HAWASSA UNIVERSITY COLLEGE OF AGRICULTURE

SCHOOL OF PLANT AND HORTICULTURAL SCIENCE

MSc Thesis On

Investigating the Biogas Production Potential of Grass Hay Co-digested with Cattle Dung

By: Zerihun Desalegn Dana

Advisor: Shemelis Nigatu (PhD)

November, 2022

Hawassa, Ethiopia

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A Thesis Submitted to the School of Plant and Horticulture Science, College of Agriculture, School of Graduate Studies, Hawassa University in Partial Fulfillment of the Requirements for the Master of Science Degree in Bioenergy Science and Technology

Advisor: ShemelisNigatu(PhD)

November, 2022

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School of Graduate Studies

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Advisor's Approval Sheet (Submission Sheet – 1)

As thesis advisor, I hereby certify that the thesis entitled ''Assessment of Biogas Production Potential by Co-digesting Cattle Dung with Grass Hay'' submitted in partial fulfillment of the requirements for the Master degree of Sciences with specialization in Bioenergy Science and Technology, Graduate program of the School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University and is a record of original research carried out by ZerihunDesalegn Dana under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that, the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

SUBMITTED BY:

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Examiners' Approval Sheet (Submission Sheet – 2)

We, the undersigned, members of the Board of Examiners of the final open defense by ZerihunDesalegn Dana have read and evaluated his thesis entitled ''Assessment of Biogas Production Potential by Co-digesting Cattle Dung with Grass Hay'', and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Masters of Science in Bioenergy Science and Technology.

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Declaration

I, the undersigned, hereby declare that this thesis is my original work prepared under the supervision ofShemelisNigatu(PhD) and has not previously been submitted either in part or in full to any other higher learning institution for the purpose of earning any degree. All resources materials used in this thesis have been duly acknowledged.

Name: ZerihunDesalegn Dana

Signature:

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ZerihunDesalegn Dana

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Acronym and Symbols

AC……………………………………………………... Ash content

AD ……………………………………….....…… Anaerobic digestion

ANOVA…………………………………………...Analysis of variance

C: N…..…………………………………….……Carbon to Nitrogen ratio

CD…………………………………………….….. Cow dung

CRD………………………………………………Completely randomized design

CSTR……………………………………………..Continuous stirred tank reactor

g …………………………………………………. gram

HRT……………………………………………..…Hydraulic retention time

kg ………………………………………………… Kilio gram

L …………………………………………………. Litre

L_N ……………………………………………….. Litre (normalized)

LSD………………………………………...….. Least significant difference

MC……………………………………………….. Moisture content

ml……………………………………………….....Milliliter

mL_N ……………………………………………... Milliliter (normalized)

MSW………………………………………………Municipal solid wastes

OC……………………………………..….………Organic carbon

ODM ………………………………………………. Organic Dry Matter

OLR……………………………….………………Organic loading rate

R…………………………………..……………Reactors

SD……………………………...………………Standard deviation

T……………………………………………………Treatment

TKN…………………………..……………………Total Kjeldahl Nitrogen

TS…………………………..………………………Total solids

UASB…………………………..……………..……….Up flow anaerobic sludge blanket

VAFs ………………………………………….....…Volatile fatty acids

VS………………………………………………..…Volatile solids

Abstract

An alternative source of renewable energy can be offered from anaerobic digestion of organic materials since bio-methane has a potential in replacing fossil fuels in both heat and power generation, thus contributing to mitigate the emissions of greenhouse gases. For the reasonsjustified as easy availability, smooth digestibility, and easy technology acquisition, the anaerobic digesters in developing world mostly rely on cattle dung as feedstock. However, the declining trend of the cattle size holding at household levelcoupled with wastage of energy rich fodder (grass silage) when feeding out call for a bio-digester systems co-digestingcattle dung with grass silage. In this regard, co-digestion of the cattle dung with wastage of fodder could be an interesting remedy for meeting both the availability of sufficient biogas and waste management.

In this study, biogas and methane yields of cattle dung alone, 50% cattle dung with 50% grass hay, and 75% cattle dung with 25% grass hay were measured and compared using in anaerobic batch digesters.The experiment was carried out in a laboratory scale batch assay at mesophilic temperature (37±2^{o}C) for 38 days, samples were characterized in terms of total solid (TS), volatile solid (VS), power of hydrogen (pH), total nitrogen and carbon to nitrogen ratio (C:N) as per the existing standards and biogas produced was characterized using biogas analyzer.

From the dry matter of the substrate, specific biogas yields of 273.3, 318.6 and 296.4 mL_N/g oDMwas obtained from cattle dung alone, 50% cattle dung with 50% grass hay, and 75% cattle dung with 25% grass hay respectively while specific methane yields were 165, 211 and 170.8 mL_NCH_4/goDMrespectively. Moreover, methane concentrations of 60, 66 and 58% were recorded for the above treatments.Therefore, optimum co-digestion of cattle dung with grass hay resulted inmore biogas and methane yields than mono digestion of cattle dung in terms of dry matter substrateand cattle dung can be co-digested with grass hay at dry matter ratio of 1:1 for yielding the highest biogas yields of 318.6mL_N/goDM.

Key Words:*Biogas, Bio-methane, Co-digestion, Cattle dung, Feedstock, Grass hay.*

1. Introduction

1.1. Background

In one hand, the global energy demand has been increased. On the other hand, the growing concerns about bio-waste disposal and environmental problem have been raised (Pourhosseini*et al.,* 2018). According to SE4ALL (2015), traditional energy use increases the rate of deforestation and land degradation, which in turn can lead to excess soil erosion and loss of soil fertility. This further contributes to the decline of agricultural productivity and production, perpetuating the vicious cycle of rural poverty. Indoor air pollution associated with kerosene and traditional fuel use is a major health concern, especially for women and children. Reliance on traditional energy sources of biomass brought threat from overuse, creating additional environmental challenges ranging from local land use to global climate change and applications in smoky kitchens. If current fuel wood utilization trends continue, most developing countries are predicted to experience severe shortage of fuel wood by 2025 (SE4ALL, 2015). In sum, it leads to depletion of tree stocks or threat to biodiversity, desertification, reduced water quality, sedimentation, dust storms, air pollution and health problems such as respiratory illnesses and allergies (Dagninet*et al.*, 2015). These facts coupled with limited fossil resources means that alternative energy resources are necessary (Di Maria *et al.*, 2018).

Renewable energy sources are being promoted all over the world as a response to the increasing climate change concern. Biogas could be a potential renewable source for heat and electricity as well as motor fuel for the foreseeable future,contributing significantly to sustainable development in terms of socioeconomic and environmental concerns. Biogas is a mixture of different gases produced by the biological decomposition of organic matter in the absence of oxygen, called an anaerobic digestion process. It mainly consist of methane (CH_4), carbon dioxide (CO_2) and a trace amount of other gases such as hydrogen sulfide (H_2S), ammonia (NH_3), hydrogen (H_2), nitrogen (N_2) and carbon monoxide (CO). Biogas can be produced by anaerobically digesting several organic matters such as agricultural wastes, municipal wastes and industrial wastes (Karlsson*et al.*, 2017) and it can be used as such for heat and electricity production or as traffic fuel after purification to biomethane.

The combination of biomass like cattle dung, bagasse, rice husks, grass fodder, sugarcane residue, oat straw, and organic fractions from kitchen waste/food waste in the anaerobic digester in biogas plants for making methane generation will be highly efficient. Biogas is a potent renewable energy, because of its characteristics of using, collecting and monitoring organic wastes, fertilizer production and slurry use in agricultural irrigation (Sidra *et al.*, 2017).

Naturally endowed with different agro-ecological zones and suitable environmental conditions, Ethiopia is a home for many livestock species and suitable for livestock production. It is believed to have the largest livestock population in Africa (Tilahun and Schmidt, 2012). An estimate indicates that the country is a home for about 70 million cattle, 42.9 million sheep, 52.5 million goats, 8.1 million camels and 57 million chickens. From the total cattle population,97.4 % are local breeds and the remaining are hybrid (2.3%) and exotic breeds (0.31%)(CSA, 2021).

Covering around 26% of world's total land area, grasslands play an important role in global agriculture (Rodriguez *et al.*, 2017). In most developing countries, grasses are the main plant species in verges along roads, dams and on river dikes, for that reason, the hectares of grassland available are difficult to quantify (Darimani and Pant, 2020). In Ethiopia, nowadays, the most important livestock feed resources are natural pasture, crop residues and grass hay (Abebe*et al.*, 2015). According to CSA (2017) report, the major feed resources in the country are green fodder (54.59%), crop residue (31.6%), hay (6.81%) and agricultural by product (1.53%).

Anaerobic co-digestion technology allows concurrent digestion of different solid and liquid wastes (Dias *et al.,* 2014). Anaerobic treatment has been considered as the waste-to-energy technology and has been largely used in the digestion of different organic wastes, like animal manure, food waste, organic fraction of municipal solid waste and sewage sludge (Kavitha*et al.*, 2015a). Co-digestion of grass silage with animal slurry, a feedstock which usually produces a lower methane yield compared to grass silage, can complement each other and enhance the longevity of stable and productive AD (Wall *et al.*, 2014).

1.2.Statement of the Problem

In Ethiopia, biogas technology dissemination has been progressing slowly though the technology has proven to be very essential to improve the livelihood of the rural people. Of the various factors hindering the mass domestication of the biodigester, the shortage of sufficient feedstock could be mentioned.Sufficient livestock size to produce adequate cow dung and generate adequate energy is essential to meet household's cooking requirements from biogas energy (Mwirigi*et al.*, 2009). Small-scale farmers frequently do not have sufficient domestic animals to produce enough manure to charge the bio-digester for production of energy required for lighting and cooking (Parawira, 2009). According to Eshete et al. (2006), the "low" technical potential for domestic biogas in the regional states including Amhara, Oromia, SNNPR and Tigray, would amount to approximately 1.1 million households. This technical estimation is only based on the availability minimum requirement of four cattle per household and water access within a walking distance of 20–30 min away from residence for daily feeding of a bio-digester (EREDPC and SNV, 2008) though Ethiopia has a huge cattle population as an aggregate. The mixed crop-livestock farming system is the dominant livestock production system in the Ethiopian highlands with 86% of the total cattle population and the number of livestock kept per household in mixed farming areas is low due to the emphasis on crop cultivation (Management Entity, 2021). Concerning the number of holdings by size of cattle, most of the holdings (about 48 %) reported as one to two cattle (24.93 %) and without cattle (23.16%) followed by about 45 % holdings possessingthree to nine cattle (CSA, 2021). Similarly, according to Asfaw et al. (2013), the cattle holding per household has shown decline trend though the total number of livestock in Ethiopia is the largest in Africa. Households largely rely on communal grazing and crop residues to feed their cattle, and this is becoming increasingly challenging for the highland herd keepers due to expanding crop areas and hence decreasing pasturelands (Asfaw*et al*., 2013). According to CSA data (CSA, 2001–09), for instance, the numbers of households exclusively cultivating crops or exclusively keeping livestock are declining rapidly. In 2001/2, about 18 % of farmers were growing crops only, but that number dropped to only 9 % in 2008 (Asfaw*et al*., 2013). Similarly, the percentage of households only keeping livestock dropped from 8% in 2001 to 5 % in 2008 (Asfaw*et al*., 2013). By contrast, the percentage of farmers with both crop and livestock holdings increased from 74 % to 86 % during the same period (Asfaw*et al*., 2013). Farmers, thus, appear to be adapting to changes by adopting a diversification strategy that allows them to mitigate both risks of crop failure and losses of livestock.

Ethiopia is implementing the national biogas programme of Ethiopia (NBPE) since 2008 with the objective of disseminating household bio-digesters in a commercially viable manner in potential areas of the country. The number of biodigesters installed in Ethiopia before 2008 was not more than 1,000 (EREDPC and SNV, 2008) and now, it has reached 39,136 digesters (NBPE annual report, 2021/22) with the functionality rate of 81% (BUS, 2020). Though the installations have shown incremental trend, it lags behind as compared to the tremendous feedstock types and potential in the country. The bio-digester dissemination mainly depends on cattle dung putting minimum of four cattle size as criteria to get sufficient amount of gas for heating & lighting. However, most households in Ethiopia rear cattle with a size of less than four. According to Haile and Getachew (2020), about 55.4%, 32%, 5.4%, and 6.8% of the households had cattle holding size ranging from 1-3, 4-5, 6-7 and > 7, respectively. The average cattle holding size was three cattle per household, which is less thanthe minimum standard set by the National Biogas Program of four cattle as criteria to get sufficient amount of gas for heating and lighting.

On the other hand, the NBPE implementation technical document recommends the elimination of mixed grass hay in the dung before feeding to the domestic bio-digester reactor because it slows the biodegradability of the substrate. The source of mixed grass hay in the dung is obtained either from the undigested cattle dung or from the poor management during storage of the grass hay before feeding to the cattle. Besides, the conversion of the organic feedstock resources (grass hay) to biogas has not been easy because of constraints including the lack of pre-treatment mechanism that can be applied at local scale.

Wastage of conserved fodder when feeding out can vary from 4 to 46% of the dry matter (DM) (Stockdale, 2010). The main factors affecting the degree of wastage were storage method, packaging method, method of feeding out, amount of fodder on offer and its palatability and/or quality and the impact of wet weather. Although the emphasis was on grass hay, the principles should also apply to silage. Thus, total storage and feed-out losses ranged from 14 to 40% of the original dry matter that was stored (Stockdale, 2010).

With the declining trend of cattle size per household (Asfaw*et al*., 2013), the sole use of cattle dung as feedstock (EREDPC and SNV, 2008) and wastage of fodder when feeding and storing (Stockdale, 2010), mass dissemination of small size bio-digester in the country will be challenged. This was the reason why the study was initiated. Thus, this study hypothesised co-digestion of the cattle dung with grass hay could be an interesting remedy for meeting both the availability of sufficient biogas and waste management and hence, mass adoption of the technology.

Co-digestion is the simultaneous digestion of homogenous mixture of two or more substrates (Wu, 2009). According to Mata (2003), the use of co-substrates usually improves the biogas yields from anaerobic digester due positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates. The main objective of this study, therefore, is to determine the biogas production potential of mixed grass hay materials with cattle dung.

1.3. Research Objectives

1.3.1. General Objective

- ❖ The overall aim of this study is to determine the biogas production potential of mixed grass hay co-digested with cattle dung.

1.3.2. Specific Objectives

The specific objectives of the study are:

- ❖ To characterize the feedstocks (dung and grass silage) in terms of their TS and VS.
- ❖ To compare the biogas yield obtained from co-digested substrates (dung and grass hay) and biogas obtained from the digestion of dung alone.
- ❖ To identify the effect of co-digested mixed grass hay substrate in the co-digestion process and in the output.

1.4.Significance of the Study

With an estimated potential of about 36 EJ contributing 6.5% of the global energy supply (WBE, 2013), bioenergy will contribute significantly in the future energy mix of the world. Biogas, which is one of the most important bioenergy, can be boldly considered in this regard given its nature including production from many different types of feedstock, great flexibility to be converted to electricity, stored as a pressured gas or cleaned and used in a gas grid or as transport fuel. Accordingly, diversification of various types of feedstocks could help to provide option to exploit and maximize the biogas potential from various sources. This study makes some important contributions to the viability of the co-digestion of grass hay with cattle dung, an area in which there is limited literature that thoroughly evaluated the optimum ratio of co-digestion of grass hay and cattle dung.

2. Literature Review

2.1.Biogas

Anaerobic digestion (AD) is a microbiological process whereby organic matter is decomposed in the absence of oxygen. This process is common to many natural environments such as swamps or stomachs of ruminants. Using an engineered approach and controlled design, the AD process is applied to process organic biodegradable matter in airproof reactor tanks, commonly named digesters, to produce biogas. Various groups of microorganisms are involved in the anaerobic degradation process which generates two main products: energy-rich biogas and a nutritious digestate (Amalia*et al.*,2014). Biogas is a mixture of gases that is composed chiefly of:methane (CH_4) with an amount of 40 - 70 vol. %, carbon dioxide (CO_2) with an amount of 30 - 60 vol. % and other gases with an amount of 1 - 5 vol. % including hydrogen (H_2) with an amount of 0 - 1 vol. % and hydrogen sulfide (H_2S) with an amount of 0 - 3 vol. % (Kossmann, *et al.,* 1999).

This conversion of complex organic compounds into CH_4 and CO_2 requires different groups of micro-organisms and is carried out in sequence of four stages: Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis. During hydrolysis organic substrate is converted into smaller components , then acidogenic bacteria use these smaller compounds and produce volatile fatty acid such as ethanol, CO_2 and H_2. Acetogenic bacteria convert these fermentation products in to acetic acid, CO_2 and H_2. Finally methanogenic archaea bacteria use hydrogen and acetate (most important substrate) and produce methane and carbon dioxide. A variety of factors affect the rate of digestion and biogas production. The most important is temperature. Biogas production is carried out at different temperatures: temperature range 45 - 60 °C is referred to as 'thermophilic,' whereas that carried out at temperature range 20 - 45 °C is known as 'mesophilic' and at low temperatures (<20 °C) is referred to as 'psychrophilic' digestion (Safley and Westerman, 1992). Once a suitable bacteria culture has been developed inside the biogas digester, biological waste is mixed with water in a 2:3 ratio and retained for approximately 50-60 days (FAO, 1997). It is estimated that 50-70 % of the raw material fed into the biogas digester is eventually converted to usable biogas, resulting in an efficiency utilization rate 5 to 7 times greater than traditional burning of biomass energy (EWB, 2004).

2.2.Anaerobic Digestion Process

Anaerobic digestion offers a valuable option for converting biodegradable feedstock into renewable energy. In this process, the conversion occurs by bacteria and archaea in the absence of oxygen, wherein a series of complex biochemical reactions determine the product output. Generally, the organic part of the biomass is utilized by the microorganism consortia and yielded to methane and carbon dioxide, while the rest is transformed into other minorities (Shiplu*et al.*, 2019). The complete digestion process takes place in four different stages called hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Schnurer and Jarvis, 2010), which are briefly overviewed in Figure 2.1.

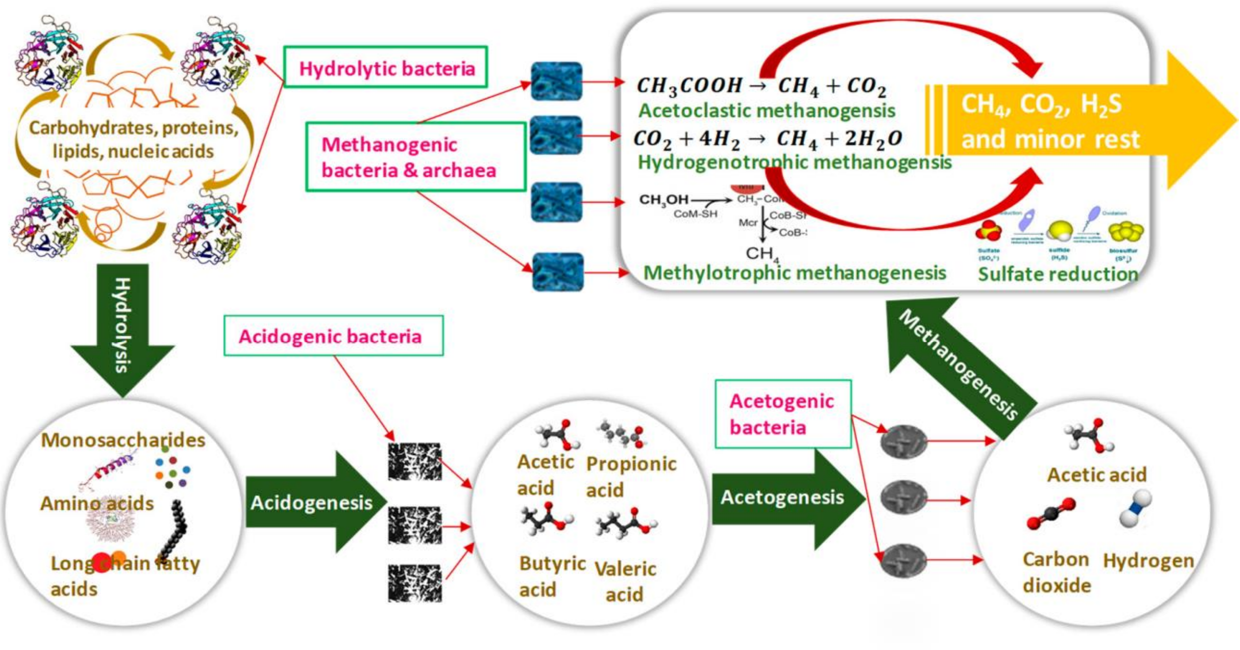

Figure 2.1: Simple schematic representation of anaerobic digestion steps (Schnurer and Jarvis, 2010).

2.2.1. Hydrolysis

In easing degradation of complex organic molecule, hydrolysis play a vital role as it involves depolymerization of insoluble complex organic hydrocarbons into soluble monomers where the principle substrate compounds (i.e., carbohydrates, lipids, and proteins) are broken down into corresponding low molecular weight monosaccharides, long chain fatty acids, and amino acids that are favorable for bacterial degradation. Hydrolysis is a complex multistep process mediated by extracellular enzymes. In the hydrolysis process, the enzymes required for hydrolysis can either be attached to microbial cells or secreted to the solution. As shown the following table, several groups of hydrolytic microorganisms are involved in the degradation of several substrate compositions, where the bacteria Bacteriods, Clostridium and Staphylococcus are significant drivers (Nayono, 2010).

Table 2.1: Bacterial groups involved in hydrolysis of different substrate components (Nayono,2010).

Primary Substrate Components	Hydrolyzed Products	Bacterial Group
Carbohydrates	Soluble sugars	Clostridium, Acitovibriocelluliticus, Staphylococcus, Bacteriodes
Lipids	Higher fatty acids or alcohols and glycerol	Clostridium, Staphylococcus, Micrococcus
Proteins	Soluble peptides and amino acids	Clostridium, Proteusvulgaris, Peptococcus, Bacteriods, Bacillus, Vibrio

The hydrolysis of lignocellulosic materials is a relatively slow process. Thus, hydrolysis is considered as a rate-limiting process in the digestion of lignocellulose (Dinh*et al*, 2020). Fortunately, this issue can be accelerated significantly by using pre-treatment processes including physical, chemical, and biological methods. The details of these methods are presented by Ariunbaatar et al. (2014).

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{Hydrolysis} nC_6H_{12}O_6 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

2.2.2. Acidogenesis

The second step (acidogenesis) transforms the products of the hydrolytic process into volatile fatty acids (VFAs) such as propionic acid, butyric acid, acetic acid and ethanol by the action of the acidogenic bacteria (Dinh*et al*, 2020). They have characteristics of strong and fast growth with a minimum doubling time of 30 min (Ramos-Suárez, 2015). Acidogenesis can be described by Eq. (2)-(5). The pH-conditions significantly influence VFA products. By stepwise shifting pH from 4 to 8, the main products changed from butyric and acetic acids to acetic and propionic acids. Moreover, the VFAs formation was strongly inhibited with pH below 4.0. The pH in the range of 5.5-6.5 was often reported as the optimal range (Dinh*et al*, 2020).

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{Acid\ forming\ bacteria} 3nCH_3COOH \quad \text{.................} (2)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H^+ + 2H_2O \quad \text{..................} (3)$$

$$C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COO^- + H^+ + 2CO_2 + 2H_2 \quad \text{..........................} (4)$$

$$C_6H_{12}O_6 \rightarrow 2CH_2CH_2OH + 2CO_2 \quad \text{..} (5)$$

2.2.3. Acetogenesis

The third step (acetogenesis) transforms most products of acidogenesis into acetic acid (CH_3COOH), hydrogen (H_2), and carbon dioxide (CO_2) as shown in Eq. (6)-(9) (Dinh*et al*, 2020). The growth kinetic of acetogenesis is slower than which of acidogenesis, with a minimal doubling time in the range of 1.5-4 days.Acetogens are strict anaerobes, the present of oxidants like oxygen or nitrate is toxic and they work better in acid-weak environment (pH from 6.0 to 6.2). Remarkably, high partial pressure of hydrogen product (≥ 10-4 atm) inhibits Eq. (6) and (7), hence hydrogen product should be released (Dinh*et al*, 2020).

$$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2 \quad \text{......................} (6)$$

$$CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \quad \text{..........................} (7)$$

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2O \quad \text{.................................} (8)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COO^- + H^+ + 2H_2O \quad \text{..} (9)$$

2.2.4. Methanogenesis

The fourth step (methanogenesis) plays the most important role in generating methane gas by methanogens. There are two basic mechanisms for methane generation including acetoclastic and hydrogenotrophicmethanogenesis. The first way, acetotrophic bacteria ferment acetic acid to CH_4 and CO_2 as in Eq. (10) (Dinh*et al*, 2020). The second one, hydrogenotrophic methanogens use CO_2 and H_2 as a food source, as given in Eq. (11). While the minimum doubling time of hydrogenotrophic bacteria is in the range of 4-12 hours, acetotrophic bacteria have a much lower maximum growth rate with doubling times of 2-3 days. Generally, methanogens are extremely sensitive to pH condition, the presence of oxygen, and other factors such as free ammonia (FAN), H_2S, and VFAs. They cannot work at pH condition lower than 6.2, even die in the pH condition under 6.0, and can be inhibited by releasing free ammonia (FAN) when pH-value is over > 7.8 (Gerardi, 2003). The optimal pH-value was often reported at neutral environment (7.0-7.2). Methanogenic process lost stable stage when FAN concentration reached 0.6-0.69 g/L, reduced 50% performance when FAN concentration was 1.45 g/L, and failed by FAN concentration of 1.7-1.8 g/L. The optimum concentrations of sulfur for the growth of methanogens were reported in the literature to vary from 16 to 160 mg-S/L. The methanogenesis was often stable with a low VFA concentration (< 200 mg/L), and inhibition occurred when acetate concentration exceeded 3 g/L. The oscillation of temperature in the reactor should be as small as possible, that is, < 1°C/d for thermophiles and 2-3°C/d for mesophiles (Dinh*et al*, 2020).

$$CH_3COOH \xrightarrow{acetociastic\ bacteria} CH_4 + CO_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (10)$$

$$CO_2 + 4H_2 \xrightarrow{hydrogenotrophic\ bacteria} CH_4 + 2H_2O \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

2.3.Reactors

2.3.1. Configuration

The way feedstock is converted in AD is greatly dependent on the way the reactor is configured. Generally, according to Kothari (2014), two broad classes of substrates, i.e., wet substrate (TS<15%) and high-solid substrate (15% < TS < 40%), are treated in a wide range of reactor designs, including anaerobic baffled stacking reactor (ABSR), anaerobic contact process (ACP), anaerobic filter (AF), anaerobic fluidized bed reactor (AFBR), agitated granular sludge bed reactor (AGSBR), anaerobic hybrid reactor (AH), anaerobic membrane reactor (AnMBR), anaerobic sequencing batch reactor (ASBR), anaerobic migrating blanket reactor (AMBR), batch system anaerobic reactor (BSAR), carrier-induced granular sludge bed reactor (CIGSBR), continuously stirred anaerobic bioreactor (CSABR), continuously stirred tank reactor (CSTR), expanded granular sludge bed reactor (EGSBR), fixed-bed reactors (FBR), plug flow reactor (PFR), submerged anaerobic membrane reactor (SAnMBR), super-high-rate anaerobic bioreactor (SAB), temperature phased anaerobic digestion reactor (TPAD), tubular reactor, up flow static media anaerobic reactor (USMAR), up flow anaerobic sludge blanket reactor(UASB), and solid-state digester.

In recent years, the process efficiency and feedstock characteristics (i.e., dry matter content) and reactor systems have been modified to meet the specific criteria of product output, and further development is expected. Broadly, digesters can either be batch or continuous. Batch configurations are straightforward, require less moving parts and are inexpensive, but they are generally employed to determine methane potential of substrates where the reactors are subject to the anaerobic environment for a long period, until a degree of digestion close to the theoretical maximum is achieved. However, in a real life scenario, constant gas production is more desirable, requiring continuous digestion) (Shiplu*etal.*, 2019).

Continuous digestion system can be designed with single or multiple digesters. In a single stage digestion system, all steps in microbial degradation, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, occur simultaneously in a single reactor vessel. For the majority of anaerobic digestion applications, single stage reactors are still dominant because of their simplicity in design and cost. So far, an array of substrates such as food waste, vegetable waste, sludge, municipal solid waste (MSW), biowaste, and livestock manure have been treated by this configuration and process performance is optimized in a vast number of cases. For example, optimum methane recovery using a single stage reactor (CSTR, PFR, UASB, ASBR, and TPAD) for livestock manure (i.e., cattle manure, pig manure, and poultry manure) was shown to be achieved and even maximized by recirculating the process liquid back into the reactor. By digestate recirculation, liquid retention time increases and the microbe wash out from the process consequently decreases (Shiplu*et al.*, 2019).

In a two-stage system, the first step involves feeding material into the first digestion tank (acidogenic reactor), where hydrolysis, acetogenesis, and acidogenesis take place. The partially digested materials from this reactor are then introduced into the second stage (methanogenic reactor), where the methane is produced. Compared to the single stage digestion, the two-stage process allows fast and efficient formation of biogas in the second stage where methane recovery of volatile solids can exceed 90% in exceptional cases. However, due to the complexity in design, two-stage reactors have a higher cost (Shiplu*et al.*, 2019).

The three-stage reactor concept was developed in the early 1990s. In this configuration, the first phase involves semi-anaerobic hydrolysis of feedstock at low hydraulic retention time and the removal of un-degraded materials to the secondary reactor for acidogenisis. From the secondary reactor, output liquids and solids are transferred to a tertiary reactor where methane-rich biogas is produced. Likewise, in a two-stage system, a part, or all of the effluent from the tertiary reactor can be recirculated back to the digester depending on the targeted HRT and process efficiency (Kim *etal.*, 2000). According to the study by Kim et al. (2000), using a three-stage reactor, COD removal efficiency of over 95% was achieved for food waste AD. In a more recent study, an 11–23% increase in methane yield for a three-stage co-digestion of food waste and horse manure was reported (Shiplu*et al.*, 2019).

In addition to the configurations mention above, AD systems have undergone several modifications over the last few decades to address the retention efficiency of slow-growing methanogens. With the development of the UASB reactor, where a dense sludge bed allows methanogenic sludge to maintain within the reactor, the improvement in retention efficiency was found to be significant. So far, numerous UASB reactor types are being used for treating waste and wastewater effluents of various characteristics. Among these, the internal circulation (IC) reactor was observed to exhibit high efficiencies in terms of considerable feasibility, robust resistance to outside accidents, high organic loading rate, and low investment cost. Membrane bioreactors (MBR) are another technique quite successfully able to retain active biomass in the system. By employing a membrane, both cells and inhibitory components within the bioreactor can be separated and as such, an efficient biological process can be achieved (Shiplu*et al.*, 2019).

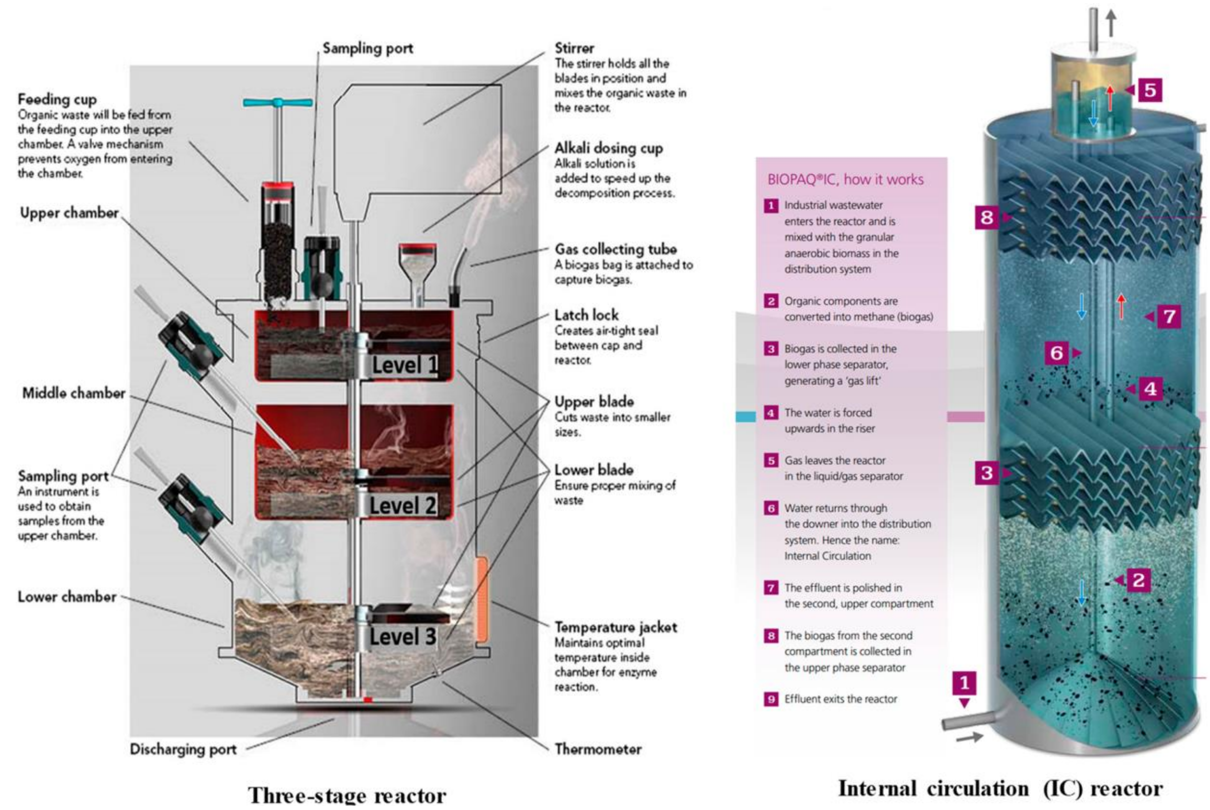

Figure 2.2: Example of two novel anaerobic reactor types(Ding & Wang, 2005).

Apart from bioreactor design, the developments in biosensor technologies and molecular biology techniques can enable understanding complex microbiological processes and potential disturbances much earlier than the conventional AD processes that do not involve any sensors for that matter (Shiplu*et al.*, 2019).

2.3.2. Mixing

It is an important operating factor for achieving digestion of organic matter. The performance and cost of an AD system is determined by mixing to a great extent. Mixing is vital for achieving uniformity among the substrate concentration, temperature, and environmental conditions to reduce the chance of scum formation and solid deposition (Muzaffar*et al.*, 2016). Adequate mixing can reduce sedimentation and foaming caused by floating fat with adhering gas bubbles or by filamentous microorganisms, such as Microthrix or Norcardia. Reactors equipped with mixing tend to produce more biogas than those without, although contrasting results do exist. Mixing can be accomplished by means of mechanical (employing a mixer), hydraulic (liquid recirculation), and pneumatic (recirculation of gases) techniques at various frequencies (continuously or intermittently with several hours or several times in an hour during a day) and intensities (gentle, intermittent, and rigorous rotation speed) depending upon the total solid concentration within the digester (Shiplu*et al.*, 2019). Ong et al. (2002) showed that employing intermittent mixing, mass transfer from the liquid phase to the gas phase greatly enhanced resulting in an increased gas release as much as 70% higher than the periods without mixing. In addition, among various mixing intensities, gentle mixing leads to the formation of aggregates and prevents methane-producing organisms from being washed out by the liquid and thereby proved to be more effective (Shiplu*et al.*, 2019).

Despite the benefits of increased gas release, mixing does have an extra cost and requires an energy input. Continuous mixing in a full-scale AD can demand as much as 50% of the total plant energy, with mixer motor start-up consuming 2.5 % additional energy. Therefore, mixing should be compromised or chosen carefully, given the feedstock type and AD technology. Strategies like switching from continuous to intermittent mixing mode could save over 25% of the energy demand and ensure higher plant productivity. Additionally, using innovative technologies such as a gas-lifting reactor (that works on biogas's rising tendency for partial recirculation and digester's agitation) can provide the effect of mixing without requiring a mixer, and has been shown to result in improved plant economies (Shiplu*et al.*, 2019).

Besides, a certain degree of mixing is necessary to obtain a good contact between the substrate and the microorganisms, but excessive mixing can reduce biogas production by disturbing aggregation and granulation of the microorganisms (Anna*et al.*, 2014). Excessive mixing can disrupt microbes, so slow mixing is preferred (Khalid *et al.*, 2011). For example did Kaparaju et al (2008) found that a minimal mixing improved methane production by 13% compared to continuous mixing during AD of cattle manure in lab-scale experiments. The effect of organic load and mixing intensities during the manure digestion in batch experiments showed that minimally mixing (thoroughly shaken by hand for about 1 minute every time a sample was taken) resulted in higher methane production. On the contrary, vigorous (110 times per minute with a 3.5 cm stroke) mixing resulted in delayed and low methane production (Kaparaju*et al.*, 2008). It has been reported that low speed mixing allowed digesters to better absorb the disturbances of shock loadings compared to reactors with high speed mixing (Stroot*et al.*, 2001; Gomez *et al.*, 2006). Thus, reduction of mixing speed may be a tool for process stabilization. In comparing continuously vs. intermittently mixed high loaded processes stable conditions are more often obtained with the intermittent systems (Stroot*et al.*, 2001). These effects are likely the result of disturbance of the syntrophic interactions among methanogens and proton-reducing bacteria.

2.4.Feedstock

2.4.1. Substrate

Feedstock for anaerobic digestions include diverse substrates originated from agricultural wastes, municipal solid wastes, industrial wastes, wastewater, aquatic biomass, and energy crops. Extracting biogas from these materials depends on the physical and chemical compositions that favour biological degradation. Not all the substrate components (carbohydrates -cellulose, hemicellulose, and lignin; lipids - fats, oil, and glycerols; and proteins) are readily degradable. For instance, lignin is highly non degradable, whereas cellulose breaks down in several weeks; hemicelluloses, fats, and proteins within a few days; and volatile fatty acids, alcohols in several hours. Therefore, the choice of appropriate feedstock concerning the amount of energy production, reactor design and waste disposal is essential (Shiplu*et al.*, 2019).

Theoretically, the biogas potential of feedstock constituents can be predicted by employing stoichiometry, as shown in Table 2.2 below.

Table 2.2: Stoichiometry of biogas potential determination from various feedstock components (Leung & Wang, 2016)

Feedstock	Methane Formation Stoichiometry	Methane Concentration, %
Carbohydrates	$(C_6H_{10}O_5)_n+nH_2O \rightarrow 3nCH_4+3nCO_2$	50
Lipids	$C_{50}H_{90}O_6+24.5H_2O \rightarrow 34.75CH_4+15.25CO_2$	69.5
Proteins	$C_{16}H_{24}O_5+14.5H_2O \rightarrow 8.25CH_4+3.75CO_2+4NH_4 + +4HCO_3$	68.8

Lipids, among the main components, have the highest methane potential (see Table 2.2); however, they can cause process problem and inhibition when hydrolyzed into long chain fatty acids (LCFA) and accumulated. Accumulated LCFA due to the adsorption on to anaerobic sludge damages bacterial cell walls, hinders nutrients and metabolic transports, and eventually results in process inhibition (Shiplu*et al.*, 2019).

A high level of proteins and fats contained in food wastes generated from slaughterhouses, food processing industries, and partly from municipal solid waste (MSW), which when used for AD, influence the production level of ammonia and sulfide. High levels of ammonia are toxic to methanogens and likely to cause process imbalances in terms of pH buffering. Additionally, substrates with high lipid content may also lead to other process problems associated with the substrate and product transport limitations, sludge floating, foaming, blockage of pipes and pumps, clogging of gas collectors and gas transport system (Shiplu*et al.*, 2019).

If lipid-rich feedstocks are co-digested in combination with substrates like municipal solid wastes, sewage sludge, paper waste, and rice husk, however, can offer an enhanced methane production due to the high methane potential. Protein-rich substrates like food waste, fish waste, algae, and energy crops have also been utilized for biogas production via AD. The major problem associated with anaerobic degradation of protein is the development of a high concentration of ammonia that often leads to process instability or inhibition. Some of the process problems related to protein-rich feedstock are suggested to be overcome by means of various approaches where reducing the feedstock particle size, keeping operating temperature to mesophilic range, increasing HRT, ammonia stripping by addition of CaOH, KOH or NaOH, and adjusting pH of bioreactor liquid by adding acidic iron and acid are commonly used options (Shiplu*et al.*, 2019).

Besides, substrates rich in carbohydrate content, such as lignocellulosic biomass, can be good sources of biogas production. Theoretical bio-methane potential of lignocellulosic biomass, such as grass, wheat, straw, and sorghum, is relatively high, but due to the high level of lignin, this class of feedstock opposes microbial hydrolysis, and hence recalcitrant to anaerobic conversion. While lignocellulosic biomass contains a rather high level of lignin, marine feedstock algae (micro and macroalgae) tend to have very low or no lignin, which makes them suitable for AD. Algae as a substrate can circumvent the issue of food vs. fuel, provide an excellent alternative to the biogas industry, and can offer a synergistic benefit of coproducing various chemicals and value added products including biogas. So far, the biomethane potential of various algae species has been documented in a number of past studies. However, a host of problems are identified, including cell wall resistance for degradation, production of toxic substances, increasing level of pH in case of high C:N ratio algae species, LCFA inhibition, and NH_3 inhibition. To overcome part of these challenges, pre-treatment, i.e., washing, maceration, thermal, sonic, and mechanical treatment along with co-digestion may be implemented (*Shiplu et al*., 2019).

Another important source of organic material used as feedstock for AD is Animal manures. Anthropogenic greenhouse gas emissions, which would have otherwise released during storage, can be reduced by using manures for biogas production. Depending on factors such as animal species, breed, growth stage, feed, amount, and type of bedding, the biomethane potential (BMP) of animal manure can vary widely. Manure provides essential nutrients (micro and macro) for bacterial proliferation, buffer capacity for degradation of low nitrogen substrates, and controlling the level of VFA during AD. Due to its many advantages, most of the anaerobic digesters in Europe are based on animal manure as a substrate. However, the biogas potential of manure is somewhat reduced by its recalcitrant material content, especially the bio-fibers or the embedded bed materials (e.g., straw). To achieve accelerated conversion of the recalcitrant fraction, manure is often suggested to undergo pre-treatment before utilization for biogas production (Shiplu*et al.*, 2019).

2.4.2. Inoculum

Setting the desired anaerobic start-up condition is determined by the choice of right inoculum type in combination with the right temperature. Generally, biogas production and the substrate utilization rate in an anaerobic digester show an inverse trend with increasing input of substrate to inoculum (S:I). The usage of too little inoculum can result in incomplete feedstock degradation and process problems associated with VFA accumulation, inhibition and slower methane production rate. For example, depending on the type of inoculum, operating temperature, and the volatile solids content of the substrate, a four-fold increase in S:I ratio could contribute to decreasing as much as 40% of biogas production from AD of cattle manure (Liu *et al.*, 2009). Additionally, a higher S:I ratio can lead to longer HRT, resulting in larger reactor working volumes. However, the use of a high amount of inoculum or a low S:I ratio might induce process instability as well as low biogas yield. Thus, a careful compromise is necessary (Shiplu*et al.*, 2019).

2.5.Co-digestion of Different Biomass

Co-digestion is the utilization and digestion of a homogenous mixture of biomass in AD for obtaining relatively higher methane yield with better conversion efficiency. This modified technique is considered economically more viable and easier to control mixed biomass compared to the traditional digestion systems. Co-digestion with some C-rich substrates has been proved for having potential to improve net C/N ratio and process efficiency (Herrmann *et al.*, 2016).According to Alemu (2016), a co-digestion of sewage waste, slaughter house waste and fruit-vegetable waste generated high biogas than individual wastes.

Ruminants manure, especially cattle, is very helpful for beginning the fermentation phase, because it already has the necessary methanogenic archaea bacteria. However, the fermentation of manure alone results in lower methane generation due to moderate anaerobic biodegradability of about 45–50 % (Rico *et al.*, 2007). Moreover, the biogas production from cattle manure is lower than that obtained from other farm animals, since cattle manure contains leftover lignin complexes from fodder that are very resistant to AD (Monteiro *et al.*, 2011). However, the high water content (70–90 %) and buffer capacity of manure have a positive effect on AD stability. Consequently, most of the farm-type anaerobic digesters are operated manure together with organic wastes (called co-digestion) (Rico *et al.*, 2014).

Mixing substrates for co-digestion have many advantages including environmental, technology and economic benefits when contrasted with a single substrate processing (Brown and Li, 2013). Over the last years, several authors investigated the effect on the performance of the AD process by simultaneously treating cattle manure as well as wastes containing high levels of organic matter or energy crops (Yangin-Gomec and Ozturk, 2013). This co-digestion method, if co-substrate is used at the appropriate rate, can give interesting results because the synergistic impact indicated by different organic substrates when processed simultaneously (Rao and Baral, 2011). For optimal carbon-to-nitrogen ratio and pH diverse substrates are mixed for best biogas production (Khan and Martin, 2016). One of the techniques for the economic sustainable of biogas systems is to improve their biogas production about 50 % by co-digesting the animal manure with more degradable wastes containing high levels of organic matter as long as such organic wastes are reachable in the digester region (Khan and Martin, 2015).

The benefits of co-digestion are evidently and clearly inexpensive, and to a simple technology and to remember ecological advantages, such as reduction in concentration toxic compounds, arranged demand of nutrients, synergistic effects of anaerobic bacteria, improved biodegradable substrate loading, increased in biogas production, hygienic stabilization and enhanced rate of digestion (Sosnowski*et al.*, 2003), adjustment percent of moisture and pH, supply of buffer capacity. All these benefits are very important for stability and performance of the anaerobic process (Esposito *et al.*, 2012). Organic-rich wastes and residues include a considerable amount of biodegradable organic carbon. The range of biogas generation from several biodegradable waste is stated as 0.20–1.11 m3 kg-1 of dry matters (CH_4 content of 57–69 %); e.g., biogas yield per unit weight of dry matters is stated as 0.31 m3 kg-1 for the cattle manure (Yangin- Gomec and Ozturk, 2013).

2.6.Pretreatmentof Feedsock

Wastes rich in lignocellulose (e.g., forestry by-products, straw) or keratinase (e.g., waste from poultry, meat, and fish industries) and wastewater sludge have significant biogas potential. However, the complex floc structures of microbial cells in sewage sludge and the recalcitrant structure of lignocellulose make hydrolysis the rate-limiting step in AD systems. Pretreatment is a well-proven approach to improve degradation of such waste and it involves the removal of non-biodegradable materials, providing effective size and uniform feedstock material and removal of such material decreases the quality of digestate (Maria and Anna, 2019).

The technique used for pretreatment depends on the type of substrate and varies to a wide degree of methods. Common pretreatment strategies comprise physical (e.g., heat/pressure, irradiation, ultrasonic), chemical (e.g., acids/bases, ozonation, oxidation), and biological (addition of fungi/bacteria/enzymes under aerobic or anaerobic conditions) methods (Maria and Anna, 2019). The primary feedstock pre-treatment categories are shown in Figure 2.3.

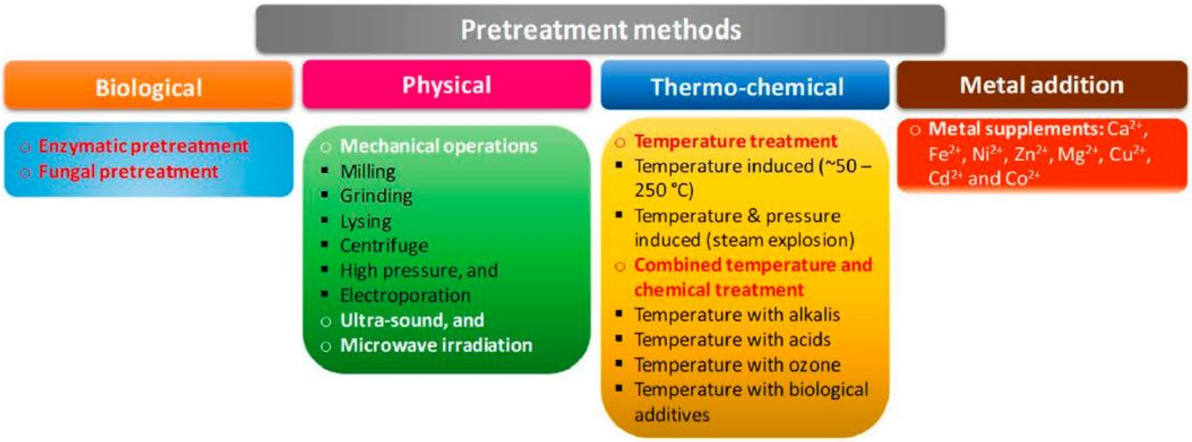

Fig.2.3. Primary feedstock pretreatment categories - modified from Ref. (Carrere *et al.*, 2016)

Physical pretreatment, unlike thermochemical pretreatment, does not require the addition of any external compounds but uses physical techniques such as physical operations (milling, grinding, lysing, centrifuge, high pressure, electroporation, ultrasound, and microwave irradiation). Mechanical pretreatment is a widely used physical method that improves bioconversion effectively, particle densification and distribution, flow properties, porosity, bulk density, and the overall conversion of lignocellulosic biomass into biogas without producing any toxic side streams (Shiplu*et al.*, 2019). Particle size reduction not only increases the rate of enzymatic degradation, it can also reduce viscosity in digesters (thus making mixing easier) and can reduce the problems of floating layers. All particle size reduction is helpful, but a particle size of 1 to 2 mm has been recommended for effective hydrolysis of lignocellulose (Schell & Harwood, 1994).

The general concept of pretreatment, therefore, is that it should improve the accessibility of the material to microbial degradation by disrupting the structure, changing the biomass porosity, and reducing the particle size to enhance the surface area that can be attacked. Many studies have investigated the effect on methane yield of pretreatment of various materials and many methods have shown improved process efficiency following pretreatment (Maria and Anna, 2019).Employing milling, such as screw press extrusion, results in an increase of up to 30% in the methane yield from dip litter (a combination of a solid fraction and straw) manure was accomplished. The combination of milling (⩽2 mm) with fungal pretreatment was observed to achieve a profound methane yield increase of up to 160% (Shiplu*et al.*, 2019).

2.7.Critical Factors Affecting Biogas Yield

Many operating parameters, individually or together, may influence the performance of anaerobic digestion towards biogas or digestate yield and quality maximization. In order for a biogas process to be effective and productive, therefore, there are a number of parameters that have to be optimized. The most preferred method of optimizing an anaerobic digestion plant often relies on how carefully the crucial parameters such as temperature, C/N ratio, pH, organic loading rate, hydraulic retention time, trace elements and pressure, are chosen.

2.7.1. Temprature

Temperature strongly affects the microbial community structure and thus also process performance and stability (Maria and Anna, 2019). The AD temperature depends on the tolerance of the microorganisms and is categorized as psychrophilic, mesophilic, thermophilic, or extremophilic established at temperature ranges of 4–25 °C, 30–40 °C, 50–60 °C, and >65 °C, respectively (Shiplu*et al.*, 2019). There is a close relationship between the biogas fermentation process and the temperature of the reactor. The higher the temperature, the more biogas is produced but when temperature is too high this can cause the metabolic processes to decline as the microorganisms cannot tolerate the conditions and enzymes become degraded (Marchaim, 1992). Although anaerobic digestion can be achieved with temperatures lower than 20 °C, below 10 °C, the degradation is three times slower than the normal mesophilic process with methanogenesis becoming the rate-limiting step (Shiplu*et al.*, 2019). With higher temperatures, such as those between mesophilic and thermophilic regimes, anaerobic digestion tends to have a perfect environment for biological degradation resulting in high rates of hydrolysis and consequently high biogas yields. The correlation between the reaction rate and the biological process with temperature is typically represented by Equation (12) below (Neshat*et al.*, 2017):

$$K_T = K_{20}\Theta^{T-20} \text{ .... (12)}$$

whereK_T denotes the reaction rate constant at temperature T, k_{20} represents the reaction rate constant at 20 °C, θ is the temperature activity constant, and T denotes the temperature.The rate of biochemical processes generally increases with temperature. As a rule of thumb, the rate is doubled for every 10 degree rise in temperature within certain limits (Q10= 2) (Jemal, 2016).

The methane production amount in thermophilic AD is almost identical to mesophilic AD, but higher temperatures improve the production rate, and reduces the requirement of high operational HRT and consequently reducing the reactor size. However, thermophilic digestion system can be energy intensive, unstable, and sensitive to inhibition, which, in addition to the robustness of the operation, is why mesophilic processes are presently the most preferred techniques implemented at industrial scale AD (Shiplu*et al.*, 2019).

For a given operational temperature type, the fluctuation of a few degrees of temperature can have a severe impact on methane yield, as microorganisms adjust to one certain temperature and readaptation corresponding to a different temperature requires an alternated microbial structure. Noticeably, a variation in mesophilic temperature of ±4 °C and thermophilic temperature of ±1 °C was found to result in a sharp decrease in biogas yield (Shiplu*et al.*, 2019).Experience from large-scale operations shows that constant temperature fluctuations should not exceed ±2–3°C in order to avoid instability (Maria and Anna, 2019). In order to avoid process collapse, temperature changes should be carefully monitored, both when increasing and decreasing the operating temperature. A temporary reduction in feed rate and prolonged retention time can be required in the event of disturbance during step-wise temperature changes (Maria and Anna, 2019).

2.7.2. pH (Hydrogen ion Concentration)

The pH value of AD system is very important factor as methanogenic bacteria are sensitive to acidic environment by which growth and gas production is inhabited. The pH value varies along the different stages of anaerobic digestion (Lee and Jahng, 2011). The pH variation is caused by volatile fatty acids, bicarbonates, alkalinity and CO_2. Chemicals like NaOH and $NaHCO_3$ are used to maintain the pH value (Muzaffar*et al.*, 2016). During the acetogenesis, the pH value declines below 5 causing mass death of methogens. This would lead to acid accumulation and digester failure. Constant pH is vital for starting the digestion, which is maintained by buffers like calcium carbonate or lime (Ray *et al.*, 2013). The methane-producing bacteria require neutral to slightly alkaline environment (pH 6.8–8.5). The hydrolysis and acetogenesis occur at pH between 5.5 and 6.5, respectively (Muzaffar *et al.*, 2016). A variation of pH in the magnitude of 0.5 can result in a substantial change in microbial metabolism influencing reaction kinetics and produced gas yield. Although feasible anaerobic digestion can be achieved within the pH of

5.5 and 8.5, methanogens are highly sensitive to the pH change and generally optimize at a pH of close to 7 (Shiplu*et al.*, 2019).

A pH under 6.3 or over 7.8 can adversely affect methanogenesis with a tendency of process failure to occur (Liu *et al.*, 2008). However, unlike methanogenesis, the other processing steps (e.g., hydrolysis and acidogenesis), can potentially optimize between pH of 5.5 and 6.5 (Shiplu*et al.*, 2019). With regard to the intermediates, specifically the VFAs produced during the acidogenesis if not metabolized into products, the pH can be significantly lowered (<3), eventually leading to process collapse. Process disturbances can also result due to high alkalinity caused by the formation of total ammonium nitrogen (TAN) during hydrolysis. Generally, the pH range of extreme acidic (~3 or less) or extreme alkaline (~12 or higher) can be inhibitory to the acidogenesis and limit the rate of hydrolysis (Shiplu*et al.*, 2019).

To control pH fluctuation and to operate AD process at the desired pH range, modern biogas plants are often installed with an automatic pH controller. The primary objective of the controller is to help balancing the system buffer (alkalinity) by adding an appropriate neutralizing agent at an appropriate concentration when necessary. Strong bases (e.g., NaOH) or carbonate salts (e.g., Na_2CO_3 and $NaHCO_3$) and acids (e.g., HCl) are primarily the components used as chemicals to achieve a pH increase or decrease. Moreover, indirect pH control can be applied by manipulating operating parameters including a reduction in organic loading rate, digestate recirculation, HRT variation and co-digestion (Shiplu*et al.*, 2019).

The controlling of pH, whether achieved by the direct addition of a buffer or by the manipulation of the operating conditions, requires a careful and sophisticated application to avoid disturbances to methanogenesis and further to biogas yield. Robust controlling of pH by incorporating multiple parameters towards an optimum methane yield using sensor technologies is a growing field of research in AD (Shiplu*et al.*, 2019).

2.7.3. Carbon to Nirogen (C/N) Ratio

It is important to note that just like any other organisim, methanogens need a number of macro- and micronutrients in order to grow. The most important macronutrients are nitrogen (N), phosphorus (P) and potassium (K). Nitrogen is used by bacteria to produce proteins. The nitrogen content is often quoted in relation to carbon, as this gives an indication of whether there is sufficient nitrogen available for bacteria.

The ratio of C and N plays a crucial role in anaerobic digestion. The carbon acts as energy source and nitrogen serves to enhance the microbial growth by providing an essential element for synthesis of amino acids. The gas production could be low due to high C/N ratio by rapid consumption of nitrogen. On the other hand, low C/N ratio causes ammonia accumulation. It has been reported that very high C/N ratio in an anaerobic digester can result in a too low nitrogen concentration for microbial growth (Banks *et al.*, 2013) as well as result in the consumption of nitrogen by the methanogens for protein production while some leftover carbon will remain unreacted, thereby leading to low biogas generation (Chandra *et al.*, 2012). In contrast, a low C/N ratio can lead to ammonia nitrogen accumulation which can result in the digestion process inhibition (Banks *et al.*, 2013). Optimum C/N ratio can be achieved by mixing substrate of low and high C/N ratio (Khalid *et al.*, 2011). C/N ratio between 20 and 30 provide sufficient nitrogen for anaerobic process (Weiland, 2006).

2.7.4. Retention Time

It is defined as the time during which feedstock remain in the reactor (Muzaffar *et al.*, 2016).Retention time is an important parameter used for design and optimization of anaerobic digestion. It refers to both hydraulic retention time (HRT) and solid retention time (SRT). HRT represents the retention time of the liquid phase, whereas SRT denotes the retention of the microbial culture in the digester. In an anaerobic reactor system where the feedstock and microbial mixed cultures are present at the same phase, the HRT equals SRT. AD of food waste, kitchen waste, and municipal solid waste are the examples for which HRT is essentially SRT and vice versa, while for the substrates like waste activated sludge and primary sludge, the interaction between solids and microbial cultures are biphasic making HRT and SRT different (Shiplu*et al.*, 2019). HRT and SRT are typically represented by Equations (13) and (14) (Yoon *et al.*, 2004):

$$HRT = \frac{V}{Q} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (13)$$

$$SRT = \frac{V.X}{Q_x .X_x} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (14)$$

where, V: Individual reactor volume [m^3]; Q: Influent flow rate [m^3/d]; X: Mixed liquid suspended solids in an individual reactor [mg/L]; Q_x: Excess biosolids removal rate [m^3/d]; X_x: Mixed liquid suspended solids in excess biosolids flow [mg/L].

It depends upon the type of substrate, environmental conditions, and intends use of digested material (Muzaffar *et al.*, 2016). In general, the choice of HRT differs based on feedstock composition, reactor volume, processes, and temperature. Substrates rich in starch and sugar can be easily digested, and as such, hydrolysis is less or not required, resulting in shorter retention times, while longer retention times are required for fiber and cellulose plant matter as hydrolysis of these substrates is slow and rate limiting. High reactor temperature increases the decomposition rate and consequently lowers HRT, which is why most thermophilic reactors are generally operated at a lower HRT than mesophilic reactors. Comparing two HRT types, shorter HRT risks bacterial mobilization including the build-up of higher molecular weight VFAs and consequently elevated stress to the methanogens. Conversely, a longer HRT increases the digester size. Therefore, the optimal operational HRT is usually neither too long nor too short and in the majority of cases lies between 10 and 25 days, although a very high HRT in the order of 50–100 or more days may be needed for digesters operated in colder climates (Shiplu*et al.*, 2019).

A short HRT and a high OLR are often desirable in commercial biogas production plants, since they allow for high-quantity waste treatment and high biogas production (if the AD can maintain efficiency). However, SRT should exceed the microbial doubling time of prevailing microorganisms, in order to avoid washout of the consortium and thus process collapse. Immobilization of microorganisms through inclusion of support material or by allowing the formation of granular sludge, flocks, or biofilms is a strategy used in high HRT systems to support and maintain organisms with lower growth rate than the solid retention time (Maria and Anna, 2019).

2.7.5. Organic Loading Rate (OLR)

It is the biological conversion of capability of a reactor. It determines the amount of volatile solids that is feasible as the input to the anaerobic digester (Muzaffar *et al.*, 2016).The amount of raw-material added to an anaerobic digester per day per unit volume is called the organic loading rate, and is typically expressed as Equation (15):

$$OLR = \frac{C}{HRT} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (15)$$

where C is the feed concentration in g·VS/L, and HRT is the hydraulic retention time.

OLR is an essential controlling parameter in AD, and its deliberate variation can determine the degree of digestion for a broad level of influent input. In a typical anaerobic digester application, a high operating OLR is usually preferred, as this allows enriched bacterial species, reduced reactor sizes, lowered heating requirement and a reduced investment cost (Shiplu*et al.*, 2019). Over loading may cause accumulation of fatty acids, acts as inhibitor, and results in low biogas yield. It would cause proliferation of acidogenesis, decrease pH, and mass death of methogenic bacteria (Muzaffar *et al.*, 2016). According to the existing literature (Nagao *et al.*, 2012), optimal OLR and SRT were reported as 22.65 kg VS/m^{3}d (160 h) for hydrogen fermentation and 4.61 kg VS/m^{3}d (26.67 days) for methane fermentation digester.

2.7.6. Dry Matter Content

The adjustment of total solid content helps in bio-digesting the material at the faster rate, and also in deciding the mixing of the various crop residues, weeds, and plants etc. as feed stocks in biogas digester (Rai, 2004). The production of biogas is inefficient if the fermentation materials are too dilute or too concentrated. With too little water, the activities of the micro-organisms will be affected and the quantity of biogas produced will be reduced. The dilution should be made to maintain an optimum total solid content. If the feed to the digester is too diluted, the solid particles will settle down into the digester and if it is too thick, the particles impede the flow of gas formed at the lower part of digester.There is also higher risk of scum formation at the top of the slurry layer and, in both case, gas production will be less than optimal (Jan and Felix, 2010).

For bacteria to be able to degrade the material, the dry matter content must not be higher than around 50%. In a biogas plant, however, it should only be around 8-10%, if it is to remain liquid enough to be pumped. A slightly higher level can be tolerated in special reactor types with a direct feed line (Jemal, 2016).

2.7.7. Addition of trace elements and Toxicity

Trace element deficiency can severely limit microbial activity and cause accumulation of fatty acids, process instability and decreased methane yield from food waste, slaughterhouse waste, crop material, stillage, and animal manure, when used as a single substrate or as co-substrate. In this regard, it is important to consider the level of sulfide, which is primarily formed through protein degradation (Maria and Anna, 2019). Sulfide forms complexes with metals, which decreases the bioavailability of trace elements essential for microbial activity (Pham *et al.*, 2016). In addition, temperature has been suggested to impact nutrient bioavailability and nutrient requirements (Maria and Anna, 2019).

Small quantity of mineral ions (e.g., sodium, potassium, calcium, magnesium, ammonium and sulphur) stimulates the growth of bacteria, while very heavy concentration of these ions leads to toxic effects. For example, presence of NH_4 from 50 to 200 mg/l stimulates the growth of anaerobic microbes, whereas, its concentration above 1500 mg/l produces toxicity. Similarly, the trace elements such as cobalt, nickel, iron, molybdenum, and tungsten are essential trace elements, especially for acetogenic and methanogenic microorganisms (Azim *et al.*, 2017) but their higher concentration has toxic effects (Moharao, 1975). Detergents including soap, antibiotics, organic solvents etc. also inhibit the activity of methane producing bacteria and hence addition of these substances in the digester should be avoided (Moharao, 1975).

2.8.Biogas Utilization

Various energy services can be obtained from biogas and its best use depends on the type of the energy needs. Traditionally biogas has been used as fuel for boilers but generally the best usage depends on several factors such as amounts of biogas produced, energy costs, energy demand of the plant and incentives (Funda, 2011). Normally in the biogas plants, during the digestion process, more gas is generated than needed in order to support the process. As illustrated in figure 2.4 below, this excess biogas is a potential to use for other functions.

2.8.1. Biogas for Heating

Utilization of heat produced from the biogas has a direct effect on the economy of a biogas plant. The gas burns with a clean, clear flame. No soot and slag are present in boilers and other equipment, and the plant lasts longer (Energy map, 2011). According to Deublein and Steinhauser (2008), the heat could be used for heating swimming pools, industrial plants and greenhouses,warmth transformation in cold,treatment of products,cleaning and disinfection of the milking equipment, and heating stables as for the breeding of young animals.

2.8.2. Biogas for electricity generation

Biogas is commonly used for power generation. This electricity can be used for operation of plant, for sale or credit for the local power utility. Electricity generation with biogas can be produced from engine generators, turbine generators, micro turbines and fuel cells (Khanal, 2008).

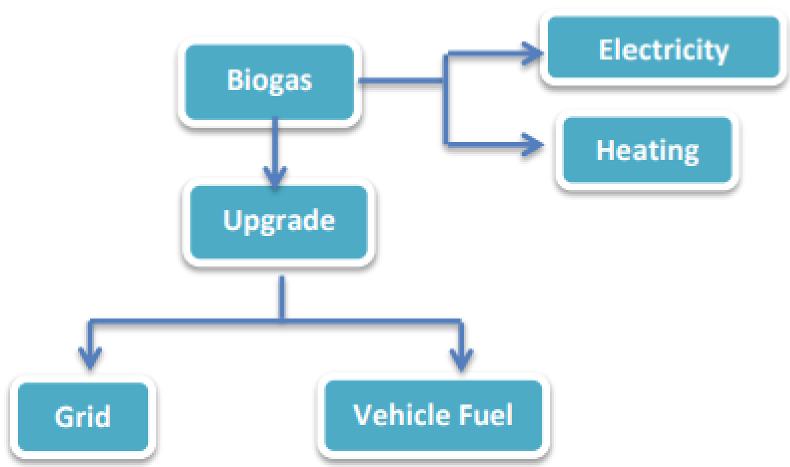

Figure 2.4: Avenues for biogas as modified from (Khanal, 2008).

2.8.3. Biogas as vehicle fuel

Biogas must be transformed to natural gas quality for use in vehicles. This process requires the removal of particulates, carbon dioxide, hydrogen sulfide, moisture and other contaminants (Khanal, 2008).

When biogas is used as vehicle fuel, it gives the lowest emissions of carbon dioxide and particles of all the fuels currently on the market (Energy map, 2011).In Sweden, according to Funda (2011), using biogas as vehicle fuel is more convenient, since there is no need to use biogas for electricity generations or heating. Replacement of petroleum fuels with biofuels have been addressed by the European Commission in the directive 2003/30/EG where the following targets were set; 2% biofuels by the end of 2005 and 5,75% biofuels by the end of 2010 (Jönsson, 2004).

Biogas utilization as fuel is in general less problematic and cheaper in comparison with the cost of feeding biogas into the natural gas network. The Swedish government has set the future goal to import oil and natural gas (Funda, 2011). In many cities it is typical to see "green" cars, working with renewable fuels. Today, between Linkoping and the city of Vesternik, situated 80km away, city buses, taxis, transporters, refuse collectors, private vehicles and even the train are driven by biogas (Deublein and Steinhauser, 2008).

2.8.4. Digester gas for cooking

Biogas is an appropriate alternative to the traditional solid and gaseous cooking fuels mostly used by communities in the developing world. Biogas is a clean-burning, renewable fuel that contains 50-60% methane and can be used in household cooking applications (Mohanty*et al.*, 2016). The biogas produced from anaerobic digestion of waste streams can be used through conventional low pressure gas burners for cooking (Khanal, 2008).Globally, roughly 50 million biogas systems are being used for cooking, mostly in Asia and especially in China and India (Putti *et al.*, 2015). Compared to other cooking fuels and technologies, therefore, biogas stoves offer a superior performance and are highly rated in terms of health, economic and climate impacts.

3. Materials and Methods

3.1.The Study Area

Before conducting the actual experiment, selection of appropriate study area was made. Accordingly, the study was conducted at School of Chemical and Bio Engineering laboratory in Addis Ababa University Institute of Technology (AAiT) located in Addis Ababa, the capital of Ethiopia.

3.2.Materials

3.2.1. Feedstock (Inputs)

The substrates used as feed stock materials for the generation of biogas in the laboratory were samples of cow dung, grass hay and different combinations of cow dung with grass which were set as different treatments. The required quantity of dung was obtained from private dairy farm located in Bella, Yeka sub city whereas the grass hay were found from Shola market, Yeaka Sub city; Addis Ababa which is supplied as cattle fodder in market. The dung samples collected were maintained in a refrigerator at 4°C to avoid microbial action until further use. Using a graduated cutting machinefrom Rural Energy Development and Technology Transformation Center Experimental Workshop under Ministry of Water and Energy, Addis Ababa, the grass hay were crushed into 2mm sized small pieces and used for digestion (physical pre-treatment to reduce particle size) as reported by Palmowski and Muller (1999). To start up the digestion process in the laboratory 1% v/v inoculum (cattledunginoculum) from active anaerobic digester found around Genesis in Bishofitu city, Oromia, was collected using plastic jar and applied as inoculum.

3.2.2. Equipment and Chemicals

Equipment including plastic bag, digital balance, oven, furnaces, crucibles, corks (stopper), bottles, water bath, graduated air bag,graduated cylinders, graduated buckets, pressure meter, gas analyzer, and PH meter were used during wet anaerobic digestion experimental analysis. Besides, water, weak acids and weak bases were chemicals used during the experiment.

Other materials used in this research work include crucible, laboratory oven, desiccator, electronic precision balance, dish tongs, wash bottles, bottle reactors, and muffle furnace.

3.3.Methodology

3.3.1. General Procedure

Laboratory-scale digestion experiments for the determination of biogas yield and the subsequent evaluation of the underlying biogas potential was performed using a discontinuous (batch) approach. The quantity of substrate and inoculum measured in each of the bio-digesters (bottles in this case) were measured in line with German Standard Procedure (VDI 4630, 2016). This procedure was preferred because it is an established test system which directly measure the sum of biochemical parameters, availsstandard protocols for test methodology, has many reference values and long-term experience, needs limited effort compared to continuous tests and is a substrate independent methodology (Weinrich*et al.*, 2018). Quantitative evaluation of the results gained in batch anaerobic digestion tests included the following steps: standardizing the volume of biogas to normal litres (1N); (dry gas, t_0=273 K, P_0=1,013hPa) and correcting the methane and carbon dioxide contents to 100% (VDI, 4630). Readings were analysed using Microsoft Excel spread sheet together with one way ANOVA.

3.3.2. Physico-Chemical properties of the feedstock

i) Total Solids (TS)

Freshly collected samples; inoculum, dung and grass hay, were weighed in a crucible using electrical balance (digital weight measuring device). The crucibles were placed inside an electric hot air-oven maintained at 105ºC.The crucibles were allowed to remain in the oven for 24 hours, then taken out, cooled in a desiccator, and weighed. The weight of the sample, which was left in the oven, gave the total solids and was represented by the formula.

$$\%TS = \frac{A-B}{\text{Weight of fresh sample}}\ x\ 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (16)$$

Where: % Ts = percentage of total solid

A = total mass of dried sample (mass of dry sample + mass of crucible)

B = mass of crucible

ii) Volatile Solids

The TS obtained was ignited at 550°C in furnace for three hours as indicated in (John *et al.*, 2000) to determine the volatile and fixed solid content of the sample. Equation 17 was employed to calculate the percentage of volatile solid content of the total solid (TS).

$$\%VS = \frac{D-C}{D}\ x\ 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (17)$$

Where: % VS = percentage of volatile solid

D = total mass of dried sample

C = total mass of fixed solid (mass of ash& fixed carbon + mass of crucible)

iii) Ash Content

The ash content refers to the weight of the mineral content of biomass remaining after dry oxidation of the total solids of a given sample at 550^0C(John *et al*., 2000). To determine the ash content, firstly the total solid was determined according to Equation 16. The obtained total solid (TS) was ignited at 550^0C in a muffle furnace for 5 hours to determine the fixed solid (ash) content of the sample (John *et al.*, 2000). These samples were cooled in desiccators to constant weight. The difference in weights in each case gave the volatile solids and the residue after ignition was the fixed solid.

$$\% \text{ Ash content} = \frac{G-F}{E-F} \times 100 \text{ ....} (18)$$

Where;

E= Weight of dried residue + crucible in g

F= Weight of only crucible in g

G= Weight of residue after ignition + weight of crucible in g

iv) Carbon to Nitrogen Ratio (C/N)

a) Carbon Determination

The carbon content of the feedstock was measured by considering the volatile solids content that expressed as a percentage and the total carbon content obtained from volatile solids data using anempirical equation 19 (Elias *et al.*, 2011).

$$\text{Carbon \%} = \frac{VS\ \%}{1.8} \text{ ....} (19)$$

b) Nitrogen Determination

The Kjeldahl method was applied to determine the total nitrogen content of the feedstock. This method has three main steps; namely, digestion step, distillation step and titration step. Two gram sampled and 15 ml of concentrated H_2SO_4 was added in reactor tube and mixed carefully.Then 5 ml of H_2O_2 was added step by step. Violent color due to reaction was observed. As soon as the violet reaction has ceased, the tube was shaken by hand. Then the digester switched on and waits until it reaches 370^0C. As the digester reached at this temperature, the rack was placed in it and the digestion continued for about 4 hours until clear solution was observed. Then the tube in the rack was transferred to the fume hood for cooling.

About 50 ml distill water was added and shaken by hand to avoid sulphate precipitation in the solution. At this time 25 ml 40% NaOH solution was added into digested and diluted solution. Then 250 ml conical flask containing 25 ml of boric acid and 25 ml of distilled water was placed under the condenser of the distiller for about 10 minutes until a total volume become between 200 ml to 250 ml. Finally, the solution was titrated using 0.1N HCl to a reddish color and the following calculation computed to get $\%N_2$.

$$\%N_2 = \frac{V_{HCl} \text{ in L} * N_{HCl}(\text{ca.}0.1) * 14.0 * 100}{W_0} \text{ ……………………….. (20)}$$

Where : %N = percentage of nitrogen

N = normality of HCl (used often is 0.1N)

V = volume of HClin L consumed to the end point of titration,

Wo = sample weight of dry matter basis and 14.0 = the molecular weight of nitrogen

Hence, the carbon to nitrogen ratio for each treatment will be calculated by dividing the % carbon with % nitrogen (Elias *et al.*, 2011).

3.4.Experimental Design and Set Up

The experimental design and set up for this particular experiment was based on German Standard Procedure (VDI 4630, 2016). The main features, as indicated by Weinrich*et al.*, 2018, of this procedure include:

- Glass bio-digester is the preferred material for the reaction,
- Manometric or volumetric (water displacement, flow meter, syringe extension) methods were used for measuring the amount of produced biogas,
- Batch bio-digesters with volumes vary from 0.1 to 2 L flasks would be used,
- Incubation of anaerobic digestion tests usually conducted under mesophilic (37 °C ± 2 °C) or thermophilic (55 °C ± 1 °C) conditions in climatic chambers or water baths,
- The batch test should be continued until the daily biogas production equals 0.5 % of the total biogas production on three consecutive days.

3.4.1. Experimental Design

The amount of substrate and seeding sludge (inoculums) weighed into the fermentation bottles were determined in accordance to German Standard Procedure VDI 4630 using the Equation (21):

$$M_s = \frac{M_i C_i}{2Cs} \quad \text{..} \quad (21)$$

Where:

M_s = Mass of substrate (g)

M_i = Mass of inoculum (g)

C_i = Concentration of inoculum (%)

C_s = Concentration of substrate (%)

The total solid (TS), volatile solid (VS), and the C:N ratio of the feed-stocks (cattle dung, grass hay and inoculum) had been determined before the anaerobic digestion process began. The process of anaerobic digestion for the generation of biogas was then conducted by varying the ratio of the substrates (cattle dung &grass hay) in four treatments with two replications for each digester, as, T_0 (inoculum), T1 (inoculum& 100% cattle dung), T2 (inoculum, 50% cattle dung &50% grass hay), and T3 (inoculum, 75% cattle dung & 25% grass hay) keeping temperature at 37^0C ± 2^0C in water bath set up. Inoculums were filled to each of the bio-digesters (only as control) to know the amount of biogas produced which would be deducted from biogas produced from the treatments. In the laboratory the water content for each sample was determined using Equation 21 to meet the recommendation for better biogas production as reported by Nijaguna (2002), i.e., a total solid (TS) of 9% in the fermentation slurry. The amount of the feed-stocks and water added to each digester is presented in Table 3.1.

The data including: date, time, volume of the gas (ml), gas temperature (^{0}C) and air pressure (mbar) were taking periodically throughout the retention period. Standard temperature (0^0C) and standard pressure (1,013.25 mbar) were employed to compute the biogas and methane yield. Gas factor was calculated and Enwuff equation was employed to calculate the fresh mass biogas and methane released; and the organic dry matter (oDM) biogas and methane released were calculated periodically throughout the retention period. Standard condition of temperature (273.15 K) and pressure (1,013.25 mbar) was used to convert the volume of gas formed. Equation (22) was used to calculate the gas factor (Entwurf, 2004).

$$F = \frac{(P - P_{H_2O}) X\, To}{(t + 273.15) X\, Po} \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (22)$$

Where:

F = Gas factor

T_o = 273.15 ^{0}C (Normal Temperature)

t = Gas Temperature in ^{0}C

P_o = 1013.25 mbar (standard pressure)

P = Air pressure.

Water vapour pressure (P_{H_2O}) depends on the gas temperature and amounts to 23.4 mbar for 20^{0}C. Equation 23 describes the water vapour pressure as a function of temperature and depicts the range between 15^{0}C and 30^{0}C.

$$P_{H2O} = y_0 + a.e^{b.t} \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (23)$$

Where:

y_o = - 4.3905

a = 9.762

b = 0.0521

t = ambient temperature (35^{0}C)

The normalized biogas volume is given as

Biogas [Nml] = Biogas [ml] x F ………………………………….... (24)

Normalised by the quantity of biogas released, the total gas that took off the control batch was calculated using Equation 25.

$$\text{Biogas [Nml]} = (\text{Biogas [Nml]} - \text{Control [Nml]}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots (25)$$

On weight basis, mass of biogas released in standard litres/kg FM (ml/g FM or 1m^3/t FM) fresh mass was calculated using Equation 26.

$$\text{Mass of Biogas Yield} = \sum \frac{Biogas\ [Nml]}{Mass\ [g]} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (26)$$

Finally, the oDM biogas produced (as a percentage of volatile solid (VS)) and the oDM methane yield weredetermined using Equations 27 and 28.

$$\text{oDM biogas} = \sum \frac{Biogas\ [Nml]}{Mass\ [g]} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (27)$$

$$\text{oDM Methane Yield} = \frac{\text{oDM } biogas\ yield\ X\ \text{CH4corr.}}{100} \ldots\ldots\ldots\ldots\ldots (28)$$

Where the corrected methane amount is determined using Equation 29.

$$CH_{4corr.} = \sum \frac{\text{CH4[vol]} X\ 100]}{(Mass\ [g] + \text{CO2 [vol\%]}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (29)$$

3.4.2. Digester Composition (Feed-stock content, water content and inoculation)

To initiate or start up the digestion process, inoculum containing methanogenic bacteria was added for all treatments in 500 ml of glass bottle digesters. For the purpose of this study the amount of inoculum (digestate) in digesters was fixed to be 157 ml (taking the digesters volume in to consideration) and then, inoculum alone (T0), inoculum with freshcattle dung(T1), and the mixture of inoculum, freshcattle dung and grass hay with different ratio (T2&T3) was added to 500 ml of glass bottle digesters.

According to Nijaguna (2002), a wet anaerobic digestion process have an optimal total solid (TS) content of 5 to 10%. When the TS values were above the optimal value water was add to obtain the optimum concentration of 9% TS, according to Nijaguna (2002). For this study the water content were adjusted according to the indicated optimal condition as recommended using Equation 30. The amount of water added was then determined by the formula and added after determining the moisture content and the total solid of inoculum, freshcattle dung and grass hay.

$$\frac{\mathrm{mTS}}{\mathrm{A+B}} = 9\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (30)$$

Where mTS = mass of total solid

A= mass of sample; x

B= mass of water;

The content of each digester were summarized in table 3.1 as follows.

Table 3.1: Composition of each batch test

Treatment	Condition/ Temperature	Substrate		Water (ml)	Inoculum (ml)	Total mass (ml)
		Cattle dung (g)	Grass hay (g)			
T0	37 ± 2^0C	-	-	-	157	157
T1	37 ± 2^0C	66	0	277	157	500
T2	37 ± 2^0C	33	6	304	157	500
T3	37 ± 2^0C	49.5	3	290.5	157	500

3.4.3. ExperimentalSet Up

Batch tests require gastight equipment (no oxygen inflow and fugitive biogas outflow) and typically consist of a digester (reaction vessel) and gas collection/measurement system. The experimental set up for this study on batch digestion consists of glass bottle with a cork/stopper. All the eight anaerobic digesters (each test with two replications) were constructed in bench-scale experiments at which the degradation of the substrate was accomplished in sealed glass bottles with a capacity of 0.5 liters. Each bottle was sealed with a rubber stopper/cork having one outlet. The outlet was attached to air tight connector; one branch connect to the digester and the other one was connected to graduated plastic air bag which was used to measure the collected gas by using water displacement method. Then plastic bottle containing stored gas was connected manually to gas analyzer to determine CH_4, CO_2, O_2 and H_2S compositions in biogas (Rajeshwari*et al.*, 2009).

The electronic weighing balance and graduated cylinder were used to determine the mass and volume of cow dung, grass (hay) and inoculum that made up the total solid for this particular fermentation. To maintain an optimum temperature for mesophilic condition a water bath was used at a temperature adjusted for $37^0C \pm 2$ and the digesters effective volume was fully immersed in this bath. Manual agitation was taken place by hand shaking the digester twice in each day to ensure contact between the substrate molecules, nutrients and microbial cells.

A digital pH meter was used to determine the pH of the fermentation slurry (samples) on the first day of the experiment as it is another factor that affects digestion of substrates in reactors. The pH measured in the range of 6.54 to 6.98 for all treatments and this is in agreement with a pH range of 6.25 to 7.5 which is conducive for methanogenic bacteria to function properly as indicated by Rai (2004). Besides, a thermometer and pressure meter was used to determine the daily ambient temperature and pressure so that average temperature and pressure were calculated.

3.5.Biogas yield and quality

The volume of gas produced in the anaerobic reactors was measured by using water displacement method with the aid of graduated vessel.According to Archimedes principle of floatation,the amount of gas produced is equivalent to the amount of water displaced in the water chambers (Mahmoud, 2007).Biogas volumes were measured starting from the fourth day after digestion set up and stayed for 38 days until biogas production was zero for consecutive three days.

Geo Tech gas analyzer from Addis Ababa University (AAiT), School of Chemical and Bio-Engineering laboratory was used to determine the quality of biogas for each treatment. The gas stored plastic bottle was directly connected to the gas analyzer for its composition analysis and the analyzer gives the result as the percent composition of CH_4, CO_2, O_2 and H_2S in the biogas.

3.6.Data Analysis

The average biogas yield and methane percentage of the four treatments, respectively were carried out using mean calculation. The data obtained from the investigation was analyzed using analysis of variance (one way ANOVA) for comparison of means of each response. In addition, the biogas yield, the percentage of methane and the energy equivalent of each treatment were presentedusing graph, bar chart and table using Microsoft Office Excel.

4. Results and Discussions

4.1.Feed-stocks Characterization

In this study the physico-chemical properties; total solid (TS), volatile solid (VS), moisture content (MC), ash content and the C:N ratio were determined for inoculum, cattle dung and grass hay. Accordingly, as presented in Tale 4.1, the total solid contents and moisture contents of fresh cattle dung were 17.67% and 82.33% while its volatile solid and ash content were 84.83% and 15.17%, respectively. For the sun dried grass hay, the total solid and moisture contents recorded were 91.62 and 8.38 while its volatile solid and ash content were 91.26% and 8.74%.

Table 4.1: Characteristics of inoculum and substrates

Parameters	Inoculum (Digestate))	Cattle dung	Grass hay
TS (%)	8.61	17.67	91.62
MC (%)	91.39	82.33	8.38
VS (as % of TS)	73.99	84.83	91.26
Ash (as % of TS)	26.01	15.17	8.74
C	41.11	47.13	50.7
N	2.18	2.46	1.99
C/N	18.86	19.15	25.47

The total solid (TS) and volatile solid (VS) content of substrates affect the performances of anaerobic digestion process. Change in total solids content will lead to change in microbial morphology of AD systems. Volatile solids are portion of percent total solids expected to be converted to the biogas and this supports the fact that most literatures present CH_4 yield in terms of volume of methane per gram VS digested.

The volatile solid content as part of the total solids of the feedstocks; cattle dung and grass hay, were 84.83% and 91.26%, respectively. The higher percentage of volatile solid, in this case the grass hay, indicates that the feedstock contains high amount of organic carbon that can be converted into biogas yield (Bayr*et al.*, 2014). Increase in volatile content increases biogas production and, hence the present investigation revealed that grass hay has the highest volatile solid content and it was the better feedstock for biogas production. On the other hand, cattle dung has relatively lower solid volatile content as compared to the grass feedstock and hence, co-digestion is required so as to yield better biogas production. In this regard, the optimum co-digestion of cattle dung with grass hay will increase its volatile content and hence, the biogas production. This may be due to modification of the physicochemical properties of the dung manure that affects growth and development of fermentative bacteria.

4.2.Carbon to Nitrogen Ratio (C/N)

In this experiment, as shown in Table 4.1, the percent degradation of organic carbon for grass hay was 50.7 and higher than the cattle dung, i.e., 47.13. Similarly, the total nitrogen content available was 2.46 and 1.99 for cattle dung and grass hay, respectively. The carbon content of the feedstock was obtained from volatile solids data using Equation 19, whereas the nitrogen content for the feed-stocks were determined in laboratory.

The results revealed that there are differences in C/N ratio between feedstocks used; 19.15% for cattle dung and 25.47% for grass hay. Table 4.1 shows that the C/N ratio of cattle dung is lower than the required optimum value while the C/N ratio of the grass hay is higher and thus it can serve as a co-substrate to improve the overall C/N ratio of the anaerobic bio-digester feed ingredientbysatisfying the recommendation of optimal C/N ratio for anaerobic digestion ranging from 20 to 30:1 (Banks *et al.*, 2013). Timothy-based grass has been reported to have carbon contents of 46% TS, corresponding to C/N ratios of 26 (Lehtom¨aki*et al.*, 2006). Cattledung has a C/N ratio of 11–14 (Hills and Roberts, 1981; Hashimoto, 1983), and addition of 30–40% of grasshay increased the ratio in the feedstock to approximately 15–25, while the optimum range for anaerobic digestion is 25–32, according to Hills and Roberts (1981). The fact that the co-digestion in T2 (50% dung with 50% grass) higher methane yields were obtained than the co-digestion in T3 (75% dung with 25% grass), even though on the basis of the batch assays the T3 test demonstrated similar co-digestion with different substrate ratio, is explained by an optimum C/N ratio in T2 digester.

4.3.The pH Value

The pH is another important parameter that must be monitored in an anaerobic digestion process. Several studies on anaerobic digestion of waste have shown that the pH of substrates have strong influence on the rate of biogas production (Tengku*et al.*, 2014). The pH of each digester were also measuredat the beginning of the digestion process and evaluated as T0=6.8, T1=6.4, T2=6.7 and T3= 6.9. Previous studies have reported that, methanogens activity is optimum within a pH range of 6.5–8.0 for mesophilic digestion (Rahman and Muyeed, 2010). Accordingly, all reactors or treatments were having pH within recommended range for anaerobic digestion process. The pH of all the treatments came down at the end of the digestion period, i.e., T_0=6.4, T1=5.5, T2=4.7, T3=5.4.It is obvious that at the initial stage of the overall process of biogas production, the pH drops and the growth of methanogenic bacteria is inhibited as a result of the accumulation of Volatile Fatty Acids (VFA) produced by acid forming bacteria. This means the microorganisms responsible for biogas production is inactivated by a lower pH. It can be seen that the pH of all the bio-digesters were not in the range of optimal level of 6.5 – 8.0 suitable for most methanogenic bacteria to function for biogas production.

4.4.Amount and Quality of biogas production

4.4.1. Cumulative Biogas Production

Biogas production and its methane content were measured for about 38 days of digestion period until gas production was stopped. All the experiments were carried out under daily mean temperature range of 37˚C ± 2˚C throughout the period of biogas production. The results showed that the blending of grass hay with the cattle dung affected the total biogas yield and onset of gas production was seen starting from the second day for all digesters except the inoculum.Maximum biogas was produced on the first four days in all the digesters. This was due to the increased availability of the biomass during the initial stages, leading to subsequent growth of the anaerobic organisms. At the beginning of the gas production, the anaerobes present in the organic material became active and began increasing in population to attain the peak of the production followed by drop in gas production due to a reduction in nutrient content (Lee et al., 2014). Daily biogas production from the cattle dung, cattle dung co-digested with grass hay in different ratios is graphically shown in Figure 4.1.

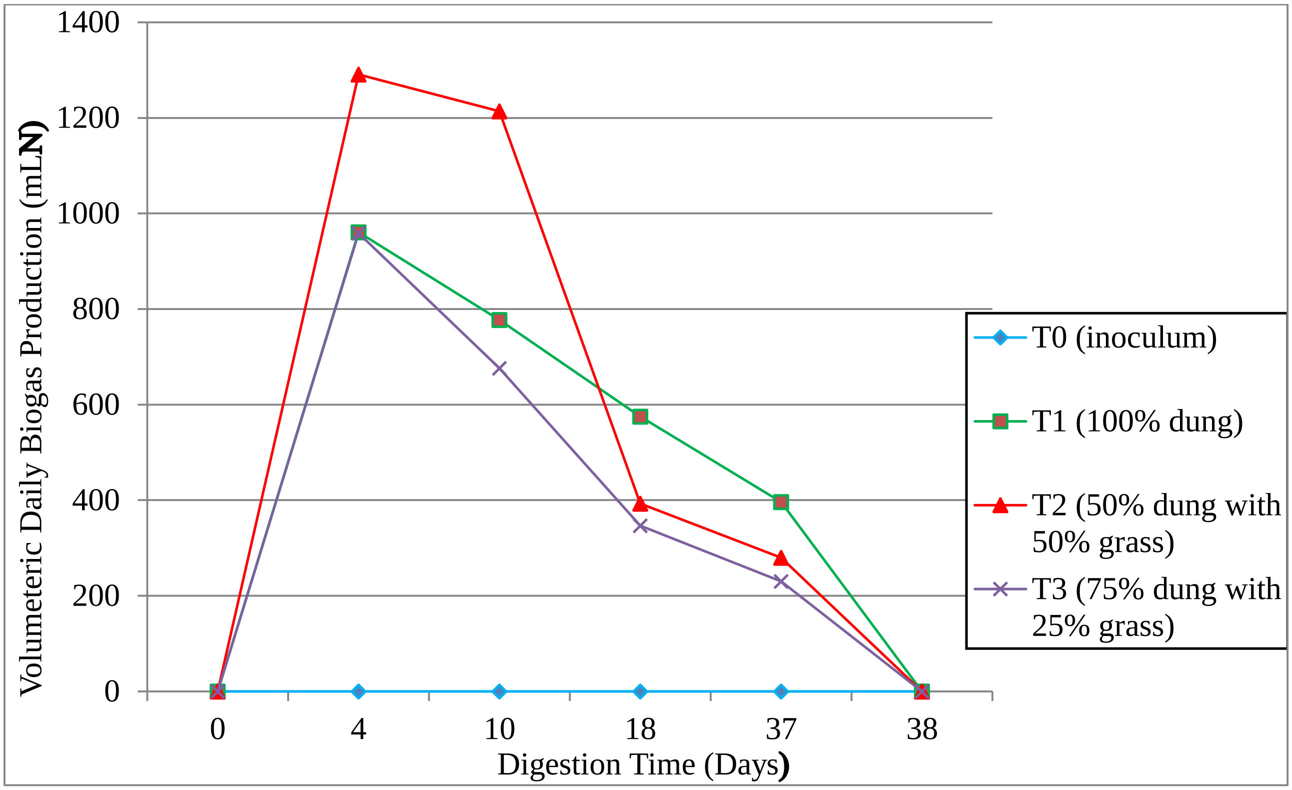

Figure 4.1: Daily biogas production of inoculum, cattle dung, cow dung co-digested with grass in different percentages.

Cumulative biogas production for all reactors over a period of thirty eight(38) days is shown by Figure 4.2. These include the fresh mass biogas yields, fresh mass methane yields, organic dry matter biogas yields and organic dry matter methane yields from the inoculum, cattle dung, co-digestion of cattle dung with grass hay in different mix ratio, respectively. Under mesophilic condition ($37^oC \pm 2^oC$), the fresh mass specific biogas yields fromT0 (only inoculum), T1 (100% cattle dung), T2 (50% cattle dung co-digested with 50% grass hay), and T3 (75% cattle dung co-digested with 25% grass hay) were found to be 0, 41, 81.5 and 61.5 mL_N/g FM respectively while the fresh mass specific methane yields for the same combinations were found to be 0, 24.7, 53.96 and 35.4 mL_NCH_4/g respectively. In the same digester, the specific biogas yields (oDM) of inoculum, cattle dung and cattle dung co-digested with grass hay at different ratios were found to be 0, 273.3, 318.6 and 296.4 mL_N/g oDM while the specific methane yields (oDM) were respectively found to be 0, 165, 211 and 170.8 mL_NCH_4/g oDM when experimented at mesophilic temperatures.

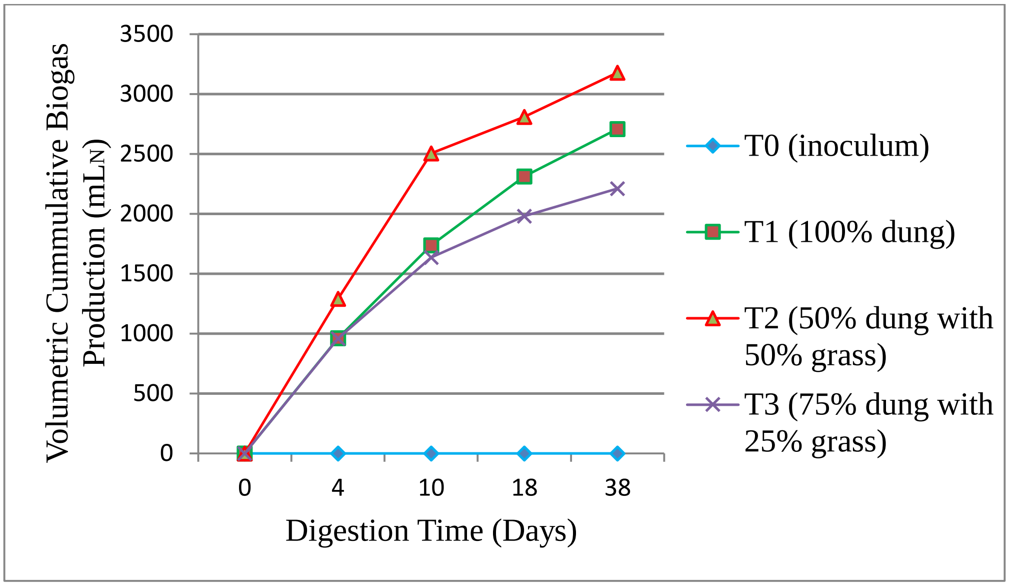

Figure 4.2:Cumulative biogas production of inoculum, cattle dung, cattle dung co-digested with grass hay in different percentages.

It was observed that the T2 produced the highest biogas yield in terms of volumetric, fresh mass and dry matter yielding 3,177 mL_N, 81.5 mL_N/g FM and 318.6 mL_N/g oDM, respectively. The T2 produced the highest volumetric biogas yield of 3,177 mL_N, followed by T1 which produced volumetric biogas yield of 2,709 mL_N and T3 which produced volumetric biogas yield of 2,212 mL_N. Substrates of batch T2 produced 17% and 43.6% more volumetric biogas yield than substrates in batch T1 and T3, respectively.

The amount of biogas with in two treatments (T1 & T3) were 2,709 ml and 2,212 ml, these indicated that the volume of biogas of the treatments were not highly affected by the C:Nand other parameters, but the treatment T2 gives the higher amounts of biogas (3,177 ml) than other treatments. Similarly, T2 produced the highest specific methane yield of 211 mL_N CH_4/g oDM, followed by T3 (170.8 mL_N CH_4/g oDM) and T1 (165 mL_N CH_4/g oDM), respectively. Substrates of batch T2 produced 23% and 28% more specific methane yield than substrates in batch T3 and T1, respectively. The measured specific methane yields for T1 (mono digestion of cattle dung) agree with previous reports of 136-239 mL CH_4g^{-1} of VS (Allen *et al.*, 2013) while the measured specific methane yields from co-digestion of cattle slurries and grass silages which are quite varied with values reported from 140 to 300 mL CH_4g-1 of VS (Lehtomaki*et al.*, 2007; Jagadabhi*et al.*, 2008) were also in line with T2 and T3.

According to this experiment, mono digestion of cattle dung produced lower yield of methane than co-digestion of the cattle dung with grass hay. This is due to the fact that the majority of energy rich substrates have already been eliminated through the digestive tract of the animal (Lehtomaki*et al.*, 2007). Besides, grass silage (hay) is also an excellent feedstock for anaerobic digester (AD) due to its high digestible organic matter content. Wall et al. (2013) found that grass silage has a much higher specific methane yield (400 L CH_4/Kg VS) than that of cattle dungs (239 L CH4/Kg VS). On a fresh weight basis, grass silage produced almost seven times more methane than slurry (107 L CH_4/Kg compared to 16 L CH4/Kg). When cattle dung and grass hay, therefore, are co-digested at the same OLR, increasing the fraction of grass silage in the feedstock should increase the methane yields. The higher specific methane yields in co-digestion compared with digestion of cattle dung alone may also be due to synergy effects owing to a more balanced nutrient composition and C/N ratio in the feedstock, i.e., an optimum condition may be created when the lower C: N ratio values of cattle dung (19.15) mixed with the higher C: N ratio of grass hay (25.47).

The result of biomethane yields from T2 (211 mL_N CH_4/g oDM) is higher than T3 (170.8 mL_N CH_4/g oDM) revealed that the higher the proportion of grass hay in the substrate mix the higher the specific methane yield (SMY) achieved. This is again in agreement with a study carried out by Lehtomäki et al. (2007) who obtained an increase in the specific methane yield (SMY) from 143 to 268 ml CH_4/g VS added when the proportion of grass silage in the feedstock increased from 10% to 30% at a VS basis when co-digested with cattledung. Compared with this study, the less increase in the SMY when increasing the grass hay VS ratio in the feedstock in the present study may be due to the loss of soluble organic matter like VFAs during drying fresh grass.

As indicated in Table 4.2 below, the average values of the biogas yield data from analytical measurements were analysed using the one way ANOVA procedure in SPSS and the analysis of the results of the co-digestion of cattledung with grass hayalso revealed that there was a significant difference between the yields at 95% level of significance.The result showed that the biogas yield varies significantly with varying levels of C:N ratio of treatments at 0.05 level.

Table 4.2: One way ANOVA of dependable biogas variable between treatments

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	931122.917	2	465561.459	595.342	.000
Within Groups	2346.019	3	782.006		
Total	933468.936	5			

According to Figure 4.1, it was observed in the first four days that the T2 produced the highest biogas yield of 2,100 mL, followed by T1 which produced biogas yield of 1,563 mL and T3 which produced biogas yield of 1,560 mL. Substrates of batch T2 produced 34% and 35% more biogas yield than substrates in batchesT1 and T3, respectively. This is probably due to the optimum C:N ratio as a result of optimum co-digestion of cattle dung and grass hay.

For all of the reactors, there is a sharp increase in the amount of biogas produced during the first 1 - 4 days of the experiment. This is then followed by a gradual decrease following the decrease in consumption of the different substrates in the reactors. This was due to the increased availability of the biomass during the initial stages, leading to subsequent growth of the anaerobic organisms. Most of the digestion happened in the first four days which shows us that microorganisms did not need time to acclimatize to the substrate due to active inoculum (Abdien, 2003). After 4 days the rate of biogas production began to decrease due to a reduction in nutrient content. The samples show that there was a decrease in pH from pH 7.0 to 5.0 over the course of the experiment for all the reactors.

4.4.2. Biogas Quality

It is obvious that the type of substrates determine the biogas quality and its composition. In this specific study, samples of the gas produced from each of the three treatments were analyzed using Geo Tech gas analyzer to know the composition of biogas produced during the anaerobic digestion process and the mean values of the composition of biogas were given in Table 4.4.

Table 4.3: Volumetric biogas and methane yields and its composition

Treatments	Biogas yield (mL_N)	Methane Composition (%)	Methane yield (mL_N)
T1	2,709	60	1,635
T2	3,177	66	2,105
T3	2,212	58	1,275

According to Table 4.3, the percentage of methane for T1, T2 and T3 were 60, 66, and 58 respectively. Treatment two (T2) stands first in terms of quality of the biogas that is the proportion of methane from the produced biogas (66%) while treatment one (T1) and treatment three (T3) follows 2nd and 3rd by 60% and 58% respectively. Rahmat et al. (2014) reported that the quality of biogas mainly depends on the percentage of CH_4 and, the better quality biogas contains 50–70 % of CH_4. Accordingly, the result of the present investigation was in line with the report by Rahmat et al. (2014).

It is also important to notice that the highest methane amount was recorded from T2 (with 2,104.5 ml) which was as a result of having high carbon/nitrogen content and high organic dry matter. It is well known that the composition of biogas as well as biogas yields depend on the substrates owing to differences in material characterization in each feed material (Stewart *et al*., 1984). Results further indicate that optimum co-digestion of cattle dung with grass was found to produce methane rich biogas than that of cattle dung alone.

It can also be seen clearly that all the treatments have very good methane content; 58% to 66%, indicating that the bio-digester used were very tight and oxygen was unable to react with the system. When selecting substrate proportion for anaerobic digestion in terms of methane yield, all the three proportion can be used for various energy application prioritizing T2 (cattle dung (50%) co-digested with grass hay (50%) as it has the highest methane content.

5. Conclusions and Recommendations

5.1.Conclusions

The major source of biogas production in Ethiopia is cattle dung which is a cheap and easily available bio-resource. Though the characteristic features including high water content and optimum buffer capacity of cattle dungfavouredit to be used solely as the prime source for biogas production, the fermentation of cattle dung alone results in lower methane generation due to relatively low anaerobic biodegradability. In relation to this situation, research continues to verify the potential of other organic sources like grass hay; either in mono-digested or co-digested fermentation, to have an enhanced effect.Accordingly, an anaerobic batch experiment was conducted to characterize and investigate the biogas production potential from cattle dung, cattle dung co-digested with grass hay in different ratiosunder mesophilic condition (37 ± 2^0C) for a period of 38 days.

In the present study, the feedstocks were characterized in order to get better proportion of the substrates during digestion. Accordingly, the result has shown that the C: N ratio of cattle dung was 19.15 andthat of grass hay was 25.47, respectively.

The present study has also shown that the optimum co-digestion of cattle dung with grass at different ratios result into an increase in both biogas and methane yields. It was revealed that the largest volume of 3,177 mL of biogas was produced from T2 when 50% cattle dung and 50% grass hay were co-digested under the conditions mentioned above. The highest specific methane yields of 211, 171 and 165 mLCH_4g−1 VS were obtained from T2 (co-digestion of 50% cattle dung with 50% grass), T3 (75% cattle dung with 25% grass hay)and T1 (cattle dung alone), respectively.

From this experiment, in summary, it can be concluded that the optimum co-digestion of cattle dung with grass hay (as in T2) has the best synergetic effect relative to mono digestion of cattle dung (T1) because the co-digested feedstock gives highest amount of biogas with quality. Additionally, it is possible to conclude that the cumulative biogas yield and its bio-methane content relatively increased with increase in grass ratio.Finally, therefore, conclusion can be drawn that co-digestion of cattle dung and grass hay has the potential in addressing the challenges related to shortage of enough feedstock for biogas production.

5.2.Recommendations

Since present research work on AD system has highly improved the optimum utilization of feedstocks, the following recommendations are suggested for future research based on this thesis work. These include:

- ❖ Since biogas yield from grass feedstock differs with its quality and maturity, composition of the grass land, the cutting management and harvesting time, studies focusing on the co-digestion of cattle dung with grass and other energy plants at various conditions would be conducted.
- ❖ Further studies should be carried out in bigger volume reactors to obtain actual results of the biogas and methane yield.
- ❖ The effects of the co-digestion on the digestate have to be investigated well so as to analyze the changes in the composition of the micro and macro nutrients of the bio-slurry produced.
- ❖ It is also recommended to make biogas users survey (BUS) to know their attitude towards applying the co-digestion of cattle dung and grass hay/silage rather applying the waste grass hay directly to the farm as organic fertilizer in composting form.
- ❖ It is known that the current bio-digester designs under application in Ethiopia majorly based on cattle dung as feedstock. Finally, therefore, it is recommended to study and select appropriate small scale bio-digester designs(2-15m^3)suiting the co-digestion of cattle dung with grass hay and/or other energy plants.

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7. Appendixes

7.1. Results of substrate characterization

						weight after dying	weight after incineration				
Sample	Analysis	Label	Cr. wt.	Cr.+samp. wt.	Samp. wt. (FM)	Cr. + sam. wt. @105ºC	Cr. + sam. wt. @550ºC	TS	MC	VS	Ash
			(g)	(g)	(g)	(g)	(g)	(% FM)	(% FM)	(% TS)	(% TS)
Inoculum	1	A	61.94	94.56	32.62	64.68	62.36	8.40	91.60	84.67	15.33
Inoculum	2	B	58.12	102.1	43.91	61.99	59.54	8.81	91.19	63.31	36.69
Dung	1	A	60.37	91.00	30.63	65.90	61.22	18.05	81.95	84.63	15.37
Dung	2	B	64.35	99.94	35.59	70.50	65.27	17.28	82.72	85.04	14.96
Grass hay	1	A	56.87	63.68	6.81	63.11	57.41	91.63	8.37	91.35	8.65
Grass hay	2	B	72.97	79.40	6.43	78.86	73.49	91.60	8.40	91.17	8.83

7.2. Sample preparation, testing and measuring results

7.3.Feed stock preparation and hand shaking of the reactor

7.4.Bio-gas and methane production results (Row and normalized)

Biogas Production (mL)		Digestion Time (Days)					Total raw Biogas Production (mL)	CH_4 %	Raw CH_4 (ml)	Biogas (mL_N)	CH_4 (mL_N)	Specific biogas yield (mL_N)	Specific methane yield (mL_N)
	0	4	10	18	37	38							
T0 (Inoculum)	0	0	0	0	0	0	0	0	0	0	0	0	0
T1 (Only dung)	0	1,563	1,265	935	645	0	4,408	60%	2661	2709	1635	41.0	24.8
T2 (Dung+grass)	0	2,100	1,975	640	455	0	5,170	66%	3424	3177	2104	81.5	54.0
T3(Dung+grass); d/t ratio	0	1,560	1,100	565	375	0	3,600	58%	2075	2212	1275	61.5	35.4

7.5.Statistical output

Statistical output between treatments (comparison by volume of biogas, mL)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
T1: Only dung(100%)	2	2708.5550	32.59055	23.04500	2415.7405	3001.3695
T2: Dung (50%) with grass (50%)	2	3177.1350	8.69034	6.14500	3099.0554	3255.2146
T3: Dung (75%) with grass (25%)	2	2212.3200	34.76137	24.58000	1900.0015	2524.6385
Total	6	2699.3367	432.08076	176.39623	2245.8957	3152.7776

Multiple Comparisons of Biogas **(**LSD)

(I) Biogas treatments	(J) Biogas treatments	Mean Difference (I-J)	Std. Error	Sig.
T1: Only dung (100%)	Dung (50%) with grass (50%)	-468.58000*	27.96438	.001
	Dung (75%) with grass (25%)	496.23500*	27.96438	.001
T2: Dung (50%) with grass (50%)	Only dung	468.58000*	27.96438	.001
	Dung (75%) with grass (25%)	964.81500*	27.96438	.000
T3: Dung (75%) with grass (25%)	Only dung	-496.23500*	27.96438	.001
	Dung (50%) with grass (50%)	-964.81500*	27.96438	.000