ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

CENTER OF RENEWABLE ENERGY TECHNOLOGY

EXPERIMENTAL ANALYSIS OF BIOGAS PRODUCTION FROM SEVEN TYPES OF FEEDSTOCKS (MOWN GRASS, FOOD WASTE, CHICKEN MANURE, CATTLE BLOOD, CATTLE RUMEN CONTENT, PIG MANURE, AND COW DUNG)

A Thesis Submitted to the School of Graduate Studies, at Addis Ababa Institute of Technology, Center for Renewable Energy, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Renewable Energy Technology.

By

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Advisor

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July 2023

Addis Ababa, Ethiopia

DECLARATION

I hereby declare that the work which is being presented in the thesis entitled "Experimental Analysis of Biogas Production from Seven Types of Feedstocks (Mown Grass, Food Waste, Chicken Manure, Cattle Blood, Cattle Rumen Content, Pig Manure, and Cow Dung)" in partial fulfillment of the requirements for the award of the degree of Master of Science in Renewable energy technology is an authentic record of my own work under the supervision of **Dr. Ebrahim Tilahun** (Assistant professor). This MSc thesis was not submitted for the purpose of receiving any other degree or professional qualification.

Where other sources are quoted, and full references are given.

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This is to certify that the thesis prepared by Henok Girma entitled **"Experimental Analysis of Biogas Production from Seven Types of Feedstocks (Mown Grass, Food Waste, Chicken Manure, Cattle Blood, Cattle Rumen Content, Pig Manure, and Cow Dung)"** it submitted in partial fulfillment of the requirements for the degree of Master of Sciences in Renewable Energy Technology (Center of Renewable Energy). Complies with the regulations of the University, and meets the accepted standards with respect to originality, and quality.

By:

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ABSTRACT

The significance and role of biogas in energy production and waste management are growing fast. The most determining factor for the gas composition and microorganism community in biogas production is the type of feedstock used. Biomass such as agricultural residues and livestock wastes are the major feedstocks for producing biogas. Ethiopia is one of the leading potential sources of agricultural and livestock wastes in Africa. Regardless of these resources, the majority of Ethiopians still lack access to clean energy. The feedstocks in most domestic biogas digesters constructed by the national biogas program of Ethiopia were mostly livestock (cattle) wastes. This study was conducted to assess the biogas potential of seven feedstocks that were as mown grass, food waste, chicken manure, cattle blood, cattle rumen content, pig manure, and cow dung under a controlled temperature of 40 ± 0.2 ℃ in a 500-mL laboratory scale batch digester. The feedstocks used in this experiment were subjected to characterization before and after digestion and the methane production was measured and normalized on a volatile solids basis. The maximum specific methane yield (mL/g VS) and methane percentages of the mown grass, food waste, chicken manure, cattle blood, cattle rumen content, pig manure, and cow dung were 231 and 79.4%, 82.12 and 67.9%, 177.99 and 77.8%, 173.06 and 68.6%, 350.3 and 77.1%, 127.05 and 77.6%, 32.88 and 69.9%, respectively. The chicken manure and cattle blood had the highest concentrations of hydrogen sulfide, producing 1249 and 1102 ppm, respectively. The grass, food waste and inoculum I also generated 805, 170, and 52 ppm of H_2S within 28 days of the HRT. The obtained results showed that utilizing feedstocks beside cow dung could increase the quality and quantity of biogas production.

Key words: Biomass, anaerobic digestion, biogas, feedstock, specific methane yield

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ABBREVIATIONS

AAAE	Addis Ababa Abattoir Enterprise
AAiT	Addis Ababa institute of technology
AAU	Addis Ababa University
ABPP	Africa Biogas Partnership Program
AD	Anaerobic Digestion
APHA	American Public Health Association
BOD	Biochemical oxygen demand
C/N	Carbon to Nitrogen ratio
CHP	Combined Heat and Power
COD	Chemical oxygen demand
DM	Dry Matter
DO	Dissolved Oxygen
DO	Dissolved oxygen
EREDPC	Ethiopian Rural Development and Promotion Centre
ESA	Ethiopian statistics agency
FAO	Food and Agriculture Organization
FM	Fresh Mass
FS	Fixed solid
SNV	Netherlands Development Organization
TOC	Total organic Carbon
TS	Total Solid
VFA	Volatile Fatty Acid
VS	Volatile Solid

SYMBOLS

C	Carbon
CH_4	Methane
$C_6H_{12}O_6$	Glucose
$CaCO_3$	Calcium carbonate
CH_3 CH_2COOH	propionic acid
CH_3 CH_2OH	Primary alcohol
CH_3COO^-	Acetate
CH_3COOH	Acetic acid
CO_2	Carbon dioxide
H_2	Hydrogen
H_2O	Water
H_2S	Hydrogen sulfide

GHG	Greenhouse gas
HRT	Hydraulic retention time
IEA	International Energy Agency
LEM-Ethiopia	Environment and Development Society of Ethiopia
LUPO	Land Use Planning and Resource Management, Oromia
NBPE	National Biogas Programme of Ethiopia
NBPE+	Biogas Dissemination Scale-Up Program
oDM	organic Dry Matter
OLR	Organic loading rate
oTS	organic Total Solid
PSA	Pressure Swing Adsorption
SNNPR	Southern Nations, Nationalities and Peoples Region

HCO_3^-	Bicarbonate
N_2	Nitrogen
NH_3	Ammonia
NH_4	Ammonium ion
O2	Oxygen
S	sulfur

UNITS

L	Liter
mg/L	Milligrams per Liter
mL/g VS	Milliliter per gram of Volatile solid
mL_N	Milliliter in normal state
PJ	Petajoule (10^{15} J)
ppm	Parts per million
hpa	Hectopascal

1. INTRODUCTION

1.1. Background

Human beings consume energy for many applications in their daily lives. The main sources of these energies are coal, oil, natural gas, uranium, and renewable sources like biomass, solar, water, geothermal, and wind. From these sources renewables are the most environmental friendly extraction and consumption of energy in a way that benefits both humans and nature [2]. Returning to renewables to assist mitigate weather problems is an incredible method that needs to be sustainable to meet the energy need of future generations [3].

Biomass, an organic matter, and the conversion of waste to bioenergy are renewable energy sources playing a great role in providing developing human energy needs around the world [4]. The anaerobic digestion of biomass such as food and agricultural waste, plant material, manure, sewage, and municipal waste produces biogas which is a combination of gases, primarily methane and carbon dioxide with some trace amount of hydrogen sulfide, moisture, siloxanes, and other compounds [5].

Biogas has many applications, especially in cooking, space heating and electricity, combined heat and power (CHP), and combustion engines. It also reduces the dependency on fossil fuels, waste reduction, and helps to achieve environmental targets. Biogas digestate can also be used as a fertilizer [1].

However, the production of biogas is affected by different parameters like Temperature, pH and alkalinity, Nutrients and trace metals, Hydraulic retention time (HRT), Organic loading rate (OLR), Substrate agitation (Mixing), Substrate-to-inoculum ratio, Nitrogen content and C/N ratio, Toxic and Inhibitory materials, and Reactor Configuration [4, 6].

The most determining factor for the gas composition and micro-organism community in biogas production is the type of substrate used. The size and the compositional structure of the raw material have a great impact on the digestion process [7]. Some lignocellulose organic plants have a large amount of lignin, which causes the anaerobic fermentation to proceed imperfectly [8]. As the digestibility of the feedstock increases its biogas yield also increases [9]. The biogas production potential is a crucial factor in determining how effectively anaerobic digestion operates [10].

In the case of Ethiopia, biogas is not a brand-new technology. The first biogas plant was ever established in 1957 at the Ambo College of Agriculture, which marked the introduction of the technology [11]. Despite the fact that the technology has been used since then, the nation with a vast quantity of resources for producing biogas couldn't develop as much as anticipated.

Ethiopia's development and effective use of this technology have been hampered by several obstacles. The most common challenges are related to capacity limitations (technical, economical, technological) and institutional flaws [12, 13]. The technology is used conventionally with the same substrates and with little or no guidance on the loading rate, retention period, and inhibitory materials, which elevates the capacity limitations [14]. This study aimed to investigate various substrates on the potential to produce biogas in a laboratory scale batch digester.

1.2. Problem Statement

In the past decade, the world's energy demand is escalating drastically as well as environmental problems. The solution for sustainable development is manipulating energy resources with low environmental impacts such as biomass.

Biogas from the anaerobic digestion of biomass provides a renewable energy source that decreases fossil fuel dependency and greenhouse gas emissions. The energy content of biogas from anaerobic digestion is chemically bounded by methane [1].

The utilization of biogas provides several benefits for developing nations like Ethiopia in terms of the cost of natural gas, waste management, and energy requirements. According to the 2021 world bank data [15], about 78% of Ethiopia's total population is located in rural areas, where agriculture with livestock is practiced as their main source of livelihood.

Gabisa [16], estimated a total of about 750 PJ per year of bio-energy potential in feedstock such as livestock waste (19%), agricultural residues (34%), and forest residues (47%) in Ethiopia which are the major constituents/feedstock of biogas production. Regardless of these resources the majority of Ethiopians still lack access to clean energy due to inefficient use of biomass.

The national biogas program of Ethiopia (NBPE) with three phases planned to install around 70,000 and achieved to put only 23,802 biogas digesters on the ground. The raw materials/feedstocks for the biogas production used were livestock wastes mostly cattle [17, 18]. Thus, using different feedstocks, such as agricultural waste, municipal solid waste, grass, food waste, and livestock waste from sheep, chickens, and other animals, for the production of biogas not only increases the amount of gas produced in the country but also serves as a method of waste management. Even though there have been several papers published on the area of anaerobic digestion in the nation, most of them failed to normalize the produced biogas from local pressure and temperature conditions. Besides, this study helps to select the potential feedstocks for co-digestion in the applications of system analysis of renewable generation from organic wastes and its conversion to protein technology in Prime Meat and Food Processing in Ethiopia: focusing on energy and nutrient supply.

1.3. Objectives

1.3.1. General Objective

The main objective of this work was to experimentally analyze the potential of biogas production from different feedstocks (mown grass, food waste, chicken manure, cattle blood, cattle rumen content, pig manure, and cow dung).

1.3.2. Specific Objectives

In order to achieve the main objective, the following specific objectives were carried out.

- To characterize each feedstock.
- To design batch experimental set-up and operational strategy for production of biogas.
- To determine the biogas volume, composition and methane yield from each types of feedstocks.

1.4. Significance and Scope of the Study

Biogas can be used for a variety of purposes, including cooking, combined heat and power (CHP), and combustion engines. Due to the limited quantity and poor quality of the biogas produced, Ethiopia is still a long way from employing the last two applications.

The potential of various substrates to produce biogas has a significant impact on preserving the quantity and quality of the gas produced. By describing the amount and methane yield of various substrates for biogas generation, the outcome of this study will help Ethiopia's biogas production process.

This work focuses on determining the effects of various substrates on the production of biogas in a labratory-scale batch digester at a constant temperature. For this purpose, it included substrate characterization, biogas production, and analyzing the composition of biogas.

2. LITERATURE REVIEW

2.1. Introduction

Biomass is any organic matter that can be used as an energy source. Crops, wood, garden, and animal waste are examples of biomass [4]. Through the process of photosynthesis, biomass stores chemical energy in carbohydrate form using carbon dioxide and solar energy combinations.

Biomass can be converted to energy through different processes. The type of conversion method is carefully chosen depending on the biomass characteristics and the type of end product needed [19]. The conversion technologies can be mainly classified as direct combustion or burning, thermochemical conversion, chemical conversion, and biological conversion [20]

Biological conversion contains anaerobic digestion that produces biogas and fermentation to transform biomass into ethanol. Biogas is mainly methane with some carbon dioxide, carbon monoxide, and some other trace gases and can be used to produce electricity and/or heat. The generation process engages bacteria which works under anaerobic conditions (without oxygen) since the oxygen inhibits the bacteria. The raw materials (substrate) that are used in the process include livestock manure, weeds, sewage, straw wastes, and so on. Mostly the substrates contain methane-producing bacteria naturally [21].

Ethiopia's available feedstock to produce biogas is tremendous and these feedstocks have significant impact on the potential of biogas production. There has been some research done in Ethiopia on the biogas potential from various substrates, but the bio-methane yield potentials reported vary significantly. Yohaness [22] investigated the biogas potential for different types of cow manure at 37 ℃ for a retention time of 42 days and found a maximum methane yield of 0.116 g CH_4/g TS. T.Moges and W.Bogale [23] with A case study of biogas for electricity generation from food waste in sadist kilo campus, Addis Ababa University, estimated the total of 90 ml/g of biogas per day which yields 59.7% of methane. Fufa, Dadi, and Birke [24] aslo investigated the biogas production potential of food waste in the Jimma region, Ethiopia. The amount of biogas yield was 64.2 ml/g with 57.86% of methane gas recorded in 35 days of HRT. As much as Ethiopia has to offer in terms of organic waste, not much has been researched about the characterization and bio-methane potential of its various substrates for biogas production.

2.2. Biochemical Processes in Anaerobic Digestion

Anaerobic digestion is a process by which organic matters converted into biogas with the help of chemoheterotrophic or methanogenic bacteria/microorganisms in the absence of oxygen. In contrary to aerobic decomposition (with oxygen), which occurs during composting, relatively little heat is produced during AD. The energy that is chemically linked to the substrate remains mostly as methane in the biogas that is generated. It mainly consists of four stages [5, 25–27]:

- Hydrolysis
- Acidogenesis
- Acetogenesis and
- Methanogenesis.

2.2.1. Hydrolysis

In hydrolysis stage, complex compounds of organic matter are converted to free sugars, alcohols and other simple compounds by addition of water. Enzymes produced by the fermentative bacteria hydrolyze the complex bio-polymers like polysaccharides, proteins, and lipids into water soluble smaller molecules like sugars, amino acids, and long chain fatty acids as shown in equation 2.1 [5, 26].

$$\text{Biomass} + H_2O \rightarrow \text{monomers} + H_2 \quad (2.1)$$

2.2.2. Acidogenesis

The results of hydrolysis are transformed into methanogenic substrates during acidogenesis by acidogenic (fermentative) bacteria. As equations 2.2 and 2.3 showed simple sugars, amino acids, and long chain fatty acids are broken down into short chain volatile fatty acids (propionate, butyrate), alcohol and directly to acetate, carbon dioxide, and hydrogen [27, 28].

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad (2.2)$$

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \quad (2.3)$$

2.2.3. Acetogenesis

In the course of acetogenesis, acidogenesis products that methanogenic bacteria cannot directly convert to methane are transformed into methanogenic substrates. Alcohols and VFA are oxidized to produce acetate, hydrogen, and carbon dioxide, which are methanogenic substrates. Methanogenesis and acetogenesis typically occur concurrently, with some methane being created at this step [5, 28].

Different bacteria and micro-organisims take part in this stage, including: Syntrophobacter wolinii(for propionate decomposer), Syntrophomonos wolfei (for butyrate decomposer) and peptococcus anaerobes, actinomyces, lactobacillus (acid formers) [26].

VFA and alcohol oxidation

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (2.4)$$

$$CH_32(CH_2)COO^- + 2H_2O \leftrightarrow 2CH_3COO^- + H^+ + 2H_2 \quad (2.5)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (2.6)$$

Hydrogen utilization by acetogens and methanogens

$$2HCO_3^- + 4H_2 + H^+ \leftrightarrow CH_3COO^- + 4H_2O \quad (2.7)$$

$$HCO_3^- + 4H_2 + H^+ \leftrightarrow CH_4 + 3H_2O \quad (2.8)$$

Acetate oxidation with hydrogenotrophic methanogenesis

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (2.9)$$

2.2.4. Methanogenesis

In methanogenesis, the final stage of AD, carbon dioxide and methane are generated from intermediate products by methanogenic bacteria like methanosarcina, methanobacterium, methanococcus, and methanobacillus. Acetic acid is converted to CO_2 and CH_4 (about 70% of the formed methane), and CO_2 and H_2 are converted to CH_4 as shown in equations 2.10 and 2.11.

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{Acetoclastic methanogens} \quad (2.10)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{Hydrogenotrophic methanogens} \quad (2.11)$$

Methanogenesis is a vital phase in AD in methane production. The operation circumstances have a significant impact on methanogenesis. Factors influencing the methanogenesis process include feedstock composition, feeding rate, temperature fluctuations, pH and a substantial entrance of oxygen can all result in the termination of the process [5, 29]. The summarized processes in the stages of AD process are shown in Figure 2.1.

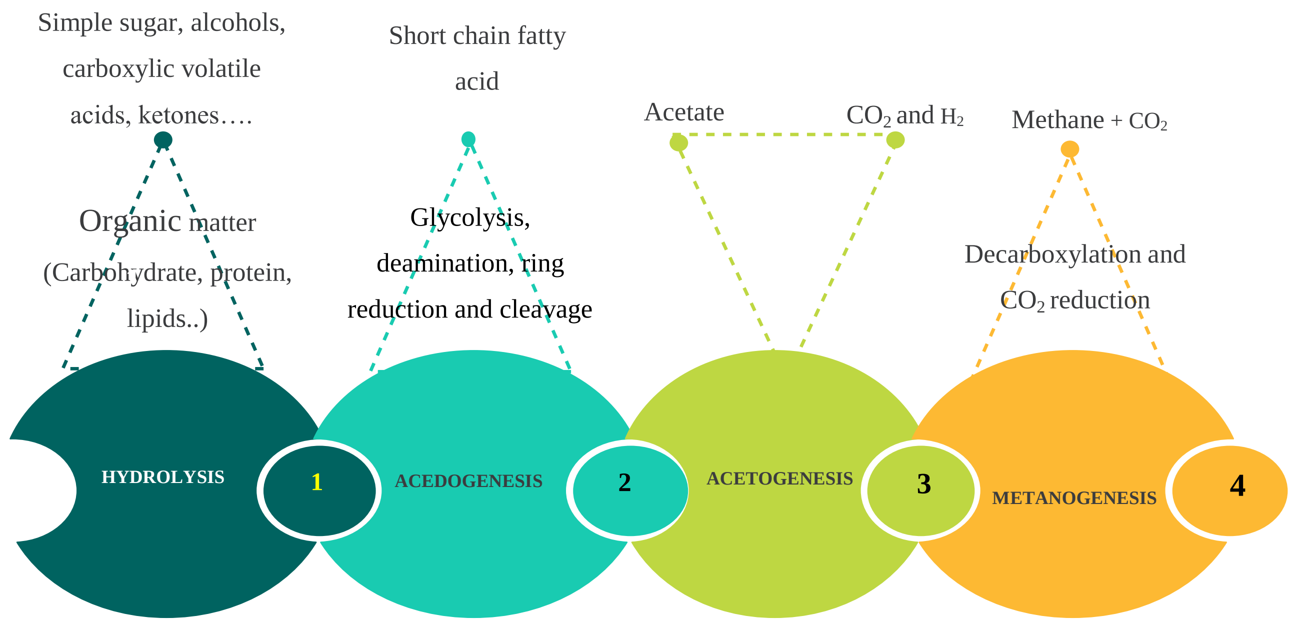


Figure 2. 1 Stages of Anaerobic digestion process [1]

2.3. Factors Affecting the Process of AD

The biogas produced from AD can be affected by a number of factors. These factors can be AD parameters (such as temperature, pH…), operational parameters (such as HRT, organic loading rate…), and feedstock characteristics.

2.3.1. Feedstock/substrate type

The substrates for anaerobic digestion (AD) affect the composition and can be classified according to different measures like origin, methane yield, dry matter (DM) content, etc. Substrates with dry matter content below 20% are used for wet digestion. And it includes animal manure, slurries, and different wet organic wastes gained from food industries. On the other hand, if dry matter content is above 35%, it is called dry digestion which is mainly used for silages and energy crops [1].

The type and content of the substrate directly affect biogas production. The amount of anaerobic substrate is frequently quantified in terms of total chemical oxygen demand (COD) or total volatile solid (VS). Manure has a lower methane production per VS or COD than an easily degradable substrate like an organic waste because it contains a large amount of water and resistant components [30, 31].

For certain feedstock to be more biodegradable, pretreatment is necessary. It is possible to combine different feedstock and co-digested. However, some combinations can reduce performance or even stop the AD process [32]. The characteristics of some substrates are shown in Table 2.1 below.

Table 2. 1 characteristics of some substrates [32–34]

Type of feedstock	Organic content	C:N ratio	DM %	VS %	Biogas yield m^3 $*kg^{-1}$ VS	Unwanted impurities
Cattle manure	Carbohydrates, lipids, proteins	6-20	5-12	80	0.20-0.30	Soil, water, wood straw, NH_4^+
Pig manure	Carbohydrates, lipids, proteins	3-10	3-10	70-80	0.25-0.50	Wood, sand, cords, disinfectants
Poultry manure	Carbohydrates, lipids, proteins	3-10	10-30	80	0.35-0.60	Feathers, sand, NH_4^+
Straw	Carbohydrates, lipids	80- 100	70-90	80-90	0.15-0.35	Grit , sand
Grass	Carbohydrates, proteins	12-25	20-25	90	0.55	Grit, Pesticides
Garden wastes	Lignocelluloses, carbohydrates	100- 150	60-70	90	0.20-0.50	Soil, Pesticides, cellulosic component
Fruit wastes	polyphenols, carbohydrates	35	15-20	75	0.25-0.50	
Food remains	carbohydrates, proteins, lipids	10-40	10	80	0.50-0.60	Plastic, bones and inorganic compounds

2.3.2. Temperature

The process of AD can be takes place at different temperatures, psychrophilic below 25 ℃, Mesophilic ranges from 25 ℃ to 45 ℃, and thermophilic ranges from 45 ℃ to 70 ℃. The optimal digestion temperature in most anaerobic digestion processes should be kept roughly constant to maintain the gas production rate. Temperature in mesophilic and thermophilic zone is considered the best condition for most of the anaerobic digestion plants. But thermophilic is a more expensive and has the effect of imbalance and ammonia inhibition to operate [5, 6, 35].

2.3.3. PH, Alkalinity and volatile fatty acid (VFA) concentration

The pH of AD affects the growth of methanogenic bacteria as well as the solubility of several important chemicals for the AD process. According to literatures, [36, 37] methane production occurs within a very restricted pH range of about 5 to 9, with an optimal interval of 6.5 to 8 for most methanogens. Acidogenic bacteria often have a lower optimal pH value. The pH is highly related to retention time and VFA concentration. It is also interdependent on temperature [38, 39].

Alkalinity is the capacity of the mixture's AD liquid to buffer the potential creation of acids created during the biological process and, thus, minimize potential pH fluctuations [40]. The non-protonated forms of VFAs and the carbonate system are the principal sources of alkalinity in the AD liquid combination. This is mostly caused by the breakdown of nitrogen-containing organic materials, which generate ammonia, which then reacts with carbon dioxide to make ammonium bicarbonate [41, 42].

Alkalinity measurements provide insight into how "safe" the reactor is in terms of absorbing acidic shock loads as well as how healthy the reactor is, as alkalinity is typically between 2000 and 4000 mg/L as $CaCO_3$/L [43].

The primary intermediates during the breakdown of organic materials in the acidogenic stage are volatile fatty acids (VFA) such as acetic, propionic, and butyric acids. Propionic acid is considerably more inhibiting than butyric acid, it has been linked to system failure [44]. A failure of AD may be brought on by acetic acid concentrations of more than 0.8 g/L or a ratio of propionic acid to acetic acid of 1.4 [43].

2.3.4. Organic loading rate (OLR)

The organic loading rate refers to the input amount of organic material per unit volume of the digester. Most commonly loading rates are expressed in volatile solids (VS) for organic sludges and solid wastes and in BOD or COD for diluted wastewater [45].

An imbalance between the stages of hydrolysis/acidogenesis and methanogenesis is often brought on by an OLR shock/organic overloading. As a result, volatile fatty acids (VFA) build up, the pH drop, and methanogenesis fails [46].

It depends on the feedstock and its concentration, the type of biogas digester, temperature, and other factors. OLRs between 0.5 and 5 kg VS/m^3/day are recommended. The thumb rule of AD states that for optimal gas production, a biogas digester needs 4 kg of fresh cow dung per m^3 [47, 48].

2.3.5. Hydraulic retention time (HRT)

The HRT indicates how long the substrate stays in the digester on average. To promote the greatest possible conversion of organic materials into biogas, substrates should be allowed to remain in the digester for an appropriate amount of time. But a longer HRT necessitates a bigger digester, which raises the digester's capital expense. On the other hand, All of the active microorganisms leave the reactor when the HRT is very low, causing the washout of the reactor [32, 49].

Process parameters, like temperature and substrate type, affect the HRT. Retention times for substrates processed in mesophilic digesters range from 15 to 30 days, whereas thermophilic digesters have retention times of 12 to 14 days [50].

2.3.6. Mixing/stirring

Mixing of AD substrate is vital to provide a uniform substrate to micro-organism concentration, dilute levels of inhibitors, and uniform temperature. It also helps to reduce scum buildup and retains inorganic materials from settling at the bottom of the digester [51, 52].

High moisture substrates usually don't need to be mixed. Recirculation of digester material using a pump or nozzle, mechanical agitation, and biogas recirculation are a few methods for mixing feedstock. Mixing necessitates a sophisticated digester design and increased operational expenses [32, 42].

2.3.7. C/N ratio

The proportion of carbon to nitrogen in organic matter is referred to as the carbon to nitrogen ratio (C/N). Anaerobic bacterial growth requires nitrogen, whereas the generation of biogas requires carbon [53]. When the C/N ratio is relatively high, the nitrogen is quickly consumed to make the required quantity of protein and fails to combine with the carbon component, which inhibits gas production. Microbial inhibition, ammonia accumulation, and reduced amount of VFA resulted from a higher C/N ratio [54].

For the production of biogas, a C/N ratio of 20–30/1 is optimum. The C/N ratio is recommended to be more than 23/1 and level less than 10/1 [51]. To operate the anaerobic digester with the best C/N ratio, biodegradable materials with a high C/N ratio must be mixed with biodegradable materials with a low C: N ratio [55].

2.4. Biogas Compositions

The biogas compositions mostly vary with the type of feedstock used. The table 2.2 below shows the main compositions for different substrates.

Table 2. 2 Composition of biogas [56]**.**

Component		Landfill	Waste water treatment	House hold waste	Industrial waste/animal manure digest
Methane, CH_4	vol.%	36–52	65	65	60–80/50–70
Carbon dioxide, CO_2	vol.%	30–41	33.5	29	20–40/30–50
Water vapour	vol.%	0.1–3.3	2	0.5	1-4
Nitrogen, N_2	vol.%	<10	<5		Not detected/<5
Oxygen, O_2	vol.%	<3	<1		Not detected/<1
Siloxane	mg/m^3	1.4–9.8	1.3	1.35	Not detected
Sulfur, S	mg/m^3	29–900	25	30	
Hydrogen sulfide, H_2S	ppm	10–1000	150–3000		<30000/<5000
Ammonia, NH_3	ppm	<5	<5	<5	<5
Halogenated compounds	mg/m^3	0–7	Not detected	Not detected	<0.1
Benzene	mg/m^3	<36	<0.3		
Toluene	mg/m^3	<287	<27		

2.5. Biogas Upgrading Technologies

Upgrading of biogas involves the removal of CO_2 in the biogas plus the cleaning of trace impurities like H_2S, water vapor…, which improves the biomethane concentration. After an upgrade is done, biogas can be served as fuel for stoves, engines, boilers, and gas turbines in order to produce electricity [57].

Various researches have been done regarding different technologies of biogas upgrading systems which deal with improving the removal efficiency of impurities from the raw biogas and the cost. Technologies for biogas purification and upgrading processes are water scrubbing, membranes separation, absorption, pressure swing adsorption (PSA), and biological upgrading technology. Also new technologies like ecological lung, in situ methane enrichment, and cryogenic Separation [57–59].

2.6. Biogas Utilization

The significance and role of biogas in energy production is growing fast. These days, many countries promote the utilization of renewable energies even by guaranteed refund or emission trading systems [60].

Since biogas is a methane rich gas, it is very feasible and has the effect of reduction of odor, and prohibiting the emission of harmful gas into the environment, which improves the quality of air. Biogas is one of the most reliable sources of energy since it is cheap and delivers an efficient fuel for cooking and lighting, Combined Heat and Power (CHP) generation, internal combustion engine (vehicles), and gas turbine engines [61–63].

Biogas can be directly used for the desired applications accepting the effects [59]. It can also be utilized by transforming into syngas [64]. Additionally, biogas with hydrogen gas can be utilized as a fuel for a Compression Ignition Engine [65] but the drawback is the need for higher pressure to liquidify the biogas [57]. But for both human and material safety applications biogas can be upgraded with different technologies.

2.7. Biogas Potential and Production in Ethiopia

In most developing countries, particularly sub-Saharan countries, their energy source is highly based on biomass like wood, residues of crops, dung, sewage, animal and agricultural waste, and municipal waste [66]. According to the 2018 world bank data [15], about 78% of Ethiopia's total population is located in rural areas, where agriculture with livestock is practiced as their main source of livelihood. In Ethiopia, a sub-Saharan country, biomass has a 92 % share of energy. Out of this percent, wood and charcoal cover 73%, dung 14%, and residue 13%.

Gabisa 2018 [16], estimated a total of about 750 PJ per year of bio-energy potential in feedstock like livestock waste (19%), agricultural residues (34%), and forest residues (47%) in Ethiopia which are the major constituents/feedstock of biogas production.

2.8. Status of Biogas Technology in Ethiopia

2.8.1. Early development

The first ever installed biogas plant for the purpose of generating energy in the process of welding agricultural tools is a batch digester unit, which allows adding the feedstock only one time and closing till it produces methane, in Ambo College of Agriculture in 1957 [11, 67]. Then in the 1970s food and agriculture organization (FAO) constructed two biogas plants as a pilot project. The first one was around Debre Zeit with a farmer and another one was at a school around Kobo in Wollo [68].

In the years between 1980 and 2000, with the help of different parties (government, non-government organizations, and private sectors) around 1000 biogas plants were built in different parts of the country with a digester size of 2.5-200 m^3. The models include an Indian floating drum, bag digester, and fixed dome type.

Sixty fixed-dome biogas plants were constructed in the year 1999 to 2002 with an Ethiopian–German development project by a German biogas expert called Christopher Kellner. He also prepared a manual for the construction of what he called Land Use Planning and Resource Management, Oromia (LUPO). A local non-government organization called LEM-Ethiopia with its awareness and promotion program built 22-25 latrine-fed biogas digesters in schools and families in regions like SNNPR, Oromia, and Amhara [18, 68, 69].

2.8.2. The National Biogas Program of Ethiopia (NBPE)

The national biogas program of Ethiopia (NBPE) started in 2008 after a feasibility study was conducted by experts from SNV (a Netherlands development organization) and Ethiopian Rural Development and Promotion Centre (EREDPC) in 2006. This program was launched to estimate the biogas potential of Ethiopia in four regional states with the highest population and livestock (SNNPR, Tigray, Oromia, and Amhara) [18, 69, 70].

2.8.3. The national biogas program of Ethiopia first phase (NBPE-I)

The first phase of the national biogas program of Ethiopia (NBPE-I) was launched and executed between 2008 and 2013. African biogas partnership program (ABPP), EREDPC, and SNV were the stakeholders in the program. After extensive consultation, the project implementation document (PID) was set to construct 14,000 household biogas plants [71]. However, the initial stage of the implementation faces several problems like a cement crisis, skilled personnel, lack of follow up, and other results in the slow development of the program. Consequently, in 2010 the ABPP revised the plan to construct only 10,000 biogas plants.

During the implementation years (2009-2013), the NBPE-I constructed 8,063 household biogas digesters in 163 woredas (districts) of four regions of Ethiopia. The constructed plants were the SINIDU model GGC 2047 and has a size of 4, 6, 8, and 10 m^3 [69, 70]. The number of installed biogas plants in the four regions shown in the table below.

Table 2. 3 The number of installed biogas plants in Ethiopia [72]

Region	Installed biogas plants
Amhara	1,892
SNNPR	1,699
Tigray	1,992
Oromia	2,480

2.8.4. The national biogas program of Ethiopia second phase (NBPE-II)

The second phase of the program (NBPE-II) was under implementation from 2014 to 2019. The target in this phase was to build 20,000 higher quality market-driven biogas plants and to ensure that the bio-digesters constructed in phase I and II continue to function effectively and efficiently [18, 73].

Unlike the first phase, the second phase succeeded in involving the private sector to the implementation program. And out of the planned 20,000 biogas plants 12,538 bio-digesters were constructed until February 2019 [14, 18].

2.8.5. Biogas Distribution Scale-up Program (NBPE+)

This program was launched in April 2017, and the second phase is in progress; with the goal of improving and scaling-up the work done in the two previous phases. It is planned to be a 5 years program and will continue until July 2022. Besides the four regional states of Ethiopia, this scale up program added another four, namely Afar, Benishangul-Gumuz, Somali, and Gambela [18].

It mainly considers 36,000 low income people in rural areas with at least four cattle for dung, a piece of land, and labor for the bio-digester. This installed number makes a total of 23,802 biogas digesters constructed in the three phases until April 2019.

2.9. Different Biomass Feedstock and their Potential for Biogas Production in Ethiopia

2.9.1. Agricultural residue

Agricultural residues are elements that remain in fields as byproducts after the main crop has been collected (mostly non-edible). These materials range from maize Stover (stalk and leaves) to other cereal grain straws made of rice, barley, wheat, etc, and other remains throughout agricultural products processing like sunflower husks, bagasse, and so on [74]. The agricultural residue amount varies from crop to crop based on their harvesting method, crop structure, and season. However, if the production is higher it is expected that the residue will also increases [75]. In the biogas context, a high share of crop residue stimulates in achieving a higher gas yield. And different crop types can be managed in a co-digestion process [76].

Ethiopia's primary and most important economic sector is agriculture. The agricultural industry accounts for around 40% of GDP, 80% of exports, and 75% of total employment in the country. Cereals are Ethiopia's most important crop, accounting for over 70% of the country's agricultural GDP [77]. Some of the agricultural residues in Ethiopia are listed in Table 2.4 below.

Table 2. 4 Ethiopia's different agricultural residues potential [78]

Agricultural residues	Crop residues	Coffee residues	Cotton Stalk residue	Chat (cash crop) residue	Sawmill residue	Energy plants(Jatropha, Castor bean, palm tree)
Amount	24.23 million tones/year	214,299 tones/year	400,301.5 tones/year	6,608 tones/year	25,000 tones/year	23.3 million hectare

Agriculture wastes are the major source of anaerobic digestion. They may either be digested alone (mono digestion), results in an excessively high C/N ratio because of the plant wastes' high carbon content, or combined with other waste (co-digestion). Most lignocellulose residues have a significant amount of lignin, which slows down and incomplete anaerobic digestion, necessitating extensive retention periods of several weeks to months [79, 80]. As a result, co-digestion of crop residue with other substrates, such as animal manure, is more common than single-stage digestion of crop residue [81].

The biogas production potential for some agricultural residues from different research work has been compiled in Table 2.5.

Table 2. 5 Biogas production potential for agricultural residues

Agricultural residues	Dry matter (DM) (%)	Volatile solids (VS) (%)	Retention time (days)	Biogas production (m^3/ton)
Maize silage	20-35	85-95	-,261-300	450-700 [82], 710 [83]
Mown grass	12-35	83-92	-,80,42	550-680 [82], 322-349 [84], 500-600 [85]
Straw (chopped)	86	90	-	210 (1 kg^{-1} VS) [86]
Coffee husk	88	89	40,270,60	336 [87], 358 [88], 159.4 (1 kg^{-1} VS)
Chat residue	30	74-90	39,39	69 [24], 65 [89]
Rice straw	92-96	87	69,75	213 [88], 222 [90]
Cotton stalk	95	91	48,23	238-260 [91], 873 [92]
Agro-Fruit peels(orange, banana, pineapple, apple, jackfruit straw)	13. 5, 77, 15, 91, 85, respectively	99, 90, 89, 90, 92	47-63	715,482, 773, 679, 614, respectively [88]

2.9.2. Livestock waste

Ethiopia had the largest livestock population in Africa in 2020, with 65 million cattle, 40 million sheep, 51 million goats, 8 million camels, and 49 million chickens, according to the Ethiopian Statistics Agency (ESA) [93]. These animals produce around 42 million tons of dry-weight dung annually, with 84% of the production coming from cattle [94].

In developing nations, like Ethiopia, collected livestock manure can be spread on farmland or made into cakes that are then dried in the sun and used for home cooking by direct combustion.

The health of anyone breathing in this air, especially women and children, is negatively affected by the smoke since it produces large amounts of particulate matter and greenhouse gases [79, 95].

Biogas production from livestock wastes could also be a way to solve the environmental and economic challenges of managing animal wastes and manure [96]. Since the country has poor experience in managing livestock wastes properly [97].

- **Cattle dung/manure**

One of the most plentiful sources of digester feedstock is cattle manure/waste. Depending on the type of diet and the feeding rate, cattle discharge 8–12 kg of dung each day. After processing and collecting waste, nearly 9 kg of manure could be collected [98]. The bacteria in the digester convert around 20-30% of volatile chemicals into biogas. Cow manure can be used as the sole feedstock for biogas production [99].

Abdallah et al. [100] investigated the biogas potential for different types of cow manure with a substrate to innoculum ratio of 0.5 at 37 ℃ and found a methane yield between 147 and 215 mL CH_4/g VS. The total amount and quality of biogas yield were not stated. In similar work, Yohaness [22] found a maximum methane yield of 0.116 g CH_4/g TS for a retention time of 42 days at 37 ℃. Using a plug flow reactor, Dong et al. [101] produced methane of 0.22 m^3/kg VS (0.39 m^3/kg VS of specific biogas) within a temperature range of 37–40 °C in 25 days of HRT.

- **Pig manure**

Every day, a fully-grown pig generates around 5 kilograms of waste. Pig dung has a high nitrogen level and low carbon content, thus using it alone is not advised. Because of its high alkalinity, it has the ability to prevent microorganisms that produce methane from growing. Methanogens favor an acidic environment [99, 102].

The study on the biogas potential of pig manure by Miroshnichenko et al. [103] results in an average specific biogas yield of 109 m^3 /t oDM and a maximum methane yield of 66.88% within 35 days at 37 ℃. Yin et al. [102] investigated the biogas production on transparent and nontransparent reactors for a retention time of 53 days finding 265.53 and 47.29 m^3 /t of oDM, respectively, at 35 °C. Its highest methane content was 51.9%. Similarly, a capacity of 370 m^3 /t of oDM from swine manure was collected by Yu, T. et al. [104] in 40 days of retention time with alkaline microwaving pretreatment.

- **Chicken manure**

An average chicken generates 150–200 g of fresh manure [105], which can be used as a feedstock for anaerobic digestion. Nevertheless, the high nitrogen and phosphorus concentrations in chicken manure make it difficult for the bacteria in a biogas plant to digest it. By combining chicken waste with carbon rich materials, such as biomass or cow dung, you may increase the quality of poultry biogas [79, 99, 106].

Chicken manures' biogas production potential has been investigated by different researchers. Böjti et al. [106] studies show a sole digestion of chicken manure can produce around 150 mL/g oTS of methane for a 16.26 g oTS/L of chicken manure and a treated chicken manure with water extraction method can produce 24% more methane in 30 days. In a comparative study of cow manure and chicken dung, Amakom et al. [107] and Alfa [108] also found 9.23 mL/g and 35 mL/g of biogas from chicken dung, and 4.2 mL/g and 32 mL/g respectively in 30 days of retention time.

- **Abattoir/Slaughterhouse wastes**

Animal byproducts that are left behind after slaughtering are referred to as abattoir wastes. One of the most significant wastes that need to be tracked is the waste from slaughterhouses [109]. For instance, in Addis Ababa Abattoir Enterprise, inedible Wastes like animal tissues and organs, different glands, the intestine, bones, lungs, and different bodily components are changed to animal (dog and cat) foods with the help of heating. The remaining stomach/intestinal contents (rumen fluid/digest), dung, cattle blood, etc. are removed by connecting to the river, which contributes to environmental pollution. Because of their abundance of organic matter and moisture content, the organic components of abattoir wastes are a potential resource for the AD process.

According to Kefalew [23], the rumen digest weight was projected to be 12% of the overall weight of the cattle, whereas the amount of blood accessible was to be 8.4% of the weight of the cattle. It indicates that around 20.5% of total cattle weight is a combination of blood and rumen digest waste.

Bauer [110] employed dilution to make the blood less potent. During a 10-day incubation period at 35 °C, digesting blood water from an abattoir in a batch reactor fed with 0.6 g VS per liter yielded 0.73 m^3/kg VS of biogas and 0.117 m^3/kg VS of methane. In a batch digester, operated at 37 °C with 14 days of retention time, Gyasi et al. [111] studied the biogas production of blood. Resulting in no measurable methane, but the suggested 1:5 ratio of blood to rumen digest could produce 0.7375 mL/g of biogas and a 26.6% methane yield.

The cattle rumen content in a 1:1 ratio with water produced 52.5 mL/kg of biogas and an average methane production of 58.77% in Sylhet City, according to a work by Muhammad Rashed Al Mamun et al. [112] in 40 days. A study by Tambuwal et al. [113] investigated the biogas production potential of cattle rumen content and the distribution of bacteria. The study found that a retention time of 56 days and a ratio of 1:2 in favor of water resulted in 9.56 mL/g of biogas production and 55-70% methane output. Annor et al. [114] examined the effect of rumen waste to water ratio in biogas production and found that a 2:1 ratio of mixing generated the highest amount of biogas, with 16.70 m^3 per kilogram of rumen material produced in 20 days. In the 1:1 ratio, 10.72 m^3/kg of biogas was produced, whereas 12.02 m^3 was produced in the 1:2 ratio. The research showed that a 2:1 mixing ratio resulted in the highest proportion of methane (56.4%).

2.9.3. Municipal solid waste

Due to rapid population growth and industrial expansion, large volumes of municipal solid waste - mostly organic fractions, papers, plastics, etc. - are produced. This makes waste management challenging [72]. One way of handling this problem is through biogas. However, some MSW may contain toxic elements that are hazardous to the AD bacteria. To prevent the bacteria from collapsing, it should be pretreated. Furthermore, non-biodegradable feed components like woody biomass with high lignin content must also be eliminated or reduced to use the digester capacity efficiently [32].

The municipal solid waste generation rates of different towns and cities in Ethiopia are summarized in below Table 2.6.

Table 2. 6 Ethiopia cities and towns Municipal solid waste generation rates [115]

Town/City	Municipal solid waste (kg/capita/day)
Dilla	0.475
Chiro	0.30
Burayu	0.29
Robe	0.26
Addis Ababa	0.45
Mekelle	0.27
Bahir Dar	0.45
Wolaita Sodo	0.47
Jimma	0.34
Laga Tafo	0.41
Debre Birhan	0.25
Jigjiga	0.49
Hawassa	0.43
Dassie	0.45

The average generation rate in Ethiopian cities and towns ranges between 0.25 and 0.49 kg/capita/day. The bulk municipal solid waste generation recorded from households [115].

- **Food waste**

According to FAO 2019 report [116], about 33% (one-third) of the 1.3 billion tons of human food consumed is wasted worldwide. The food loss per capita in sub-Saharan Africa is 6 to 11 kg per year. Food waste is the major portion of the organic fraction in the MSW. It is a promising feedstock for AD and has high energy content. Food waste may be combined with other substrates in order to stabilize the digestive process, even though it can and is frequently digested on its own [79].

T.Moges and W.Bogale [23] with A case study of biogas for electricity generation from food waste in sidist kilo campus, Addis Ababa University, estimated the total of 90 mL/g of biogas per day which yields 59.7% of methane. Fufa, Dadi, and Birke [24] also investigated the biogas production potential of food waste in the Jimma region of Ethiopia, recording 64.2 mL/g biogas yield with 57.86% methane gas in 35 days of HRT. Furthermore, Hussaro et al. [117], investigated biogas production by feeding 105.28 kg fresh mass of food waste, which produced 1152.45 L of biogas in 40 days with the highest methane percentage at 55%.

2.10. Summary of the literature review

MSW and agricultural waste, in addition to livestock manure, offer benefits for energy production through anaerobic digestion. Agricultural waste is not always available, unlike animal or food waste. It is produced around the harvest period and must be stored properly to provide a consistent supply year-round. The agricultural residues are finely chopped and compressed to release oxygen [79].

In Ethiopia, cattle manure is used as the primary substrate for biogas generation since it is easily accessible on agricultural fields. Despite having several advantageous qualities for AD, livestock manure generates less biogas than other feedstock since the animal intestine has already predigested them. However, due to its beneficial properties, it is frequently used as a basic substrate and digested along with other feedstock [32].

Therefore, using various substrates for the production of biogas, such as agricultural waste, municipal solid waste, grass, food waste, and livestock waste from sheep, pigs, chickens, and other animals, not only increases the amount of gas produced in the nation but also serves as a method of waste management.

Although biogas production has been evaluated for some feedstock, there are still many that have not been thoroughly examined. Ethiopia has a vast range of feedstock available for biogas production, and the quality and quantity of the gas produced is significantly impacted by these feedstocks. This study aimed to investigate the quantity and quality of methane yield from various substrates for biogas generation.

3. MATERIAL AND METHOD

3.1. Study Area and Period

The study was conducted at the Addis Ababa Institute of Technology (AAiT) environmental laboratory, Ethiopia. The data collection and experiments were conducted from January 2023 to June 2023. The experiments took place in two batch categories, from February 14 to March 13 for the first batch and the second from March 26 to May 6, 2023, which has 28 and 40 days of hydraulic retention time for each batch, respectively. Before the two batches run, an adaptation experiment was done.

3.2. Substrates and Inoculum

The feedstocks for the biogas production in the laboratory batch digester were samples of mown grass (S_2), food waste (S_3), chicken manure (S_4), cattle blood (S_6), cattle rumen content (S_7), pig manure (S_8) and cow dung (S_9) as shown in Figure 3.1. The cow dung was used for comparing the potential of biogas production from the rest of the feedstocks.

The grass (local name Q'etema, Poaceae family), common type of grass in most parts of Ethiopia, and food wastes used in this study were collected from the AAiT 5 kilo campus background and the student restaurant, respectively. Whereas the pig and chicken manure were obtained from the micro and small enterprise pig and chicken farm in Ferensay Legasion, Addis Ababa.

The cow blood and rumen content were collected from Addis Ababa Abattoir Enterprise (AAAE), which is the biggest Abattoir in the city that provides more than half of the city's meat demand with the average daily capacity of slaughtering 1,200 animals both in main and Akaki Abattoir. The number increases up to 5000 cattle per day in holidays [118].

The inoculum I (S_1), inoculum II (S_5), and cow dung were collected from Zagol Dairy Farm, a long-running biogas plant in Chancho City. It has a productive 80 m^3 biogas plant connected to an electricity-generating biogas generator.

To avoid contamination, the inoculum and substrates (1 kg each) were collected before having contact with any other type of waste and stored at 4 ℃ until use.

Figure 3. 1 Substrate used in the experiment: a. Inoculum, b. Chicken manure, c. Rumen content, d. Grass, e. Cattle blood, f. Food waste, g. Pig manure and h. Cow dung

3.3. Sample Preparation

In order to boost anaerobic degradation, the size of the substrates has been reduced aiming at particle sizes of around 10-20 mm. The size reduction was performed by separating interferents (inorganic materials comes with the substrate and large grain sizes) out and later using crushing and grinding.

After the desired size was obtained with a grade of 10-20 mm, the substrates were homogenized by stirring. The inoculum and liquid substrates were well mixed before being introduced to the digester in order to maintain a representative sampling. The steps shown in Figure 3.2 are used to prepare the substrates for the AD test.

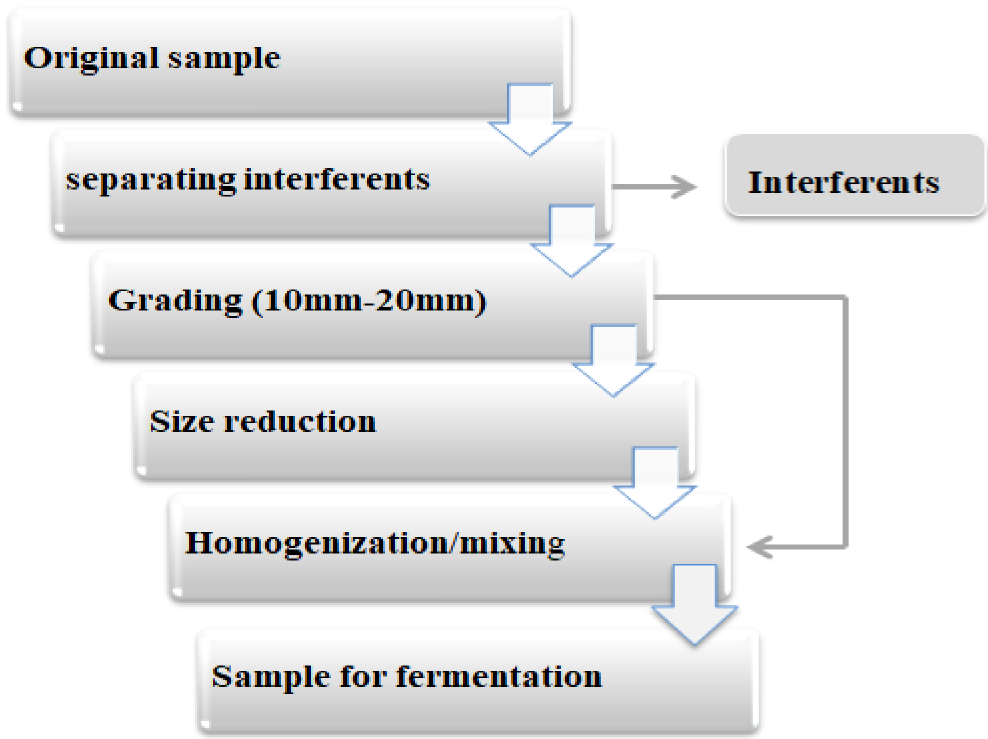


Figure 3. 2 Procedure of sample preparation

3.4. Equipment and Chemical Used

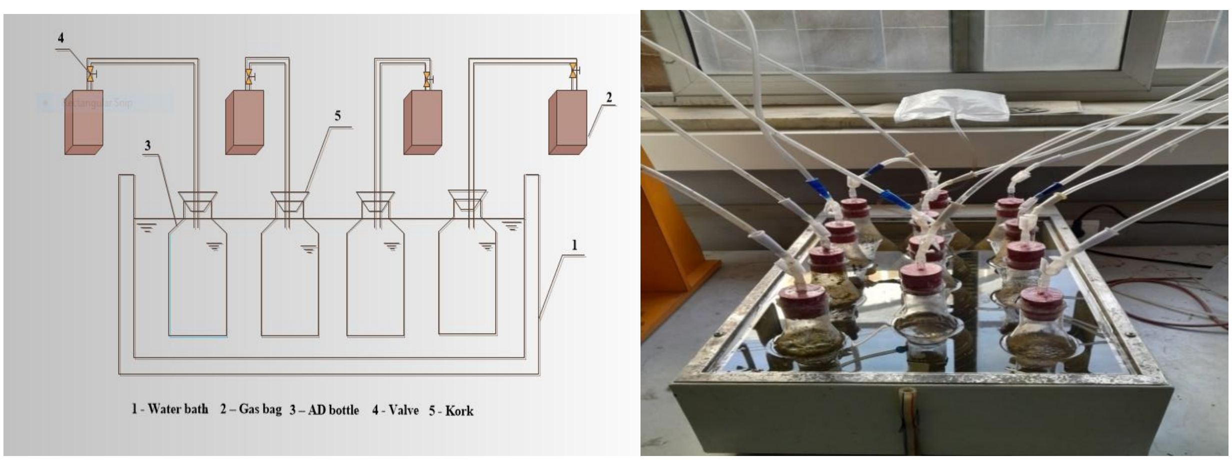
The major equipment and chemicals used in the experiment are listed in Table 3.1 below.

Table 3. 1 Equipment and chemicals used in the experiment

Materials		Institution/ Organization	Purpose in the Project
Chemicals			
i.	Nitric acid, sodium chloride and phenyl		• For barrier solution in the volume measurement
ii.	Mercury sulfate, potassium dichromate, and sulfuric acid.		• Reagents for COD analysis
Equipment/labratory tools			
i.	Water bath	AAU/AAiT	• To keep samples at a constant temperature.
ii.	Measuring cylinders (2000 mL) and flask (500 mL)		• For measuring the volume of biogas (liquid displacement method) and AD digester
iii.	Biogas analyzer (GeoTech Biogas 5000)		• For gas composition analysis
iv.	Gas bag/urine bag (2000 mL)		• For storing produced biogas
v.	Rubber Pipes, korks and valves		• For fluid flow control
vi.	Oven, Furnace and crucibles		• For proximate analysis
vii.	DO (dissolved oxygen) meter, incubator, COD digester multi-photometer and 3510 pH meter		• For BOD and COD determination, and pH measurement
viii.	Data logger (DL220THP)		• For recording temperature, humidity and pressure

3.5. Experimental Setup and Operation

The anaerobic digestion experiment was conducted in reference to VDI standard procedure 4630 [119]. The reactor mainly consists of batch scale mesophilic anaerobic digestion reactors (500 mL pyrex glass flask), biogas collection device (urine bag), and temperature control device (water bath) as shown in Figure 3.3. The substrate and inoculum were added to the reaction flasks in accordance with the calculated loading rate. The total loading volume was adjusted to 500 mL by adding water. For each treatment, triplicate samples were used in two categories of the batch experiment. After the substrates, inoculum, and water were mixed well, the flasks were closed with a rubber kork and sealed with silicon to ensure an anaerobic environment. The reactors were placed in a water bath at a mesophilic temperature of 40 ± 0.2 ℃ for 28-40 days. The gas volume and composition was measured at the same time every three days using the liquid displacement method and gas analyzer (GeoTech Biogas 5000), respectively. The cumulative gas production was calculated at the end of anaerobic digestion. To avoid scam formation the flasks were manually stirred twice a day during the experiment.



(a) **(b)**

Figure 3. 3 Experimental setup of the batch AD: (a) Schematic and (b) image

3.6. Characterization of the Feedstock

3.6.1. Proximate analysis

In this study the proximate analysis used APHA, 1999 (American Public Health Association) standard method for the examination of water and wastewater [120]. The section used for determining total solid, volatile solid, fixed solid was 2540 A-G; for BOD_5 was 5210 and COD was 5220.

- **Total Solid**

Total solid refers to the remaining solids in the sample following the loss of moisture/water molecules or it is the amount of solid material residue that is still in the crucible after the sample has evaporated and has been dried at 105°C in a laboratory oven.

The procedures followed for the analysis of total solid are;

i. The crucibles were thoroughly cleaned, dried for one hour at 105°C in the laboratory oven, and then chilled in a desiccator.

ii. The crucibles were weighed using an electronic precision balance. And the result was recorded before use.

iii. The substrate samples were mixed properly and loaded (duplicate for each sample) to the crucibles and re-weighed.

iv. A temperature of 105°C was allowed to build up in the laboratory oven after it was turned on. The experiment was conducted at this temperature.

v. The crucibles with the substrates samples were placed in the laboratory oven and left to dry for 24 hours.

vi. To maintain the temperature, the crucibles with the substrate samples were allowed to cool in a desiccator.

vii. The crucibles with the substrate samples were weighed and the percentage of total solids was calculated using equation (3.1) below.

$$\%TS = \frac{W_{cd} - W_c}{W_{cw} - W_c} \times 100 \quad (3.1)$$

Where,

$\%TS$ = Total solids percentage

W_{cd} = Weight of crucible with the dried substrate at 105 ℃

W_c = Weight of crucible

W_{cw} = Weight of crucible with the substrate(wet) before placed into the laboratory oven

- **Volatile Solid**

The volatile solid is the fraction of the total solid remaining after it is ignited at 600°C.

The procedures followed for the analysis of volatile solids are;

i. Using a muffle furnace, the remains produced from the total solids determination were burned for two hours at 600°C.

ii. Before being moved to the desiccator for complete cooling, the crucible and burned solids were permitted to cool by turning off the muffle furnace.

iii. The crucibles with the substrate samples were weighed and the percentage of volatile solids was calculated using equation (3.2) below.

$$\%VS = \frac{W_{cd} - W_{cv}}{W_{cd} - W_c} \times 100 \tag{3.2}$$

Where,

$\%VS$ = Volatile solids percentage

W_{cd} = Weight of crucible with the dried substrate at 105 ℃

W_{cv} = Weight of crucible with the substrate after removed from the muffle furnace

W_c = Weight of crucible

- **Fixed Solid**

The fixed solids are represented by what's left of the sample after ignition. The percentage of fixed solids was calculated using equation (3.3) below.

$$\%FS = \frac{W_{cv} - W_c}{W_{cd} - W_c} \times 100 \tag{3.3}$$

Where,

$\%FS$ = Fixed solids percentage

W_{cd} = Weight of crucible with the dried substrate at 105 ℃

W_{cv} = Weight of crucible with the substrate after removed from the muffle furnace

W_c = Weight of crucible

- **Moisture Content**

Water content was determined by using oven dry at a temperature of 105±1ºC for 24 hours and the weight difference was measured using analytical balance.

$$\%MC = \frac{W_{wet} - W}{W_{dried}} \times 100 \quad (3.4)$$

Where,

MC = Moisture content

W_{wet} = Weight of wet sample

W_{dried} = Weight of dried sample

- **Biochemical Oxygen Demand (BOD_5)**

The 5-day biochemical oxygen demand (BOD_5) test was carried out for the raw materials using as stated in the standard method, the major apparatuses used were a DO (dissolved oxygen) meter and an incubator set at 20±1 ºC. The initial dissolved oxygen and the final dissolved oxygen after 5-day incubation were measured and recorded. The BOD was then calculated in the given equation 3.5 below.

$$BOD(mg/L) = \frac{D_1 - D_2}{P} \quad (3.5)$$

Where,

D_1 = is the initial dissolved oxygen of the raw dung (mg/L)

D_2 = is the final dissolved oxygen after 5 − day incubation of the dung (mg/L)

P = is the decimal volumetric fraction of the sample used

- **Chemical Oxygen Demand (COD)**

The chemical oxygen demand determined using section 5220D closed reflux (calorimetric) method using reagents like mercury sulfate, potassium dichromate, and sulfuric acid.

1 mL of each samples to be examined were diluted with 5 mL of distilled water. After diluting the sample, 2 mL of it was added to COD reagent and digested for two hours in a digestion tank that was heated to 150 °C. Following digestion, the liquid was chilled and put into vials for reading by a multi-photometer.

COD removal efficiency was calculated using Equation 3.6 below.

$$\mathrm{COD}_{\mathrm{Removal\ efficency}} = \frac{\mathrm{COD}_{\mathrm{BD}} - \mathrm{COD}_{\mathrm{AD}}}{\mathrm{COD}_{\mathrm{BD}}} \times 100\% \tag{3.6}$$

Where,

$\mathrm{COD}_{\mathrm{BD}}$ = Chemical oxygen demand before AD

$\mathrm{COD}_{\mathrm{AD}}$ = Chemical oxygen demand after AD

3.6.2. Ultimate analysis

- **Nitrogen content (Total kjeldahl nitrogen)**

The total Kjeldahl nitrogen was determined using section 4500-Norg. 1 g of every sample was loaded in the Kjeldahl flask, and then concentrated sulfuric acid was added to help break down the nitrogen in the sample. Then the solution was distilled using a 1.5N NaOH solution capable of producing ammonia from the ammonium salt. By using sample titration in boric acid, the sample's ammonia content was determined. The ammonia gas is captured by the boric acid. In the end, using the measured concentration of ammonia ions in the receiving boric acid solution, the quantity of nitrogen in a sample has been determined.

- **Total organic carbon (TOC)**

The total organic carbon was determined using section 5310 B. high temperature combustion method. The sample was blended and diluted as necessary and a portion of the sample is placed into a heated reaction chamber at 105±1 ºC containing cobalt oxide, an oxidative catalyst. Then the remaining TOC is measured by excluding the inorganic carbon.

3.7. C/N Ratio

The carbon to nitrogen ratio of each sample was calculated by dividing the values of carbon to nitrogen that has been calculated in the ultimate analysis as an equation 3.7.

$$\text{C: N ratio} = \frac{\text{C\%}}{\text{N\%}} \quad (3.7)$$

3.8. Organic Loading

In the batch fermentation, a discontinuous test in which organic substrates are subjected to ferment under defined anaerobic conditions, the weight of the organic mass of the inoculum was taken in the range of 2% to 2.5% (minimum to maximum) of the total volume of the digester. The percentage has an equivalent value of 10 g VS to 12.5 g VS for a 500 mL digester (assumption 1 g = 1 mL).

For determining the amount of the fresh mass (FM) of inoculum and substrate loaded into a fermentation batch, the following equations have been used.

$$\text{Inoculum FM (g)} = \frac{\text{12.5 g VS}}{(\text{\%TS}\times\text{\%VS})_{inoculum}} \quad (3.8)$$

$$\text{Substrate VS (g)} = 0.5 \times 12.5 \text{ g VS} = 6.25 \text{ g VS} \quad (3.9)$$

$$\text{Substrate FM (g)} = \frac{\text{Substrate VS (g)}}{(\text{\%TS}\times\text{\%VS})_{substrate}} \quad (3.10)$$

In this study, the organic mass of the substrate had about 50% of that of the inoculum (inoculum to substrate ratio was 2:1) to prevent inhibition as shown in equation (3.9) [119]. In order to compare the feedstock potential at the same ORL, all substrates were set to 6.25 g VS and 12.5 g VS for the cow dung and the inoculums, respectively. Table 3.2 below shows the fresh mass (FM) of inoculum and substrates loaded into a fermentation batch.

Table 3. 2 Fresh mass (FM) of inoculum and substrates loaded into a fermentation batch

FIRST BATCH			SECOND BATCH		
Sample	Inoculum (g)	Substrate (g)	Sample	Inoculum (g)	Substrate (g)
S_{11}	203.75		S_{51}	165.1	
S_{12}	203.59		S_{52}	165	
S_{13}	203.59		S_{53}	164.84	
S_{21}	203.54	21.13	S_{61}	165.02	47.78
S_{22}	203.52	21.13	S_{62}	165.04	47.71
S_{23}	203.56	21.13	S_{63}	165	47.7
S_{31}	203.51	20.33	S_{71}	165.04	75.34
S_{32}	203.55	20.32	S_{72}	165.07	75.31
S_{33}	203.58	20.32	S_{73}	165.08	75.3
S_{41}	203.54	24.44	S_{81}	165.03	34.7
S_{42}	203.52	24.44	S_{82}	165.11	34.71
S_{43}	203.55	24.45	S_{83}	164.88	34.75
			S_{91}	165.2	101.8
			S_{92}	165	101.67
			S_{93}	165.1	101.6

3.9. Temperature and pH

The experiment was done under a mesophilic temperature of 40 ± 0.2 ℃ using water bath. The room temperature of the Environmental Science Laboratory was monitored during the HRT with 10 minute gap using Data logger (DL220THP) and the pH of influent and effluent was recorded using 3510 pH meter as shown in Figure 3.4.

(a) **(b)**

Figure 3. 4 Temperature and pH measurement: (a) Data logger (DL220THP) and (b) 3510 pH meter

3.10. Gas volume and Composition

The biogas quantity and quality are expressed in volume and composition, respectively. These are one of the performance evaluator in the field.

3.10.1. Gas Volume

For the volume measurement of the biogas, a laboratory scale liquid displacement gas volume measuring device was constructed using a 2L plastic measuring cylinder as shown in Figure 3.5. In order to minimize the biogas's carbon dioxide dilution in the displacing liquids, a saturated acidified brine solution (NaCl and citric acid) was used as a barrier solution [121]. For ease of reading, a phenol red color indicator with a pH range of 6.6 (yellow) to 8.4 (red) was used.

Figure 3. 5 laboratory scale liquid displacement gas volume measuring device

3.10.2. Gas composition

The gas composition of the biogas stored in the gas bags was measured using a portable biogas analyzer (GeoTech Biogas 5000) shown in Figure 3.6. The reading is expressed as percentages for methane, carbon dioxide, oxygen, and trace elements (nitrogen, hydrogen..), and as ppm(parts per million) for hydrogen sulfide.

Figure 3. 6 portable biogas analyzer (GeoTech Biogas 5000)

3.11. Analytical Method

3.11.1. Normalization

To standardize the biogas and methane production, the fermentation gas volume was normalized to standard conditions. This was calculated using the following equation based on the readings taken during each period [119]:

$$V_0^{tr} = V \cdot \frac{(P - P_w) \cdot T_0}{P_0 \cdot T} \quad (3.15)$$

Where,

V_0^{tr} volume of the dry gas in the normal state, in mL

V volume of the gas as read off, in mL

P pressure of the gas phase at the time of reading, in hPa

P_w vapour pressure of the water as a function of the temperature of the ambient space, in hPa

T_o normal temperature; $T_o = 273$ K

P_o normal pressure; $P_o = 1013$ hPa

T temperature of the fermentation gas or of the ambient space, in K

The vapour pressure of the water was obtained by equation 3.16 [122].

$$P_w = 6.1094 \exp\left(\frac{17.625T}{T + 243.04}\right) \quad (3.16)$$

Where, T Temperature in ℃ and

P_w vapour pressure in hPa

3.11.2. Specific methane yield (SMY)

The specific methane yield is the accumulative volume of methane produced at the end of HRT, divided by the mass of inlet VS content.

$$SMY = \frac{\sum V_m (ml)}{VS (g)} \quad (3.17)$$

4. RESULT AND DISCUSSION

4.1. Characteristics of the Inoculum and Substrates

Characterizing the feedstock for the influent and effluent improved the outcome of anaerobic digestion and optimized the process at both the scientific and industrial levels in the anaerobic digestion process [123]. In the batch digestion, the mean values (triplicate) of TS, VS, MC, carbon to nitrogen ratio (C/N), TKN, pH, biological oxygen demand (BOD), and chemical oxygen demand (COD) before and after AD are shown in Table 4.1. All the values of the triplicate samples are also shown in Appendix I.

The TS and VS of the mown grass were 34.22%±0.22 and 86.82%±0.9, respectively. These values agreed with a study done by Hidosa et al. [124], who reported a TS value of 34.59% and a VS of 89%. For food waste, studies [125, 126] in Addis Ababa obtained 30.9% of TS and 89% of VS which was lower than the current study. These variations could be a result of a waste combination (injera and the stew). As shown in Table 4.1 the chicken, cow, and pig manure values have agreed with the values in literature [32–34]. The cattle rumen's TS and VS results were 9.97% and 83.31%, respectively, that has a slight variation with results reported by Tsegaye [127] and Abdeshahian et al. [128], with a 16% TS and 84.85% VS, because of factors that include the way of sampling, feeding type of animal and type of sanitation system. Nazifa et al. [129] reported a blood TS of 10.8–23% and VS of 91–96.6%, as well as the cattle blood in this study, fell into the interval.

Jyi LAY et al. [130] suggested the moisture content of substrates increases the bio-methane production by facilitating the methanogenic activity; consequently, the range of moisture content in this study (52.14%±0.24 to 92.77%±0.01) indicated that the moisture content may increase the activity of the bacteria.

The ratio of BOD to COD indicated the biodegradability of organic matter. Typical values range from 0.3 to 0.8. If the ratio is 0.5 or greater, the waste is considered to be easily treatable by biological means. Similarly, a ratio below about 0.3 indicates toxic components and very high organic matter presence [131, 132]. The ratio is relevant for wastewaters like the blood, rumen content, and inoculum.

Table 4. 1 Characteristics of inoculum and substrates

sample	C/N	TS (%)		VS (%)		MC (%)		COD (mg/L)		BOD (mg/L)		pH	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Inoculum I (S_1)	17.25	7.23	3.11	85.30	41.90	92.77	96.89	3922.5	907.00	1842.33	311.27	6.73	6.96
Inoculum II (S_5)	16.60	8.83	2.60	83.53	29.58	91.17	97.40	3700.7	921.89	1776.9	164.90	7.08	7.25
Grass (S_2)	21.18	34.22	14.29	86.82	41.54	65.78	85.71	4885	993.00	2298.5	349.47	7.08	7.47
Food waste (S_3)	17.86	47.86	19.26	64.30	37.56	52.14	80.74	3728	765.11	1406.13	166.11	7.12	7.57
Chicken manure (S_4)	4.47	29.61	11.72	86.56	31.96	70.39	88.28	4808	1002.56	2726.3	315.74	8.44	7.79
Cattle blood (S_6)	10.60	13.72	5.16	95.47	39.54	86.28	94.84	3790	970.89	2152.62	346.27	7.36	7.85
Rumen content (S_7)	14.48	9.97	3.83	83.31	31.02	90.03	96.17	5866	1428.89	2426.8	148.46	7.26	7.15
Pig manure (S_8)	10.67	23.95	9.86	66.64	31.05	76.05	90.14	4327	1146.56	1754.4	142.77	7.76	7.45
Cow dung (S_9)	22.61	15.18	6.89	81.14	35.18	84.82	93.11	6917	2182.00	3104.4	144.98	7.31	7.12

From this experiment, the amount of the BOD/COD was 0.47, 0.47, 0.38, 0.57, 0.48, 0.57, 0.41, 0.41, and 0.45 for inoculum I, grass, food waste, chicken manure, inoculum II, cattle blood, cattle rumen content, pig manure, and cow dung, respectively. The results showed that the samples contained an easily degradable organic matter.

The optimal C/N ratio for AD processes reported by literature [32–34], which are compiled in Table 2.1, indicated the C/N ratio results of this experiment were compatible with the ranges. The samples' C/N ratio ranges from 4.47 to 22.61. An inadequate C/N ratio could inhibit microbial activity and affects the degrading processes. The minimum 4.47 ratio was for the chicken manure which was the result of higher nitrogen content [133]. Lin et al. [134] also describe the optimum C/N ratio for AD processes such as cow dung to be 20-30 but also 10-20 also can give a noble biogas yield.

4.2. Biogas production potential and Composition

4.2.1. Cumulative biogas production

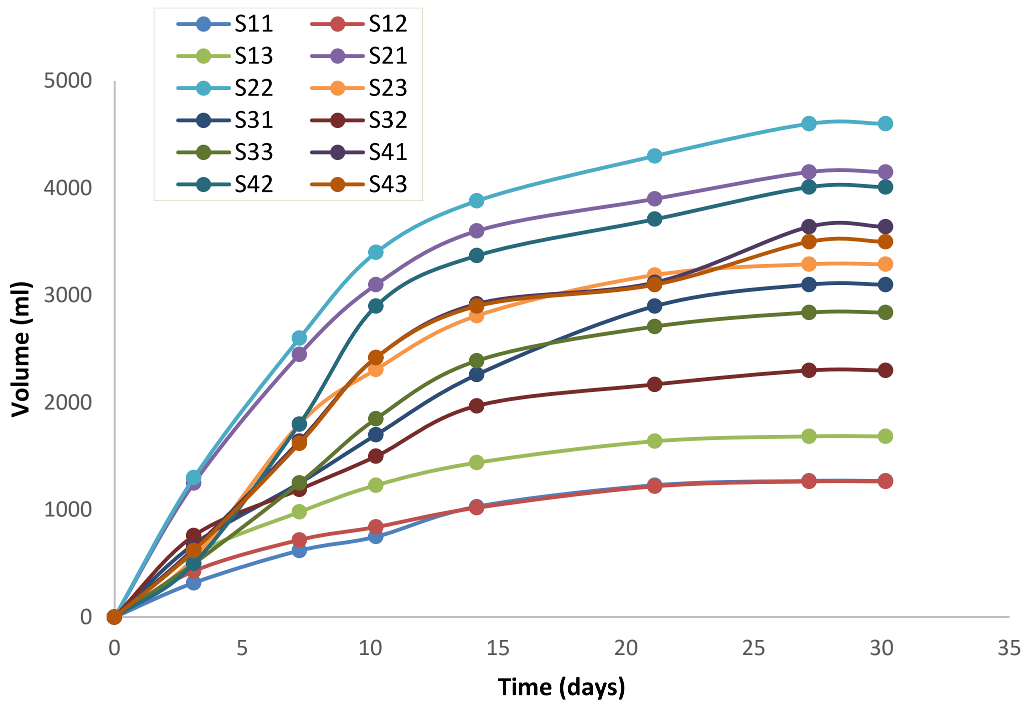
The cumulative biogas production of the substrates with their triplicate samples from the two batch experiments is shown in Table 4.2. The patterns of the cumulative biogas production are also shown in Figure 4.1. Although the sample's patterns were different, each one of them adhered to the bacterial growth curve [119].

For the two batch experiments, every sample except the control (inoculum II) for the second batch started producing biogas on the first day. The remaining sample began producing gas after two days. The interval for the gas volume measurement was every three days. Unless the samples produce measurable gas; it could go to 5 or 6 days (mostly at the end of the HRT).

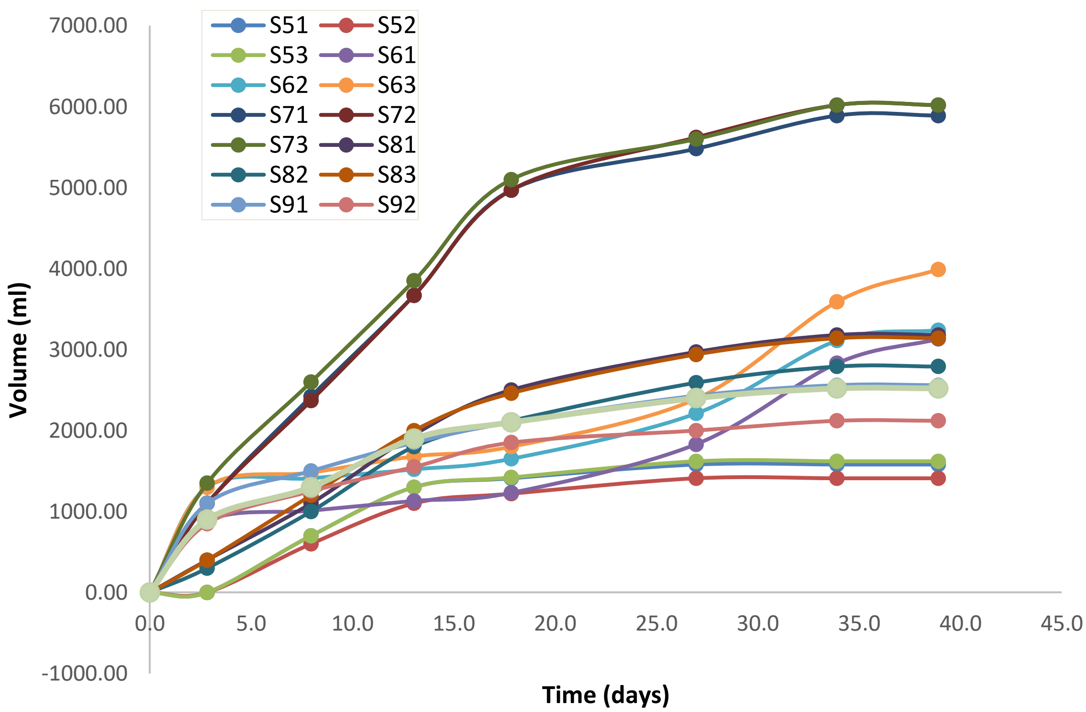
Table 4. 2 Cumulative biogas and methane production

Sample	Cumulative biogas (mL)	Cumulative methane (mL)	Sample	Cumulative biogas (mL)	Cumulative methane (mL)
S_{11}	1270	787.08	S_{51}	1580.00	920.70
S_{12}	1265	710.385	S_{52}	1410.00	818.44
S_{13}	1685	986.51	S_{53}	1620.00	1001.94
S_{21}	4150	2567.7	S_{61}	3130.00	1858.72
S_{22}	4600	2963.28	S_{62}	3240.00	1983.46
S_{23}	3290	2056.54	S_{63}	3990.00	2501.84
S_{31}	3100	1590.48	S_{71}	5890.00	4039.82
S_{32}	2300	1183.85	S_{72}	6020.00	4031.55
S_{33}	2840	1545.62	S_{73}	6020.00	4022.17
S_{41}	3640	2270.54	S_{81}	3180.00	2034.84
S_{42}	4010	2492.05	S_{82}	2790.00	1862.15
S_{43}	3500	2151.2	S_{83}	3140.00	1996.16
			S_{91}	2560.00	1505.26
			S_{92}	2120.00	1406.42
			S_{93}	2520.00	1493.78

The biogas production began at different rate for samples since the microorganisms need different intervals in order to adapted their new environment before actively engaged in the process [111].



(a)



(b)

Figure 4. 1 Cumulative biogas productions: (a) First batch and (b) Second batch

Through the first 9 to 12 days the gas production went steeper for S_{21}, S_{22}, S_{71}, S_{72}, and S_{73} which showed the substrates contain easily convertible substances [119, 135]. Consequently, they had also the highest biogas producer among the feedstocks used in the study. The mown grass (S_2) stopped producing measurable gas after 28 days while the rumen content (S_7) took 34 days. Although the production of S_{61}, S_{62}, and S_{63} highly decreased between day 3 and day 18 which could be caused by pH variation or accumulation of fat results in difficulty in degradation, the production of gas continued with normal trend after day 18 until they cease their production at day 40.

The output of the cow dung (S_{91}, S_{92}, and S_{93}) and chicken manure (S_{41}, S_{42}, and S_{43}) gradually increased during the period of the first 9 days, but then steadily dropped until they were completed producing gas on days 28 and 34, respectively.

Until day 12, the production of pig manure (S_{81}, S_{82}, and S_{83}) was increasing. After that, it began to decline. It took 34 days to finish producing gas. Meanwhile, the production of food waste (S_{31}, S_{32}, and S_{33}) was much less when compared to other substrates from the study. It started decreasing from day 3. The food waste samples were influenced by the higher protein and stew content through the first batch and adaptation experiments [136].

The feedstock's cumulative biogas productions vary from 2120 mL (cow dung) to 6020 mL (rumen content). The maximum cumulative biogas production for the two batches of inoculum was 1685 mL (first batch) and 1620 mL (second batch). The different amounts of biogas produced by the samples indicated that the type of feedstock used can have a significant impact on biogas production. Figure 4.2 shows the cumulative biogas production of the samples with their corresponding methane production.

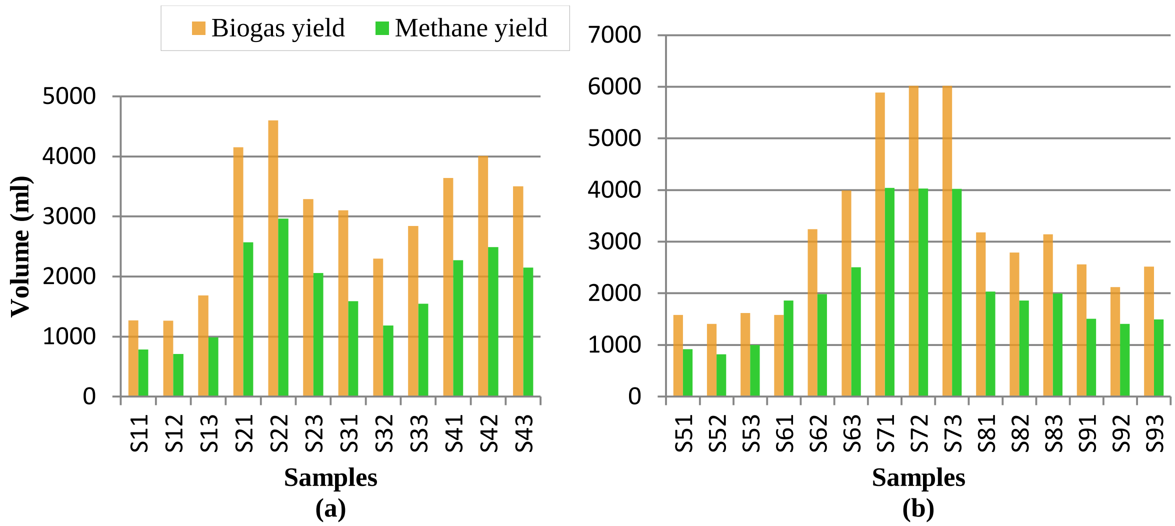


Figure 4. 2 Cumulative biogas and methane production of samples: (a) First batch (b) Second batch

4.2.2. Biogas composition

- **Methane composition**

The grass (S_2) produced the highest methane gas percentage of 79.4% among the substrates. The highest and average methane production for S_3, S_4, S_6, S_7, S_8, and S_9 was 67.9% and 49.78%, 77.8% and 58.77%, 68.6% and 54.34%, 77.1% and 62.81%, 77.6% and 61.2%, 69.9% and 59.89%, respectively. Similarly, the inoculum's methane percentage was 69.9% and 54.3% for the first batch, and 71.6% and 52.64% for the second batch. The biogas methane, CO_2 and H_2S composition for the samples is shown in Figure 4.4.

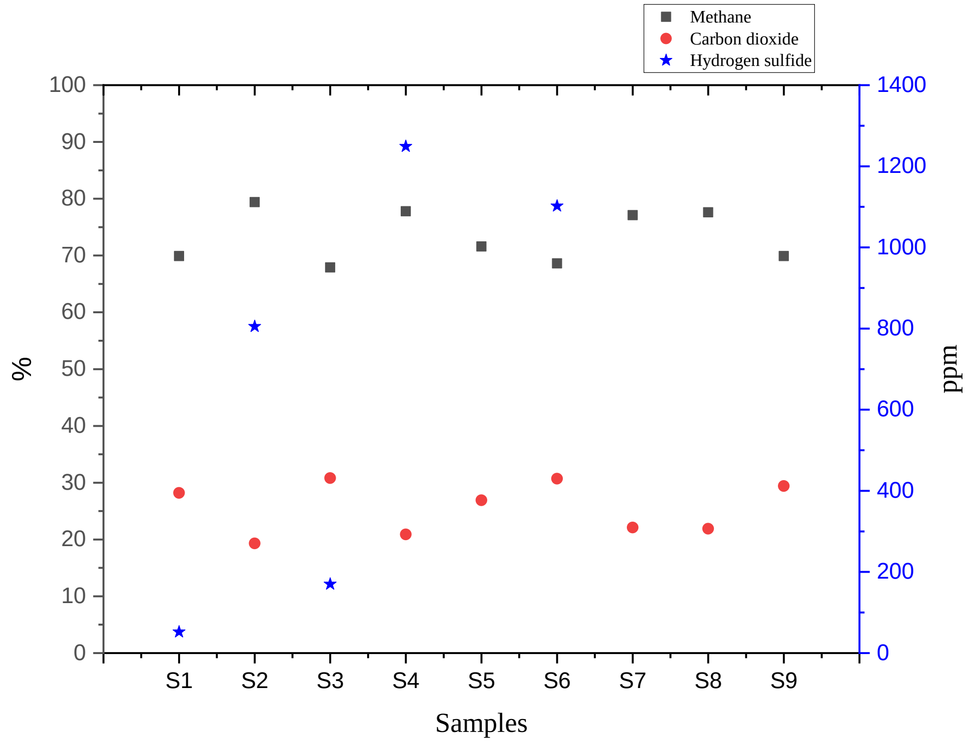


Figure 4. 3 Methane, Carbon dioxide and hydrogen sulfide concentrations of the biogas produced by the samples

Biogas typically contains between 45% and 75% methane by volume and the majority of the remaining gas is CO_2 [1]. When compared to other studies, the experiment's highest methane content was relatively high. Chiumenti et al. [137] and Unpaprom and Ramaraj [135] reported a maximum methane composition of 55.2% and 70.2%, respectively for a mowed grass silage AD; which was lower than 79.4% from this study. The reason for the highest amount of methane percentage for the grass could be the inoculum used, which has also a relatively higher percentage of methane. Additionally, the food waste also obtained a relatively higher composition of 67.9% than the values of 57.86% reported by Fufa, Dadi, and Birke [24] and 43.5% by Jiménez-Ocampo et al. [138]. The highest methane percentage obtained by Huang et al., Wedwitschka et al., and Buivydas et al. [140–142] (75.7%, 68%, and 63.2%, respectively) suggested that the chicken manure also produced higher methane content as well.

The maximum methane percentage in the cattle blood was 68.6%, which was a significantly higher figure than that of Gyasi et al.'s [111] work, which only generated 26.6%. M. R. Al Mamun et al.'s [112] attempt to recycle domestic animal rumen material from a slaughterhouse in 2018 produced biogas with the highest methane level at 62.2%. Similarly, Tsegaye [127] attained 54% of methane from AAAE rumen waste. A pig dung co-digestion with pineapple peels was investigated by Ofomatah et al. [142], and the sole digestion of pig dung produced 71% methane content, and also a close result was reported by Hanum et al. [143] from co-digestion of pig manure and fermented liquid feed. Both findings were less than what the experiment obtained. Furthermore, in comparison to the 69.9% found in this study, Gemechu [144] and Alvarez et al. [145] reported maximum methane compositions of 66.9% and 57±4%, respectively, for cow dung fermentation. The raw biogas volume and gas composition is shown in Appendix III.

- **Carbon dioxide composition**

Carbon dioxide (CO_2) made up 14.3% (minimum) to 66.4% (maximum) of the biogas produced. The percentage increased as methane content decreased at the end of the HRT. The presence of CO_2 has adverse effects on a number of parameters, including fuel conversion efficiency, combustion enthalpy, and volumetric energy density [58]. The average CO_2 production for samples S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, and S_9 was 44.23%, 39.01%, 48.75%, 39.59%, 45.36%, 45.92%, 35.19%, 36.8%, and 38.37%, which was in line with the range of values reported by a biogas handbook review [1].

- **Hydrogen sulfide**

The highest concentrations of hydrogen sulfide were produced by the chicken manure and cattle blood, which produced 1249 and 1102 ppm, respectively. The concentration of H_2S in chicken manure agreed to the results found by Konkol et al. [146], which ranged from 1000 to 35000 ppm for the anaerobic digestion of chicken manure and maize silage. Along with the two, the grass also generated 805 ppm of cumulative H_2S within 28 days of the HRT. The inoculum I and food waste both contained trace levels of H_2S, with the inoculum I 52 ppm and the food waste 170 ppm. For inoculum II, rumen content, pig dung, and cow manure, no measurable H_2S was produced.

The hydrogen sulfide (H_2S) in biogas is treated as impurity, that has a corrosive property which damages the instruments used in the biogas production, is a health hazard and also has a fetid odor like a rotten egg which is not suitable when it is used for cooking [58]. Nonetheless, an extraction of sulfur from the H_2S can help for the conversion of power to protein, a proposed future work depending on these results.

4.3. Normalization of the Biogas Production

Biogas production must be given at comparable pressure and temperature conditions, which are often at local or standard conditions, in order to compare the results from various published literature. There have been several papers published on the area of anaerobic digestion that failed to adjust gas production from local pressure and temperature conditions [147]. This is also our country's problem which makes it difficult to compare the results.

In this study, the biogas and methane production was normalized to the standard conditions (T= 273 K and P= 1013 hPa) [119] and the values are displayed in Appendix V and VI.

4.3.1. Net biogas production

The amount of biogas that a desired substrate could generate is shown by the net biogas production. The volume of the biogas produced by the inoculum was normalized and subtracted from the substrate's gas production to determine the amount of net biogas production and shown in Table 4.3 and Figure 4.3.

Table 4. 3 Normalized net biogas production of the feedstocks

Samples	Net biogas volume (mL)	Normalized net biogas volume (mL_N)
S_2	2606.67	1758.53
S_3	1340	903.502
S_4	2310	1558.789
S_6	1350	1309.015
S_7	4400	3034.98
S_8	1500	1024.006
S_9	803.33	592.9918

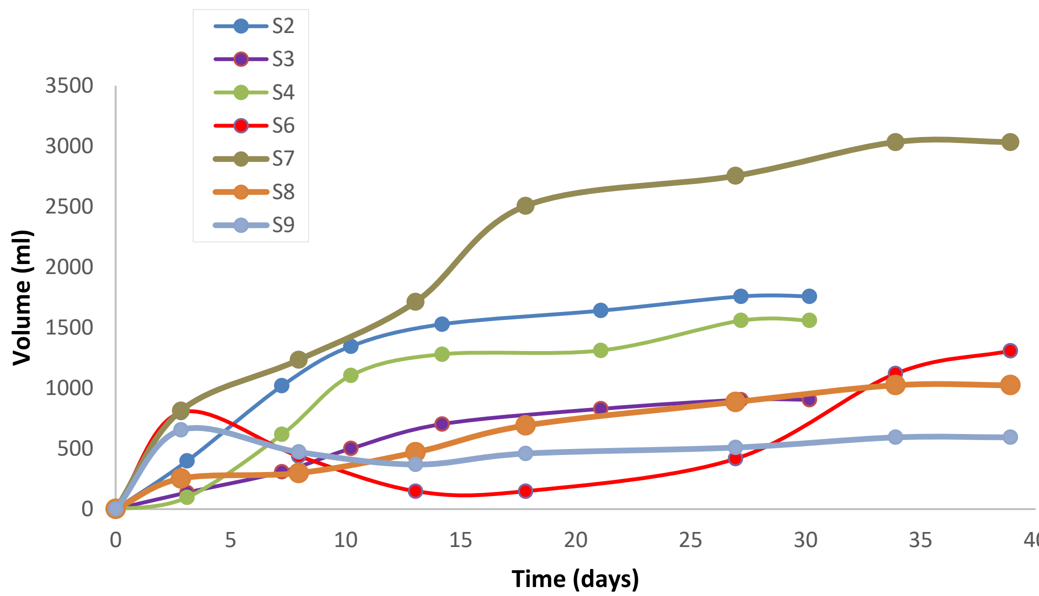


Figure 4. 4 Net cumulative biogas production of substrates

The net biogas production from the substrates S_7, S_2, S_4, S_6, S_8, and S_3 made up more than 74 %, 65 %, 62 %, 55.5 %, 49.5 %, and 49 %, respectively of the total biogas quantity of a sample. Whereas the cow dung (S_9) made up only 36 % which showed the inoculum's best quality. Due to its high carbon content and the diverse microbial community that has grown in the cattle intestines; the rumen of cattle may produce the highest amount of net biogas. The inoculums for the two batches produced a biogas production of 948.56 mL_N (S_1) and 1050.07 mL_N (S_5).

Pagés Díaz et al. [148] reported some typical shapes for the net biogas cumulative frequency curves. Easily convertible substances can be converted quickly into biogas, and the resulting curve is a sharp increase in the quantity of accumulated biogas categorized under a normal pattern. The grass (S_2) exhibited a normal trend for the net biogas cumulative frequency curve. Those materials that degrade slowly (for instance, those that include a few lipids or lignin) show a retarded gas formation curve. This curve's form may potentially be the result of minor inhibition. Samples S_8 and S_3 showed a retarded gas formation curve. On the other hand, stairway steps like curve trends also exist which is a result of degradation that occurs in two stages, or diauxia. Samples S_4, S_6, S_7, and S_9 exhibited a diauxic gas formation curve. When there is a complete inhibition the gas formation curve goes downward in the negative X-axis which results in a negative net biogas production. There were no substrates that showed a negative net biogas production for this experiment.

4.3.2. Specific methane yield

The Specific methane yield or the volume of methane gas produced per the mass of the volatile matter added is the most common interpretation for the performance of AD. This experiment also obtained promising results of SMY for the substrates as shown in Table 4.3.

In this study, the grass has achieved the maximum SMY of 231 mL_N CH_4/g VS, which was a close result obtained by Janejadkarn and Chavalparit [149] (242 mL CH_4/g VS), who applied a continuously stirred tank reactor for AD of napier grass at room temperature. The work of Sonwai et al. [150] in AD of grass under thermophilic condition and Ulukardesler [151] in the co-digestion of grass silage with cow manure with extended HRT of 65 days obtained SMY of 199 and 206 mL CH_4/g VS, respectively was lower than this experiment result. The food waste fermentation maximum SMY 82 mL_N CH_4/g VS agreed with the result obtained by Jiménez-Ocampo et al. [138] (87 mL CH_4/g VS), who used a feedback control strategy in a sequencing batch reactor. Yong et al. [152] have reported 281 mL CH_4/g VS for a canteen food waste AD in china and a higher 392 mL CH_4/g VS result when co-digested with straw, which was higher than this study. This difference could be a result of the food waste combination.

The SMY of chicken manure reported by Wedwitschka et al. [140] was 135 ± 20 mL CH_4/g VS and 127 ± 12 mL CH_4/g VS with and without structure material, which was lower compared to the findings of this study (177.99 mL_N CH_4/g VS). Other authors such as Wang et al. [153] and Huang and Shih [139] published considerable higher methane yields of 437 mL/g VS and 280 mL/g VS, which used two-stage AD with an intermediate membrane contactor in order to extract ammonia and methanogenic culture initiated by incubation at 55 ℃, respectively.

Most works on cow blood mono-digestion failed to produce methane due to inhibition. For instance, efforts to anaerobically digest slaughterhouse blood waste by Tsegaye [127] and Ozturk [154] failed to produce methane. In contrast to Bauer [110], who reported a maximum SMY of 117 mL CH_4/g VS, this experiment obtained a SMY of 173 mL_N CH_4/g VS. Similarly, this work obtained a SMY of 350 mL_N CH_4/g VS for the rumen content. Under mesophilic conditions, Ware and Power's [155] experiment in Ireland's slaughterhouse waste (paunch) produced a maximum methane output of 229 mL CH_4/g VS. Similarly, Fonoll et al.'s [156] study, which employed cow rumen material as an inoculum and co-substrate with cow dung, produced an SMY of 120 mL CH_4/g VS. Both results were less than the experiment's findings.

Table 4. 4 Maximum specific biogas and methane yield for the two batches

	Samples								
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9
SBY (mL_N/g VS)	90.87	345.57	182.42	278.64	90.33	268.24	490.29	179.33	57.23
SMY (mL_N/g VS)	53.19	231.00	82.12	177.99	55.89	173.06	350.30	127.05	32.88

The pig manure produced a maximum SMY of 127 mL_N CH_4/g VS. The result was lower than Hanum et al. [143] (238 mL CH_4/g VS) and Xie et al. [157] (279 mL CH_4/g VS), who investigated anaerobic co-digestion of pig manure and fermented liquid feed in a continuous digester under mesophilic conditions and a batch experiments for anaerobic co-digestion of grass silage and pig manure, respectively.

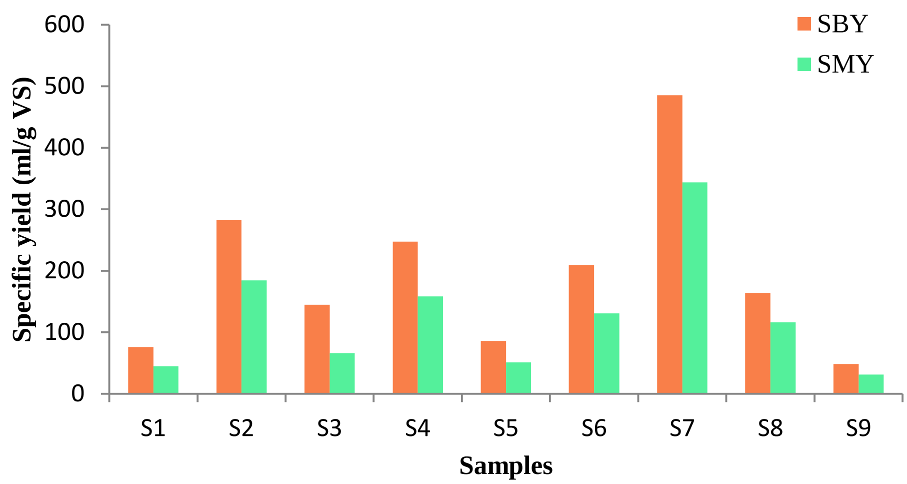


Figure 4. 5 Specific biogas and methane yield of the samples

The cow dung has achieved the maximum SMY of 32 mL_N CH_4/g VS, which agreed with the result obtained by Alvarezet al. [145] (33.61 mL CH_4/g VS), who employed a parallel reactor set-up incorporating ten laboratory scale biogasifiers for cow and llama manure AD at 11 °C in bolivia. The result was developed to 49 mL CH_4/g VS when the temperature raised from 11-35 °C. Aili Hamzah et al. [158] investigated the effect of organic loading on the AD of cow dung at mesophilic condition and reported a maximum SMY ranges from 31-63.45 mL CH_4/g VS.

Rumen content obtained the study's highest SMY result, while cow manure was the lowest. The results obtained demonstrate that the substrates' SMY has a comparable outcome to previous studies.

4.4. Organic Matter Removal Efficiency

The main purpose of implementing the AD approach is to transform the organic components of bio-wastes into biogas. Consequently, the removal efficiency of organic wastes was applied as an indicator for evaluating the AD performance. VS reduction is usually used to evaluate the reactor performance and stability of the substrate [159]. The variation of VS and COD removal efficiency is shown in Table 4.4 and Figure 4.6.

Table 4. 5 VS and COD removal efficiency of samples

	VS (%)		VS removal	COD (mg/L)		COD Removal
Samples	Influent	Effluent	efficiency (%)	Influent	Effluent	efficiency (%)
S_1	85.30	41.90	50.87	1122.50	907.00	79.67
S_2	86.82	41.54	52.16	885.00	993.00	79.48
S_3	64.30	37.56	41.59	1028.00	765.11	79.15
S_4	86.56	31.96	63.08	1108.00	1002.56	75.09
S_5	83.53	29.58	64.58	1000.70	921.89	76.88
S_6	95.47	39.54	58.58	1990.00	970.89	74.38
S_7	66.64	31.05	53.40	967.33	1428.89	75.64
S_8	83.31	31.02	62.76	1327.00	1146.56	73.50
S_9	81.14	35.18	56.64	2017.00	2182.00	69.33

The highest VS% removal efficiency was obtained from chicken and pig manure (63.07% and 62.76%, respectively), which agree the result reported by Hanum et al. [143] for the pig manure and Buivydas et al. [141] for the chicken manure. According to Li et al. [160], high VS removal was associated with the availability of organic matter in providing an adequate microbial pool and a buffering capacity. The cow dung 56.64% VS removal efficiency was higher than that of Aili Hamzah et al. [158] (28%) and Li, Jha and Bajracharya [161] (48.59%).

The food waste VS removal efficiency of 41.59% was the lowest in this study. Other literatures reported that, the higher protein and fat content in the organic matter highly affects the removal efficiency [134]. Likewise, the rumen content (53.4%) and grass (52.1%) had lower values when compared with the other substrates. In hemicellulose's hydrolysis the solubility of oligomers plays a significant role in controlling degradation [162].

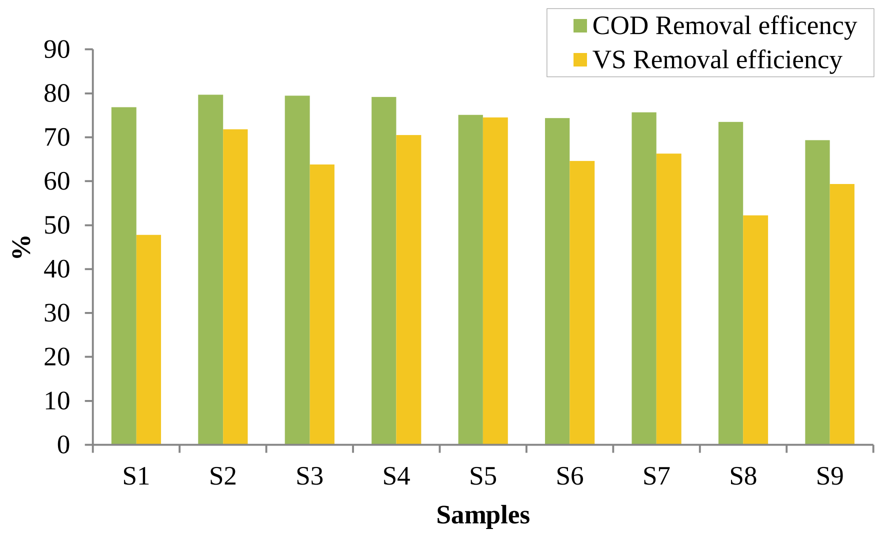


Figure 4. 6 VS and COD removal efficiency of the samples

For a typical stable anaerobic digestion system, COD removal is a critical parameter showing the effectiveness of anaerobic digestion of the liquid substrate [163]. There are a lot of contaminants in a slaughterhouse that need to be removed. The COD removal efficiency in the cattle blood and rumen content was 74.38% and 75.64%, respectively. For a cattle slaughterhouse wastewater AD Musa and Idrus [164] obtained a COD removal efficiency ranged from 71-97% for different HRT using a hybrid up flow anaerobic sludge blanket reactor at 35 °C.

According to Li, Jha and Bajracharya [161], the maximum COD removal efficiency of cow dung and pig manure, respectively, was 50.86% and 55.44%. This is lower than the study's outcomes for cow dung and pig manure, respectively, of 69.33% and 73.5%. The COD removal efficiency from chicken manure (75.08%) result supported the findings of Sakar et al.'s [165] review who reported values ranged from 32 to 78%. With the other substrates, the grass (79.47%) and food waste (79.14%) likewise exhibited nearly comparable results. This result agrees with Cheong et al. [166] work on anaerobic Co-digestion of food waste and sewage sludge with a 81.5% COD removal efficiency.

In this study, the VS and COD removal efficiency ranges from 41.59 to 63.07% and 69.33 to 79.47%. Hence it can be conclude that the performance of the batch experiment tested in this study was higher.

4.5. Effect of room temperature on the biogas volume

The temperature of the digester needs to be managed for improved biogas output. Since methane formers are temperature variation sensitive, it is even more crucial to maintain a steady temperature [167]. This study was performed under a controlled temperature of 40±0.2 ℃ using a water bath, which is an optimum mesophilic temperature for maximum biogas yield [168, 169].

Figure 4.7 shows that during the HRT for anaerobic digestion tested, the room temperature varied from 13.5 ℃ to 23.9 ℃. Through these temperature ranges, the normalized biogas volume showed a variation from the raw (not standardized to normal conditions) as shown in Table 4.3.

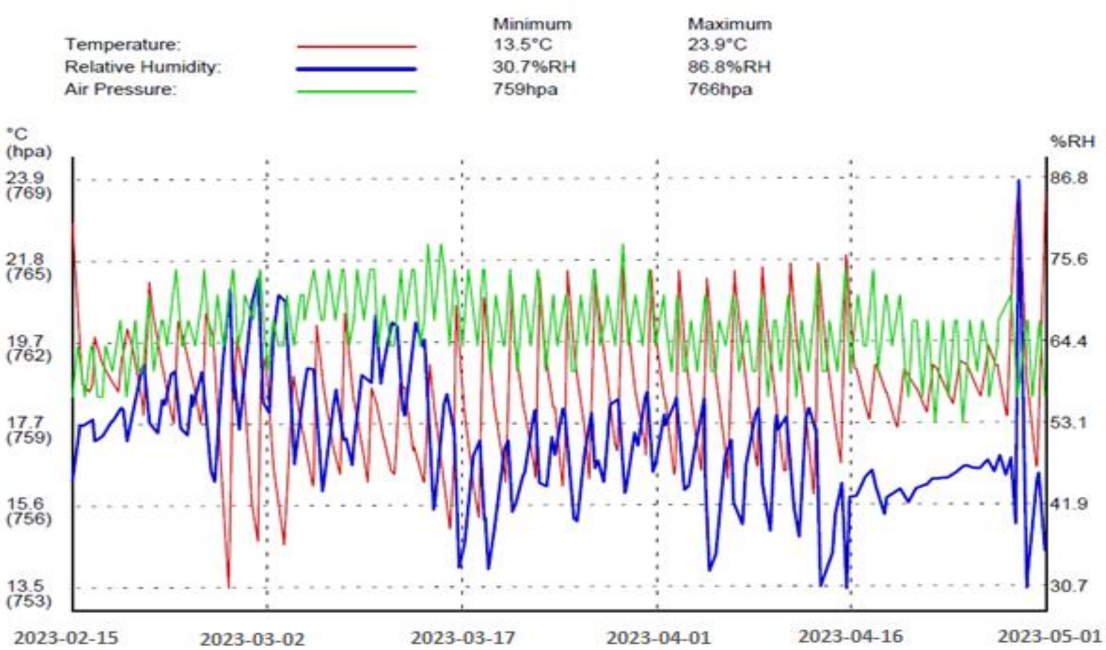


Figure 4. 7 Temperature, Air pressure and relative humidity during AD test

4.6. Effect of pH on biogas production

Anaerobic digestion occurs at predefined pH values, where all biological activities took place. Fast acidification from volatile fatty acid (VFA) formation lowers the pH value in the reactor, which is an major challenge to the anaerobic digestion process [170].

The pH values in the batch reactors before and after digestion were shown in Figure 4.8. The AD of the samples S_2, S_3, S_5, S_6, S_7, and S_9 started at a pH level close to neutral. S_4 and S_8 samples began with a more basic pH, which was in accordance with the prediction made by Buivydas et al. [141] that a high TAN concentration would be the reason. While S_1 was the only sample under an acidic range, which could be caused by VFA accumulation [38]. The pH values of samples S_1, S_2, S_3, S_5 and S_6 slightly increased after digestion, but the pH values of the other samples decreased. Since the pH remained within the ideal range of 6.96 to 7.85, the samples' natural buffering ability was great.

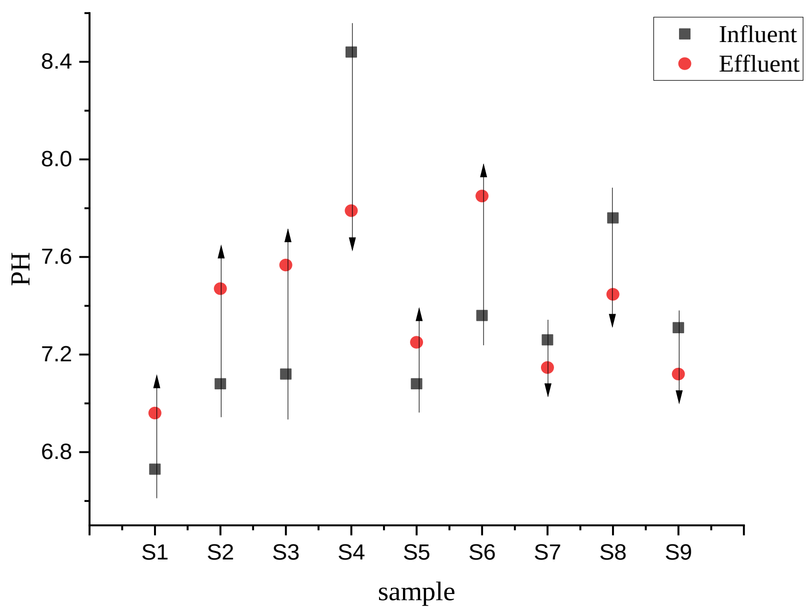


Figure 4. 8 pH values of the samples before and after AD

According to Veeken et al. [39], the optimal pH range for methane production is between 6.5 and 8. If the pH value drops below 6, the production of methane is considerably restricted and stopped altogether due to the negative impact it has on the methane-producing bacteria's activity. The results of the experiment also support these findings.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The lab-scale anaerobic digestion process successfully utilized various feedstocks including grass, food waste, chicken manure, cattle blood, cattle rumen content, pig manure, and cow dung for biogas production within 28-40 days of batch experiments. Most of the biogas production took place within the first 12 days of the process. The net biogas production of the grass, food waste, chicken manure, cattle blood, cattle rumen content, pig manure, and cow dung were 1758.5, 903.5, 1558.7, 1309, 3034.9, 1024, and 592.9 mL_N, respectively. The cattle rumen content had the highest SMY, while the grass had the highest percentage of methane. The feedstocks had COD removal efficiency between 66.33% and 79.15% and VS removal efficiency between 41.95% and 63.07%. Cattle blood and chicken manure had higher concentrations of hydrogen sulfide compared to other feedstocks. These two feedstocks have the potential to be used in co-digestion in Ethiopian prime meat and food processing applications of system analysis of renewable generation from organic wastes and its conversion to protein technology.

5.2. Recommendation and Future Work

- Institutions and industries (such as AAAE, micro and small enterprise and so on) with wastes similar to those in the study could implement a pilot AD and test its feasibility.
- To further evaluate the efficiency of the product, it is important to conduct studies on the optimum process and operational parameters (such as temperature, pH, OLR…), microbiological analyses, and monitoring VFA and ammonia nitrogen levels. Since inhibition had a significant impact on both food wastes and cattle blood AD.
- To treat several wastes at once (for instance a slaughterhouse waste could include livestock manure, blood, rumen content, food waste from a restaurant, and grass from a playground), the anaerobic co-digestion to each other should be studied.
- Modeling of methane production kinetics for estimation of the SMY at infinite time, quality control and interpretation of substrate degradation is needed for universal bases comparison.
- A study on the evaluation and characterization of the cattle feed might be helpful for improving biogas output.

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APPENDIX

APPENDIX I: Characterization of the feedstock

TS, VS and FS before AD (Influent)

Substrate	Sample weight	Crucible weight	Crucible + sample	After dry (105 ℃)	After Ignition (550 ℃)	%TS	%VS	%FS
Inoculum I (S_1)	50	44.7161	98.5308	48.612	45.2916	7.239472	85.22806	14.77194
	50	44.726	98.5526	48.6122	45.292	7.21985	85.43564	14.56436
	50	44.7163	98.5459	48.6151	45.2917	7.242855	85.24161	14.75839
Grass (S_2)	5	39.5577	44.5678	41.2781	39.7904	34.33864	86.47408	13.52592
	5	39.5768	44.5869	41.2783	39.7897	33.9614	87.48751	12.51249
	5	39.5576	44.5679	41.2789	39.7899	34.35523	86.50439	13.49561
Food Waste (S_3)	5	31.3447	36.3544	33.7498	32.2073	48.00886	64.13455	35.86545
	5	31.3546	36.3548	33.7488	32.207	47.88208	64.39729	35.60271
	5	31.3543	36.3745	33.7491	32.2075	47.70328	64.37281	35.62719
Chicken Manure (S_4)	5	31.3391	36.3586	32.8256	31.5387	29.6145	86.57249	13.42751
	5	31.3386	36.3584	32.8242	31.5383	29.5948	86.55762	13.44238
	5	31.3384	36.3578	32.8246	31.5385	29.60912	86.53613	13.46387
Inoculum II (S_5)	30	57.641	87.701	60.2981	58.0711	8.839321	83.81318	16.18682
	30	56.525	86.5252	59.1621	56.9678	8.790275	83.20883	16.79117
	30	57.431	87.3347	60.0827	57.8667	8.867465	83.56903	16.43097
Blood (S_6)	30	28.7601	59.419	32.9661	28.9507	13.71869	95.46838	4.531621
	30	28.7603	59.4192	32.9667	28.9508	13.72	95.47119	4.528813
	30	28.7605	59.4195	32.9669	28.9506	13.71995	95.4807	4.519304

Rumen content (S_7)	30	32.1741	62.2286	35.1705	32.6743	9.969888	83.30663	16.69337
	30	32.1743	62.2285	35.1703	32.6742	9.968657	83.31442	16.68558
	30	32.174	62.2287	35.1707	32.6745	9.97082	83.29829	16.70171
Pig manure (S_8)	5	25.3336	30.6954	26.6174	25.7619	23.94345	66.63811	33.36189
	5	25.3335	30.6953	26.6175	25.7619	23.94718	66.63551	33.36449
	5	25.3337	30.6951	26.6177	25.7619	23.94897	66.65109	33.34891
Cow dung (S_9)	30	57.591	87.599	62.1452	58.4496	15.17662	81.14707	18.85293
	30	55.847	85.847	60.4003	56.7069	15.17767	81.1148	18.8852
	30	55.889	85.894	60.4419	56.7472	15.1738	81.15048	18.84952

TS, VS and FS after AD (Effluent)

Substrate	**Sample weight**	**Crucible weight**	**Crucible + sample**	**After dry (105 ℃)**	**After Ignition (550 ℃)**	**%TS**	**%VS**	**%FS**
S_{11}	30	31.8629	61.0651	32.7896	32.39773	3.173391	42.28661	57.71339
	30	31.863	61.0657	32.7901	32.3978	3.174706	42.31474	57.68526
	30	31.8627	61.0649	32.7886	32.39776	3.170652	42.2119	57.7881
S_{12}	30	31.8629	60.699	32.9663	32.4995	3.826454	42.3056	57.6944
	30	31.863	60.6991	32.9662	32.4992	3.82576	42.3314	57.6686
	30	31.8627	60.6993	32.9664	32.4999	3.827428	42.26692	57.73308
S_{13}	50	48.1932	97.3947	49.3382	48.867	2.327165	41.15284	58.84716
	50	48.1933	97.3945	49.3384	48.8674	2.327382	41.13178	58.86822
	50	48.1936	97.3949	49.3385	48.8675	2.326971	41.13896	58.86104
S_{21}	50	46.4624	96.1177	53.9271	50.7842	15.03304	42.1035	57.8965
	50	46.4627	96.1174	53.9274	50.7844	15.03322	42.10484	57.89516

	50	46.4626	96.1178	53.927	50.7843	15.03246	42.10251	57.89749
	50	41.4296	90.809	47.9605	45.2987	13.22596	40.75702	59.24298
S_{22}	50	41.4295	90.8092	47.9607	45.2988	13.22649	40.75668	59.24332
	50	41.4294	90.8089	47.9611	45.2988	13.22755	40.75968	59.24032
	30	39.5551	88.2661	46.6679	43.698	14.60204	41.7543	58.2457
S_{23}	30	39.5552	88.2663	46.6675	43.6984	14.60098	41.74599	58.25401
	30	39.5554	88.2665	46.6683	43.6979	14.60222	41.76074	58.23926
	50	50.3159	100.7783	59.4497	56.1998	18.10021	35.58103	64.41897
S_{31}	50	50.3157	100.784	59.4508	56.1994	18.10067	35.59239	64.40761
	50	50.3248	100.7841	59.4516	56.1999	18.08745	35.62804	64.37196
	50	47.6523	97.2337	56.9974	53.4799	18.848	37.64005	62.35995
S_{32}	50	47.6525	97.234	56.9976	53.4811	18.84796	37.62935	62.37065
	50	47.6522	97.2335	56.9973	53.4797	18.84803	37.64112	62.35888
	50	49.1932	98.3947	59.4399	55.3985	20.82599	39.44099	60.55901
S_{33}	50	49.1933	98.3945	59.4403	55.3987	20.82673	39.44179	60.55821
	50	49.1936	98.3949	59.4411	55.3994	20.8277	39.44084	60.55916
	50	50.3369	99.6009	56.1073	54.262	11.71322	31.97872	68.02128
S_{41}	50	50.3364	99.601	56.1072	54.2617	11.71389	31.97997	68.02003
	50	50.3367	99.6011	56.1077	54.2614	11.71434	31.99272	68.00728
	50	44.7332	93.8296	50.20891	48.4979	11.15298	31.24727	68.75273
S_{42}	50	44.7335	93.8298	50.2087	48.4972	11.15196	31.25913	68.74087
	50	44.7337	93.8294	50.2091	48.4978	11.1525	31.25434	68.74566
	50	46.4416	95.437	52.4615	50.4964	12.28666	32.6434	67.3566
S_{43}	50	46.4414	95.4372	52.4618	50.4966	12.28758	32.64235	67.35765
	50	46.4419	95.4372	52.4613	50.4967	12.28567	32.6378	67.3622
	50	42.6471	91.8324	43.9434	43.5815	2.635544	27.91792	72.08208
S_{51}	50	42.6283	91.8315	43.94341	43.5816	2.672814	27.51177	72.48823
	50	42.6393	91.8313	43.94342	43.5815	2.651081	27.75205	72.24795
	50	44.9531	95.7999	45.7967	45.6034	1.659101	22.9137	77.0863
S_{52}	50	44.9595	95.8401	45.7969	45.60342	1.645814	23.10485	76.89515
	50	44.9592	95.8409	45.7973	45.603438	1.647154	23.13113	76.86887

	50	41.1325	91.0557	42.8974	42.2271	3.53523	37.97949	62.02051
S_{53}	50	41.1327	91.0558	42.8977	42.2273	3.535438	37.983	62.017
	50	41.1325	91.0555	42.898	42.2277	3.536446	37.96658	62.03342
	50	33.776	83.5473	36.7657	35.4987	6.006875	42.37883	57.62117
S_{61}	50	33.776	83.5474	36.7656	35.50002	6.006662	42.33275	57.66725
	50	33.7762	83.5476	36.7659	35.4977	6.006863	42.41897	57.58103
	50	45.9458	95.0063	48.4796	47.42065	5.164644	41.79296	58.20704
S_{62}	50	45.9463	95.006	48.4794	47.42	5.163301	41.82227	58.17773
	50	45.9462	95.0057	48.4797	47.4201	5.164137	41.82356	58.17644
	50	46.3638	95.541	48.4895	47.7585	4.322532	34.38867	65.61133
S_{63}	50	46.3689	95.541	48.4895	47.75851	4.312608	34.4709	65.5291
	50	46.3687	95.5408	48.4897	47.75852	4.313422	34.47336	65.52664
	50	56.3597	106.0598	61.4571	59.8176	10.25632	32.16346	67.83654
S_{71}	50	56.3597	106.0594	61.4572	59.8175	10.2566	32.16675	67.83325
	50	56.3598	106.0595	61.4574	59.8176	10.2568	32.16808	67.83192
	50	50.9344	100.6211	55.7924	54.3135	9.777264	30.44257	69.55743
S_{72}	50	50.9343	100.6209	55.7922	54.3134	9.777083	30.44114	69.55886
	50	50.9344	100.621	55.7923	54.3132	9.777083	30.44731	69.55269
	50	30.1541	80.5604	34.96245	33.4941	9.539185	30.5375	69.4625
S_{73}	50	30.1545	80.5604	34.96244	33.4931	9.538447	30.5607	69.4393
	50	30.1544	80.5605	34.96244	33.4938	9.538607	30.5455	69.4545
	50	44.7866	94.5532	46.6159	46.0744	3.675758	29.60149	70.39851
S_{81}	50	44.7863	94.553	46.6158	46.0742	3.676153	29.60372	70.39628
	50	44.7865	94.5531	46.6159	46.0744	3.675959	29.59987	70.40013
	50	66.0196	115.1768	67.95311	67.3349	3.93332	31.97346	68.02654
S_{82}	50	66.0195	115.1849	67.95312	67.3348	3.932888	31.97733	68.02267
	50	66.0193	115.1835	67.95314	67.3344	3.933431	31.99541	68.00459
	50	49.1446	98.4728	51.0547	50.4535	3.872227	31.47479	68.52521
S_{83}	50	49.1444	98.4724	51.0548	50.4533	3.872851	31.48555	68.51445
	50	49.1445	98.4726	51.0548	50.4535	3.872641	31.47673	68.52327
S_{91}	20	30.5329	50.0049	31.8154	31.3882	6.58638	33.30994	66.69006

	20	30.5326	50.0048	31.8154	31.388	6.587853	33.31774	66.68226
	20	30.5325	50.0044	31.8155	31.3882	6.588982	33.30475	66.69525
	20	26.2647	46.7165	27.7835	27.2479	7.426241	35.26468	64.73532
S_{92}	20	26.2651	46.7164	27.7838	27.2428	7.425934	35.62257	64.37743
	20	26.2652	46.7164	27.7839	27.2429	7.42597	35.62257	64.37743
S_{93}	20	46.6733	66.7632	47.9835	47.4927	6.521685	37.45993	62.54007
	20	46.674	66.763	47.9838	47.4922	6.519986	37.53245	62.46755
	20	46.6739	66.7632	47.9839	47.4923	6.520884	37.52672	62.47328

COD, BOD, C/N and PH before AD

sample	COD (mg/L)	BOD (mg/L)	C/N	pH	BOD/COD
Inoculum I (S_1)	3922.5	1842.33	16.60	6.73	0.469683
Grass (S_2)	4885	2298.5	21.18	7.08	0.470522
Food waste (S_3)	3728	1406.13	17.86	7.12	0.377181
Chicken manure (S_4)	4808	2726.3	4.47	8.44	0.567034
Inoculum II (S_5)	3700.7	1776.9	17.25	7.08	0.480152
Blood (S_6)	3790	2152.627	10.60	7.36	0.567975
Rumen content (S_7)	5866	2426.807	14.48	7.26	0.413707
Pig manure (S_8)	4327	1754.4	10.67	7.76	0.405454
Cow dung (S_9)	6917	3104.4	22.61	7.31	0.448807

COD, BOD, COD $_{removal\ efficiency}$ and PH after AD

sample	COD (mg/L)	BOD (mg/L)	COD $_{removal\ efficiency}$	pH
S_{11}	832	400	78.78904	6.91
	834	400.49	78.73805	
	837	401	78.66157	
S_{12}	797	136.98	79.68133	6.98
	798	138.4	79.65583	
	802	137.5	79.55386	
S_{13}	1093	396.1	72.13512	6.99
	1082	394.8	72.41555	
	1088	396.16	72.26259	
S_{21}	959	416.4	80.36847	7.49
	962	414.2	80.30706	
	964	416.34	80.26612	
S_{22}	1031	289.3	78.89458	7.42
	1034	290.1	78.83316	
	1037	289.45	78.77175	
S_{23}	987	344.5	79.79529	7.5
	980	344.9	79.93859	
	983	340	79.87718	
S_{31}	696	149	81.33047	7.59
	697	142	81.30365	
	698	147	81.27682	
S_{32}	822	194	77.95064	7.55
	823	190	77.92382	
	845	158	77.33369	
S_{33}	766	188	79.45279	7.56
	769	167	79.37232	
	770	160	79.34549	
S_{41}	942	394.6	80.40765	8.07

	946	394.66	80.32446	
	949	395.3	80.26206	
S_{42}	1017	316.8	78.84775	7.54
	1014	316.38	78.91015	
	1018	314.6	78.82696	
S_{43}	1049	235	78.1822	7.76
	1043	238.4	78.30699	
	1045	235.9	78.26539	
S_{51}	927	169	74.95069	7.28
	924	166	75.03175	
	923	168.5	75.05877	
S_{52}	932	172.9	74.81558	7.23
	933	173	74.78855	
	937	174	74.68047	
S_{53}	907	153.6	75.49112	7.25
	906	153.4	75.51815	
	908	153.7	75.4641	
S_{61}	991	317	73.85224	7.82
	993	316.5	73.79947	
	992	315	73.82586	
S_{62}	973	328	74.32718	7.71
	971	326.3	74.37995	
	972	326.8	74.35356	
S_{63}	949	395	74.96042	8.02
	948	395.8	74.98681	
	949	396	74.96042	
S_{71}	1354	156.8	76.91783	7.22
	1359	157	76.83259	
	1362	155.4	76.78145	
S_{72}	1455	143.5	75.19605	7.14
	1457	144	75.16195	

	1457	143	75.16195	
S_{73}	1469	145.45	74.95738	7.08
	1475	145.5	74.8551	
	1472	145.45	74.90624	
S_{81}	1082	132.7	74.99422	7.44
	1086	133	74.90178	
	1085	132.73	74.92489	
S_{82}	1204	152.5	72.17472	7.39
	1206	151.52	72.1285	
	1205	151.5	72.15161	
S_{83}	1152	144	73.37647	7.51
	1150	143.5	73.42269	
	1149	143.5	73.44581	
S_{91}	2518	154.8	63.59694	7.1
	2519	155	63.58248	
	2522	154.85	63.53911	
S_{92}	1816	134.8	73.74584	7.14
	1860	135.6	73.10973	
	1857	134.85	73.1531	
S_{93}	1994	153	71.17247	7.12
	1999	151	71.10019	
	2005	150	71.01345	

APPENDIX II: OLR for the inoculum and substrate

	Min	Max
Batch volume	2.0%	2.5%
500 mL	10.0 g VS	12.5 g VS

Inoculum	
TS (%)	7.2
VS (%)	85.3
Inoculum FM (g)	203.5

Grass	
TS (%)	34.2
VS (%)	86.2
Substrate VS (g)	6.3
Substrate FM (g)	21.2

Food waste	
TS (%)	47.9
VS (%)	64.3
Substrate VS (g)	6.3
Substrate FM (g)	20.3

Chicken manure	
TS (%)	29.9
VS (%)	86.3
Substrate VS (g)	6.3
Substrate FM (g)	24.2

Inoculum	
TS (%)	8.9
VS (%)	83.8
Inoculum FM (g)	168.1

blood	
TS (%)	13.7
VS (%)	95.5
Substrate VS (g)	6.3
Substrate FM (g)	47.7

Rumen	
TS (%)	10.0
VS (%)	83.3
Substrate VS (g)	6.3
Substrate FM (g)	75.3

Pig manure	
TS (%)	23.94
VS (%)	66.6
Substrate VS (g)	6.3
Substrate FM (g)	34.7

Cow dung	
TS (%)	15.2
VS (%)	81.2
Substrate VS (g)	12.5
Substrate FM (g)	101.5

APPENDIX III: Biogas volume and composition (raw data)

Sample	Date of measurement	Time of measurement	Volume (mL)	CH_4 (%)	CO_2 (%)	H_2S (ppm)
S_{11}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	320	63.7	24.3	38
	21-Feb	17:30:00	300	55.1	43.2	
	24-Feb	17:30:00	130	55.4	43.3	
	28-Feb	16:00:00	280	64.8	33.7	3
	7-Mar	15:20:00	200	43.8	55.1	
	13-Mar	16:10:00	40	32.1	66.7	
Sum			1270			41
S_{12}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	430	64.2	33.8	50
	21-Feb	17:30:00	290	54.1	44.2	
	24-Feb	17:30:00	120	69.9	28.8	
	28-Feb	16:00:00	180	53.9	44.6	2
	7-Mar	15:20:00	200	40.1	58.8	
	13-Mar	16:10:00	45	36.3	62.5	
Sum			1265			52
S_{13}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	580	60.3	37.7	11
	21-Feb	17:30:00	400	59.9	38.4	5
	24-Feb	17:30:00	250	66.9	31.8	
	28-Feb	16:00:00	160	52.1	46.4	3
	7-Mar	15:20:00	250	53.1	45.8	
	13-Mar	16:10:00	45	31.8	67	
Sum			1685			19
S_{21}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	1250	65.2	32.8	4
	21-Feb	17:30:00	1200	67.1	31.2	1

	24-Feb	17:30:00	650	65.5	33.2	440
	28-Feb	16:00:00	500	55.8	42.7	4
	7-Mar	15:20:00	300	40	58.9	
	13-Mar	16:10:00	250	49.1	49.7	
	Sum		4150			449
S_{22}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	1300	63.4	34.6	3
	21-Feb	17:30:00	1300	65.2	33.1	1
	24-Feb	17:30:00	800	79.4	19.3	798
	28-Feb	16:00:00	480	54.3	44.2	3
	7-Mar	15:20:00	420	56.7	42.2	
	13-Mar	16:10:00	300	52.5	46.3	
	Sum		4600			805
S_{23}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	550	66.6	31.4	4
	21-Feb	17:30:00	1250	63.8	34.5	3
	24-Feb	17:30:00	510	59.4	39.3	445
	28-Feb	16:00:00	500	67.8	30.7	4
	7-Mar	15:20:00	380	54	44.9	
	13-Mar	16:10:00	100	45.6	53.2	
	Sum		3290			456
S_{31}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	680	42.7	55.3	168
	21-Feb	17:30:00	570	65.3	33	
	24-Feb	17:30:00	450	67.5	31.2	2
	28-Feb	16:00:00	560	53.7	44.8	
	7-Mar	15:20:00	640	40.1	58.8	
	13-Mar	16:10:00	200	33.4	65.4	
	Sum		3100			170
S_{32}	14-Feb	0	0	0	0	
	17-Feb	14:30:00	760	48.1	49.9	70

	21-Feb	17:30:00	430	64.4	33.9	2
	24-Feb	17:30:00	310	54.4	44.3	
	28-Feb	16:00:00	470	52.2	46.3	
	7-Mar	15:20:00	200	40.1	58.8	
	13-Mar	16:10:00	130	36.3	62.5	
	Sum		2300			72
	14-Feb	0	0	0	0	
	17-Feb	14:30:00	500	44.3	53.7	50
	21-Feb	17:30:00	750	66.3	32	
S_{33}	24-Feb	17:30:00	600	67.9	30.8	
	28-Feb	16:00:00	540	46.1	52.4	
	7-Mar	15:20:00	320	39.6	59.3	
	13-Mar	16:10:00	130	33.7	65.1	
	Sum		2840			50
	14-Feb	0	0	0	0	
	17-Feb	14:30:00	640	54.6	43.4	524
	21-Feb	17:30:00	1000	61.2	37.1	68
S_{41}	24-Feb	17:30:00	780	77.8	20.9	653
	28-Feb	16:00:00	500	71.7	26.8	4
	7-Mar	15:20:00	200	32	66.9	
	13-Mar	16:10:00	520	53.8	45	
	Sum		3640			1249
	14-Feb	0	0	0	0	
	17-Feb	14:30:00	500	48.1	49.9	330
	21-Feb	17:30:00	1300	53.2	45.1	66
S_{42}	24-Feb	17:30:00	1100	76.9	21.8	407
	28-Feb	16:00:00	470	77.7	20.8	4
	7-Mar	15:20:00	340	58.4	40.5	
	13-Mar	16:10:00	300	50.1	48.7	
	Sum		4010			807
S_{43}	14-Feb	0	0	0	0	

	17-Feb	14:30:00	620	58.6	39.4	440
	21-Feb	17:30:00	1000	67.2	30.8	68
	24-Feb	17:30:00	800	74.4	23.6	574
	28-Feb	16:00:00	480	61.1	36.9	4
	7-Mar	15:20:00	200	48.3	49.7	
	13-Mar	16:10:00	400	32.7	65.3	
	Sum		3500			1086
S_{51}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	0	0	0	
	5-Apr	12:00:00	700	59.2	38.8	
	10-Apr	15:00:00	600	65.5	32.5	
	15-Apr	17:00:00	110	40.1	57.9	
	24-Apr	12:00:00	170	40.7	57.3	
	Sum		1580			
S_{52}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	0	0	0	
	5-Apr	12:00:00	600	58.7	39.3	
	10-Apr	15:00:00	500	67.7	30.3	
	15-Apr	17:00:00	120	42.8	55.2	
	24-Apr	12:00:00	190	40.2	57.8	
	Sum		1410			
S_{53}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	0	0	0	
	5-Apr	12:00:00	700	62.9	35.1	
	10-Apr	15:00:00	600	71.6	26.4	
	15-Apr	17:00:00	120	40.7	57.3	
	24-Apr	12:00:00	200	41.6	56.4	
	Sum		1620			
S_{61}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	880	56.6	41.4	>1000
	5-Apr	12:00:00	130	48.8	49.2	55

	10-Apr	15:00:00	120	44	54	17
	15-Apr	17:00:00	100	33.3	64.7	10
	24-Apr	12:00:00	600	53.8	44.2	
	1-May	15:00:00	1000	69.3	28.7	
	6-May	14:00:00	300	65.1	32.9	
	Sum		3130			1082
S_{62}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	1300	61.1	36.9	>1000
	5-Apr	12:00:00	110	46.6	51.4	60
	10-Apr	15:00:00	110	42.1	55.9	20
	15-Apr	17:00:00	130	36.7	61.3	11
	24-Apr	12:00:00	560	66.2	31.8	11
	1-May	15:00:00	900	66.1	31.9	
	6-May	14:00:00	130	60.2	37.8	
	Sum		3240			1102
S_{63}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	1300	62.1	35.9	>1000
	5-Apr	12:00:00	180	49	49	44
	10-Apr	15:00:00	200	46.5	51.5	22
	15-Apr	17:00:00	120	35.5	62.5	10
	24-Apr	12:00:00	590	68.6	29.4	
	1-May	15:00:00	1200	68.4	29.6	
	6-May	14:00:00	400	61.3	36.7	
	Sum		3990			1076
S_{71}	28-Mar	0	0	0	0	
	31-Mar	16:00:00	1100	63	35	
	5-Apr	12:00:00	1320	75.5	22.5	
	10-Apr	15:00:00	1250	76.9	21.1	
	15-Apr	17:00:00	1300	71.9	26.1	
	24-Apr	12:00:00	510	57.8	40.2	
	1-May	15:00:00	410	38.9	59.1	

	Sum		5890		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	1100	65.3	32.7
	5-Apr	12:00:00	1270	75	23
S_{72}	10-Apr	15:00:00	1300	77.1	20.9
	15-Apr	17:00:00	1300	77	21
	24-Apr	12:00:00	650	46.1	51.9
	1-May	15:00:00	400	37.2	60.8
	Sum		6020		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	1350	64	34
	5-Apr	12:00:00	1250	74.4	23.6
S_{73}	10-Apr	15:00:00	1250	75.1	22.9
	15-Apr	17:00:00	1250	72.6	25.4
	24-Apr	12:00:00	500	42.7	55.3
	1-May	15:00:00	420	40.1	57.9
	Sum		6020		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	400	59.3	38.7
	5-Apr	12:00:00	700	70.6	27.4
S_{81}	10-Apr	15:00:00	850	76.1	21.9
	15-Apr	17:00:00	550	68	30
	24-Apr	12:00:00	470	50.9	47.1
	1-May	15:00:00	210	41.6	56.4
	Sum		3180		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	300	61.1	36.9
S_{82}	5-Apr	12:00:00	700	72.8	25.2
	10-Apr	15:00:00	800	77.6	20.4
	15-Apr	17:00:00	320	69.2	28.8
	24-Apr	12:00:00	470	52.3	45.7

	1-May	15:00:00	200	40.6	57.4
	Sum		2790		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	400	60.5	37.5
	5-Apr	12:00:00	800	63.6	34.4
S_{83}	10-Apr	15:00:00	800	74	24
	15-Apr	17:00:00	460	66.8	31.2
	24-Apr	12:00:00	480	54.6	43.4
	1-May	15:00:00	200	42	56
	Sum		3140		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	1100	56	42
	5-Apr	12:00:00	400	63.4	34.6
S_{91}	10-Apr	15:00:00	350	67.6	30.4
	15-Apr	17:00:00	250	64.8	33.2
	24-Apr	12:00:00	330	56	42
	1-May	15:00:00	130	40.2	57.8
	Sum		2560		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	850	69.6	28.4
	5-Apr	12:00:00	400	69.8	28.2
S_{92}	10-Apr	15:00:00	300	69.9	28.1
	15-Apr	17:00:00	300	64.8	33.2
	24-Apr	12:00:00	150	52	46
	1-May	15:00:00	120	44.6	53.4
	Sum		2120		
	28-Mar	0	0	0	0
	31-Mar	16:00:00	900	68.1	29.9
S_{93}	5-Apr	12:00:00	400	62.3	35.7
	10-Apr	15:00:00	600	63.3	34.7
	15-Apr	17:00:00	200	64.8	33.2

	24-Apr	12:00:00	300	53.8	44.2	
	1-May	15:00:00	120	42.4	55.6	
	Sum		2520			

Appendix IV: Room temperature at the time of Measurement

Record date and time	Temperature (ºC)	Relative Humidity (%)	Air Pressure (hPa)	Water vapor pressure (P_w) (hPa)
17-02-2023 14:27:58	21	41.8	761	24.81888399
21-02-2023 17:27:58	20.8	29.1	761	24.51553604
24-02-2023 17:27:58	21.6	37.4	760	25.74876358
28-02-2023 15:57:58	21.3	52.3	762	25.28007622
07-03-2023 15:17:58	21.3	43.6	759	25.28007622
13-03-2023 16:07:58	19.1	67.9	759	22.06556646
16-03-2023 15:57:58	19.2	62.3	759	22.20349163
31-03-2023 11:57:58	17.8	71.2	763	20.33975571
05-04-2023 14:57:58	18.3	71	761	20.98896245
10-04-2023 16:57:58	18.6	70.1	762	21.38715812
15-04-2023 11:57:58	18.9	65.2	759	21.79197007
24-04-2023 14:57:58	20.9	63.7	763	24.66680117
01-05-2023 13:57:58	18.8	65.1	759	21.65629187
06-05-2023 13:57:58	19.5	65	763	22.62181719

Appendix V: Normalized biogas and methane volume

First batch cumulative normalized biogas volume (mL_N)

Samples	HRT (days)						
	0.00	**3.10**	**7.23**	**10.23**	**14.17**	**21.14**	**27.17**
S_{11}	0.00	215.94	418.61	505.93	694.83	829.20	856.40
S_{12}	0.00	290.17	486.09	566.69	688.12	822.50	853.10
S_{13}	0.00	391.40	661.62	829.54	971.22	1105.59	1136.19
S_{21}	0.00	843.53	1654.20	2090.80	2428.11	2629.68	2799.66
S_{22}	0.00	877.27	1755.50	2292.85	2616.67	2898.86	3102.83
S_{23}	0.00	371.15	1215.61	1558.16	1895.48	2150.79	2218.79
S_{31}	0.00	458.88	843.95	1146.21	1524.00	1954.01	2089.99
S_{32}	0.00	512.87	803.36	1011.58	1328.66	1463.03	1551.42
S_{33}	0.00	337.41	844.08	1247.09	1611.39	1826.40	1914.78
S_{41}	0.00	431.89	1107.45	1631.36	1968.68	2103.05	2456.61
S_{42}	0.00	337.41	1215.64	1954.49	2271.57	2500.01	2703.98
S_{43}	0.00	418.39	1093.95	1631.30	1955.12	2089.50	2361.46

Second batch Cumulative normalized biogas volume (mL_N)

Samples	HRT (days)							
	0.0	**2.8**	**8.0**	**13.0**	**17.8**	**27.0**	**33.9**	**38.9**
S_{51}	0.00	0.00	479.24	889.92	964.79	1079.88	1079.88	1079.88
S_{52}	0.00	0.00	410.77	753.01	834.69	963.32	963.32	963.32
S_{53}	0.00	0.00	479.24	889.92	971.60	1107.00	1107.00	1107.00
S_{61}	0.00	605.66	694.66	776.80	844.86	1251.08	1932.07	2136.71
S_{62}	0.00	894.73	970.04	1045.33	1133.81	1512.95	2125.83	2214.51
S_{63}	0.00	894.73	1017.96	1154.86	1236.53	1635.98	2453.16	2726.02
S_{71}	0.00	757.08	1660.78	2516.37	3401.19	3746.47	4025.68	4025.68
S_{72}	0.00	757.08	1626.55	2516.37	3401.18	3841.25	4113.64	4113.64
S_{73}	0.00	929.14	1784.92	2640.51	3491.30	3829.81	4115.82	4115.82
S_{81}	0.00	275.30	754.54	1336.34	1710.68	2028.89	2171.89	2171.89

S_{82}	0.00	206.48	685.71	1233.29	1451.09	1769.29	1905.49	1905.49
S_{83}	0.00	275.30	823.00	1370.58	1683.67	2008.64	2144.84	2144.84
S_{91}	0.00	757.08	1030.93	1270.49	1440.65	1664.07	1752.60	1752.60
S_{92}	0.00	585.02	858.87	1064.21	1268.40	1369.95	1451.67	1451.67
S_{93}	0.00	619.43	893.28	1303.96	1440.09	1643.20	1724.91	1724.91

First batch cumulative normalized Methane volume (mL_N)

Samples	HRT (days)						
	0.00	**3.10**	**7.23**	**10.23**	**14.17**	**21.14**	**27.17**
S_{11}	0.00	180.74	292.42	340.79	463.19	522.05	530.81
S_{12}	0.00	186.29	292.28	348.62	414.07	467.96	479.07
S_{13}	0.00	236.01	397.88	510.22	584.03	655.38	665.11
S_{21}	0.00	549.98	1093.94	1379.91	1568.14	1648.76	1732.22
S_{22}	0.00	556.19	1128.80	1555.45	1731.29	1891.29	1998.37
S_{23}	0.00	247.19	785.95	989.43	1218.13	1356.00	1387.00
S_{31}	0.00	195.94	447.39	651.42	854.29	1026.72	1072.14
S_{32}	0.00	246.69	433.77	547.04	712.55	766.44	798.52
S_{33}	0.00	149.47	485.40	759.04	926.98	1012.12	1041.91
S_{41}	0.00	235.81	649.25	1056.86	1298.71	1341.71	1531.93
S_{42}	0.00	162.30	629.51	1197.69	1444.06	1577.47	1679.66
S_{43}	0.00	245.18	699.15	1098.94	1296.80	1361.70	1450.63

Second batch Cumulative normalized Methane volume (mL_N)

Samples	HRT (days)							
	0.0	2.8	8.0	13.0	17.8	27.0	33.9	38.9
S_{51}	0.00	0.00	283.71	552.71	582.73	629.57	629.57	629.57
S_{52}	0.00	0.00	241.12	472.82	507.78	559.49	559.49	559.49
S_{53}	0.00	0.00	301.44	595.49	628.73	685.06	685.06	685.06
S_{61}	0.00	341.00	384.43	420.57	443.23	661.78	1133.70	1266.93
S_{62}	0.00	543.80	578.89	610.59	643.06	894.05	1299.17	1352.55
S_{63}	0.00	552.70	613.08	676.74	705.73	979.75	1538.70	1705.97
S_{71}	0.00	474.44	1156.74	1814.69	2450.87	2650.44	2759.05	2759.05
S_{72}	0.00	491.76	1143.87	1829.91	2511.22	2714.09	2815.42	2815.42
S_{73}	0.00	591.51	1228.21	1870.76	2488.43	2632.98	2747.67	2747.67
S_{81}	0.00	162.39	500.73	943.48	1198.04	1360.00	1419.50	1419.50
S_{82}	0.00	125.49	474.37	899.30	1050.01	1216.43	1271.73	1271.73
S_{83}	0.00	165.68	514.01	919.22	1128.37	1305.80	1363.00	1363.00
S_{91}	0.00	421.73	595.35	757.29	867.56	992.67	1028.26	1028.26
S_{92}	0.00	405.02	596.17	739.70	872.02	924.83	961.27	961.27
S_{93}	0.00	357.99	528.60	788.56	876.77	986.04	1020.69	1020.69

Appendix VI: Specific biogas and methane yield (SBY and SMY)

First batch cumulative normalized SBY (mL_N/g VS)

Samples	HRT (days)							
	0.00	**3.10**	**7.23**	**10.23**	**14.17**	**21.14**	**27.17**	**VS added (g)**
S_{11}	0.00	17.26	33.45	40.43	55.53	66.26	68.44	12.51
S_{12}	0.00	23.21	38.87	45.32	55.03	65.78	68.23	12.50
S_{13}	0.00	31.30	52.91	66.34	77.67	88.42	90.87	12.50
S_{21}	0.00	87.32	181.60	233.68	263.62	274.39	296.93	6.23
S_{22}	0.00	92.73	197.85	266.09	293.86	317.57	345.57	6.23
S_{23}	0.00	11.55	111.24	148.24	178.18	197.58	203.76	6.23
S_{31}	0.00	25.52	51.44	81.85	118.15	165.40	182.42	6.26
S_{32}	0.00	34.17	44.97	60.36	86.97	86.97	96.40	6.25
S_{33}	0.00	6.11	51.48	98.02	132.18	145.07	154.50	6.25
S_{41}	0.00	21.07	92.91	158.30	187.93	187.93	239.37	6.30
S_{42}	0.00	6.07	110.08	209.59	236.01	250.94	278.64	6.30
S_{43}	0.00	18.92	90.73	158.23	185.70	185.70	224.18	6.30

Second batch cumulative normalized SBY (mL_N/g VS)

Samples	HRT (days)								
	0.0	2.8	8.0	13.0	17.8	27.0	33.9	38.9	**VS added (g)**
S_{51}	0.00	0.00	39.04	72.50	78.60	87.97	87.97	87.97	12.27
S_{52}	0.00	0.00	33.48	61.38	68.04	78.53	78.53	78.53	12.27
S_{53}	0.00	0.00	39.10	72.61	79.28	90.33	90.33	90.33	12.26
S_{61}	0.00	96.78	38.07	-10.78	-12.60	32.12	140.93	173.63	6.26
S_{62}	0.00	143.17	82.19	32.17	33.62	74.07	172.14	186.33	6.25
S_{63}	0.00	143.20	89.88	49.71	50.07	93.78	224.57	268.24	6.25
S_{71}	0.00	121.01	192.51	267.27	396.00	430.99	475.62	475.62	6.26
S_{72}	0.00	121.06	187.11	267.37	396.16	446.32	489.88	489.88	6.25
S_{73}	0.00	148.59	212.46	287.26	410.63	444.55	490.29	490.29	6.25
S_{81}	0.00	44.01	47.66	78.66	125.81	156.47	179.33	179.33	6.26

S_{82}	0.00	33.00	36.64	62.17	84.29	114.94	136.71	136.71	6.26
S_{83}	0.00	44.00	58.59	84.11	121.45	153.19	174.96	174.96	6.26
S_{91}	0.00	61.68	46.80	34.72	42.12	50.02	57.23	57.23	12.27
S_{92}	0.00	47.69	32.81	17.93	28.10	26.08	32.74	32.74	12.27
S_{93}	0.00	50.54	35.65	37.51	42.14	48.40	55.06	55.06	12.26

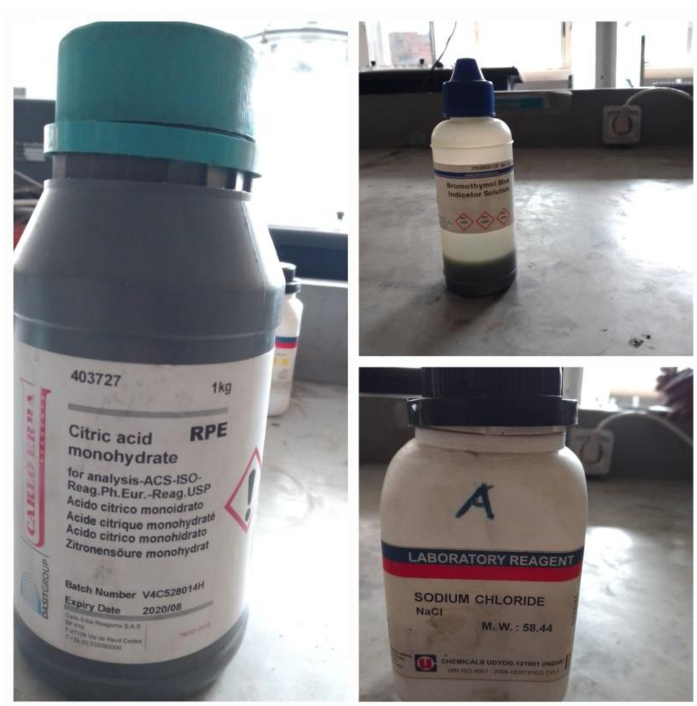
First batch cumulative normalized SMY (mL_N/g VS)

Samples	HRT (days)						
	0.00	**3.10**	**7.23**	**10.23**	**14.17**	**21.14**	**27.17**
S_{11}	0.00	14.44	23.37	27.23	37.01	41.72	42.42
S_{12}	0.00	14.90	23.38	27.88	33.12	37.42	38.31
S_{13}	0.00	18.88	31.82	40.80	46.71	52.41	53.19
S_{21}	0.00	55.98	122.94	157.21	173.41	176.50	188.30
S_{22}	0.00	56.97	128.53	185.37	199.58	215.40	231.00
S_{23}	0.00	7.41	73.54	94.57	117.26	129.54	132.93
S_{31}	0.00	-0.81	19.16	40.20	58.68	76.43	82.12
S_{32}	0.00	7.30	16.99	23.53	36.05	34.85	38.41
S_{33}	0.00	-8.24	25.24	57.43	70.34	74.14	77.32
S_{41}	0.00	5.52	51.07	104.28	128.83	125.91	154.54
S_{42}	0.00	-6.15	47.93	126.64	151.90	163.33	177.99
S_{43}	0.00	7.01	58.96	110.92	128.47	129.03	141.58

Second batch cumulative normalized SMY (mL_N/g VS)

Samples	HRT (days)							
	0.0	**2.8**	**8.0**	**13.0**	**17.8**	**27.0**	**33.9**	**38.9**
S_{51}	0.00	0.00	23.11	45.03	47.47	51.29	51.29	51.29
S_{52}	0.00	0.00	19.66	38.54	41.39	45.61	45.61	45.61
S_{53}	0.00	0.00	24.60	48.59	51.30	55.90	55.90	55.90
S_{61}	0.00	54.49	17.42	-19.14	-20.75	5.92	81.33	102.62
S_{62}	0.00	87.02	48.56	11.24	11.20	43.10	107.93	116.47
S_{63}	0.00	88.46	54.04	21.83	21.23	56.83	146.29	173.06
S_{71}	0.00	75.84	140.87	203.69	300.15	323.79	341.15	341.15
S_{72}	0.00	78.64	138.87	206.21	309.92	334.10	350.30	350.30
S_{73}	0.00	94.60	152.37	212.77	306.31	321.17	339.52	339.52
S_{81}	0.00	25.96	36.02	64.45	99.91	117.54	127.05	127.05
S_{82}	0.00	20.06	31.80	57.37	76.22	94.57	103.40	103.40
S_{83}	0.00	26.48	38.13	60.55	88.74	108.85	117.99	117.99
S_{91}	0.00	34.36	26.06	17.67	23.99	29.98	32.88	32.88
S_{92}	0.00	33.02	26.15	16.25	24.37	24.46	27.44	27.44
S_{93}	0.00	29.18	20.64	20.23	24.76	29.45	32.28	32.28

Appendix VII: Sampling, Instruments, chemicals, and Laboratory apparatus used during the study




Gas (urine) bag
Crucible
Sealant
Kork with gas outlet valve
AD digester (500 ml)
Analytical balance
Furnace
BOD incubator
Oven
Water bath
THERMOSTAT WATER BATH
HH-6
WISE TEAM