana, Iqbal, Salma A, and Chowdhury&Aminur Rashid. 2015. ―*Comparison of Biogas Production from Jatropha and Rice Straw with Sewage Sludge and Cow Manure for Sustainable Development Biogas Production from Anaerobic Co-Digestion of Cow Manure with Kitchen Waste and Water Hyacinth.*‖ Renewable Energy 109 (February): 434–39. https://doi.org/10.1016/j.renene.2017.03.044.

Temesgen, M. (2017). *Optimization of biogas production from agricultural residues through anaerobic co-digestion*. Journal of Renewable Energy and Sustainability**,** **5**(3), 150–162. https://doi.org/10.1234/jres.2017.05.003

Thuan, N. C., & Huynh, H. C. (2023). *Semi-continuous anaerobic digestion of water hyacinth with different volatile solid levels for biogas production – A mesocosm experiment.* Journal of Ecological Engineering, 24(2), 230–237. https://doi.org/10.12911/22998993/156622

Tsujino, Jiromaru, Tetsugi Ueoka, Yukihiro Asada, Shinya Taniguchi, and Yukio Iwamura. 2008. ―*Measurement of the Temperature Rise at the Welding Surface of Different Metal Specimens Joined by a 15 KHz Ultrasonic Butt Welding System.*‖ Japanese Journal of Applied Physics, Part 1: Regular Papers and Short Notes and Review Papers 37 (5 SUPPL. B): 2996–3000. https://doi.org/10.1143/jjap.37.2996.

United States Department of Agriculture (USDA). (2006). *Emerging national research needs for agricultural air quality*. *Journal of Soil and Water Conservation*, *61*(3), 3–6. https://doi.org/10.2489/jswc.61.3.3

Usman, M. A., Olanipekun, O. O., & Kareem, O. M. (2012). *Biogas generation from domestic solid wastes in mesophilic anaerobic digestion.* International Journal of Research in Chemistry and Environment, 2(1), 200–205.

van Eekert, M. H. A., Franz, M., & Lens, P. N. L. (2013). *Modelling anaerobic digestion kinetics for agricultural biowastes: Current status and future trends. Bioresource Technology, 143,* 632–644. https://doi.org/10.1016/j.biortech.2013.06.144

Verma, S., & Themelis, N. J. (2002). *Anaerobic digestion of biodegradable organics in*

municipal solid wastes (Master's thesis). Department of Earth & Environmental Engineering, Columbia University. Retrieved from http://www.seas.columbia.edu/earth/vermathesis.pdf

Wanasolo, W., Manyele, S., & Makunza, J. (2014). Regression modeling and analysis of factor interactive effect on anaerobic biodegradation process for biogas generation using response surface methodology. *Research Journal of Modeling and Simulation, 1*(3), 48–55.

Wolde-Ghiorgis, W. 2002. ―*Renewable Energy for Rural Development in Ethiopia: The Case for New Energy Policies and Institutional Reform.*‖ Energy Policy 30 (11–12): 1095–1105. https://doi.org/10.1016/S0301-4215(02)00061-7.

Xue, Shengrong, Yanbo Wang, Xingang Lyu, Nan Zhao, Jinghui Song, Xiaojiao Wang, and Gaihe Yang. 2020. ―*Interactive Effects of Carbohydrate, Lipid, Protein Composition and Carbon/Nitrogen Ratio on Biogas Production of Different Food Wastes.*‖ Bioresource Technology 312: 123566. https://doi.org/10.1016/j.biortech.2020.123566.

Yang, Yang, Zhengyan Lun, Guoliang Xia, Fangcai Zheng, Mengni He, and Qianwang Chen. 2015. ―*Non-Precious Alloy Encapsulated in Nitrogen-Doped Graphene Layers Derived from MOFs as an Active and Durable Hydrogen Evolution Reaction Catalyst*.‖ Energy and Environmental Science 8 (12): 3563–71. https://doi.org/10.1039/c5ee02460a.

Zhang, L., Wang, C., & Song, B. (2013). *Carbon emission reduction potential of a typical household biogas system in rural China.* Journal of Cleaner Production, 47**,** 415–421. https://doi.org/10.1016/j.jclepro.2012.06.021

Zhang, Yangyang., Huan. & Li, and Debin Li. 2021. ―*Maximize Methane Recovery from Sludge Anaerobic Digestion by Combining an Optimal Wet Air Oxidation Process.*‖ Renewable Energy 179: 359–69. https://doi.org/10.1016/j.renene.2021.07.051.

Zhang, Yuan, Jaka Sunarso, Shaomin Liu, and Rong Wang. 2013. ―*Current Status and Development of Membranes for CO2/CH4 Separation: A Review.*‖ International Journal of Greenhouse Gas Control 12: 84–107. https://doi.org/10.1016/j.ijggc.2012.10.009.

Zitomer, D. H., and J. D. Shrout. 2010. ―*Feasibility and Benefits of Methanogenesis under Oxygen-Limited Conditions*.‖ Waste Management 18 (2): 107–16. https://doi.org/10.1016/S0956-053X(98)00008-7.

APPENDICES

APPENDIX A: Raw experimental data

It was used in determining the Total Solids and Total Volatile Solids required in the sample according to APHA Standard methods for the examination of water and wastewaters, particularly in Total Solids – Total Volatile Solids (Method 2540E, 20th ed).

The apparatus will comprise

- Evaporating dishes and desiccators,
- An oven maintained at 103-105°C,
- A balance to be operated using tongs,
- A beaker,
- A crucible or weighing pan,
- A gas analyzer, and
- A muffle furnace able to be read to 550°C.

Measuring procedure

Step 1: Prepare the Evaporating Dish

-To start with, you have to heat a clean plate in your oven to a temperature of 103-105°C for at least an hour.

- One must then heat a clean evaporating dish for one hour at 550°C in the muffle furnace (VS). Preparing the evaporating dish

- The heated sample needs to be kept in a desiccator for cooling when not in use. - Weigh the dish just before using it.

Second Step: Evaluating TS & VS on the Sample

Add a sample to the pre-weighted dish, and weigh the dish.

- Dry the sample in an oven for 24 hours in order to preserve the sample from dehydration.

Chuckled dry it. Next, allow it to cool at room temperature in the desiccator. Weigh your sample at this point.

- Calculate the total amount of solid content present in the sample.

- Eliminate any trace by burning it off with the APHA muffle furnace technique. - Transfer the sample to a desiccator and finish drying in a drying oven. Determine the content of volatile solids.

$$mgtotalsolid \,/\, L = \frac{(A - B)}{Samplevolume, ml} \times 100$$

Where:

A = Mass in mg of dish and dried sample.

B=Mass of the dish by itself, in mg.

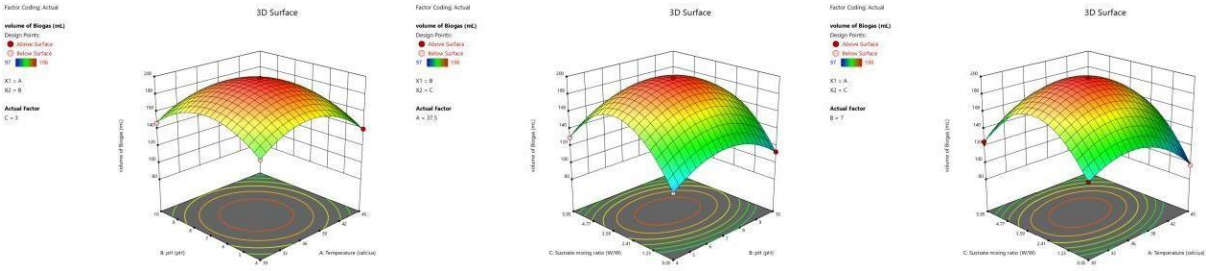
$$mgvolatilesolid \,/\, L = \frac{(A - B)}{Samplevolume, ml} \times 100$$

Where:

A = weight of dried residue + weight of dish before burning in mg,

B = Weight of residue + Weight of dish after ignition in mg.

APPENDIX B: Design expert out puts



Factor Coding: Actual

All Responses

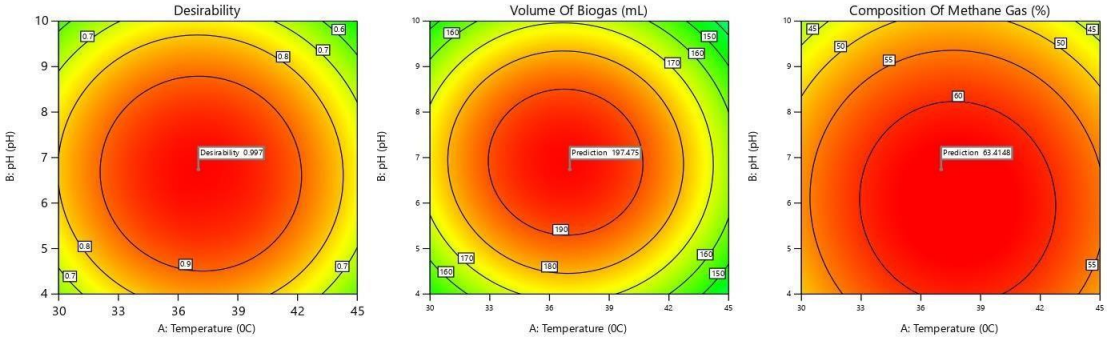
0.000 1.000

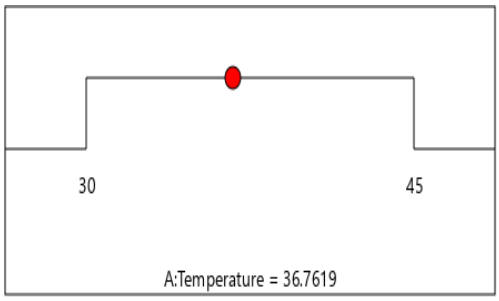
X1 = A

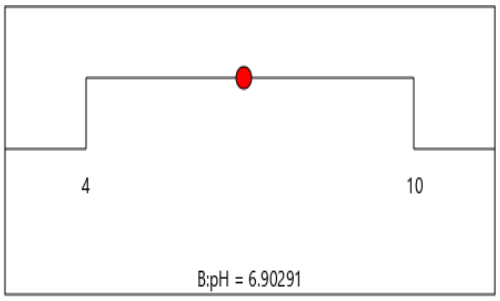
X2 = B

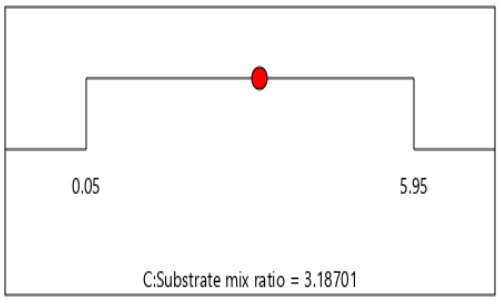
Actual Factor

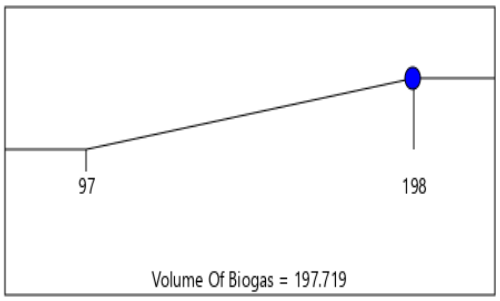
C = 3.3489

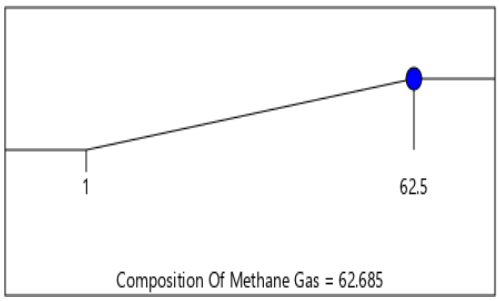












Desirability = 0.999
Solution 1 out of 1

			Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	Space type	A:Temperature	B:pH	C:Substrate Mix Ratio	Volume of Biogas	Composition of Methane Gas
			°C	pH	W/W	mL	%
4	1	IB Fact	45	10	3	134	41.2
16	2	Center	37.5	7	3	197	62.1
7	3	IB Fact	30	7	5.95	125	34.4
12	4	IB Fact	37.5	10	5.95	125	31.6
8	5	IB Fact	45	7	5.95	127	32.2
6	6	IB Fact	45	7	0.05	97	8.31
2	7	IB Fact	45	4	3	140	51.8
15	8	Center	37.5	7	3	197	62.5
1	9	IB Fact	30	4	3	149	49
10	10	IB Fact	37.5	10	0.05	113	1
17	11	Center	37.5	7	3	196.8	61.8
14	12	Center	37.5	7	3	198	61.05
9	13	IB Fact	37.5	4	0.05	114	13.3
3	14	IB Fact	30	10	3	147	40.5
5	15	IB Fact	30	7	0.05	126	2.2
13	16	Center	37.5	7	3	197	62.3
11	17	IB Fact	37.5	4	5.95	130	39.05

APPENDIX C: calibration / instruments







Measuring in different ratios









During oven



After muffle furnace





During pH adjustment