BIOGAS PRODUCTION FROM *Guizotia scabra* SUB-SPECIES *scabra* LEAVES CO-DIGESTED WITH COW DUNG

M.Sc. THESIS

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Biogas Production from *Guizotia scabra* Sub-Species *scabra* leaves Co-Digested with Cow Dung

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By

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APPROVAL SHEET

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DEDICATION

I dedicate this thesis to my beloved Parents for their patience and special care.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

The author was born on October, 1995 (GC) from her Father Ato Chala Leta and her Mother W/Ro Workinesh Borena in Bodji Dirmeji Woreda, West Wollega Zone of Oromiya Regional State. She attended her elementary and secondary school education at Bodji Dirmeji Elementary and Secondary School, respectively. After Completing her secondary school at Bodji dirmeji secondary school, in 2014 she joined Wollega university and graduated on June 2016 (GC) with B.Sc. degree in Biology. Then, after serving as Graduate assistant for one year in Wollega University, she joined postgraduate program of Haramaya university in 2017, as a government sponsored student to pursue her M.Sc. study in Biotechnology at the school of Biological Sciences and Biotechnology.

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ACRONYMS/ABBREVIATIONS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
APHA	American Public Health Association
CD	Cow Dung
C/N	Carbon/Nitrogen
EPA	Environmental Protection Agency
GHG	Green House Gas
GS	*Guizotia scabra*
HRT	Hydraulic Retention Time
IEA	International Energy Agency
M.A.S.L	Meters above Sea Level
MC	Moisture Content
MDS	Mass of Dry Sample
NBP	National Biogas Program
OLR	Organic Loading Rate
SPSS	Statistical Package for Social Sciences
SRT	Solid Retention Time
TS	Total Solid
VFA	Volatile Fatty Acid
VS	Volatile Solid

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ABSTRACT

Alternative energy sources have recently become more and more attractive due to the increasing demand for energy, global warming, depletion of fossil fuels and high prices of petroleum-based fuels. One of the alternative sources of energy is Biogas. In this study, Guizotia scabra sub-species scabra was evaluated for its potential of biogas production co-digested with Cow dung (CD) in five mix ratios under mesophilic condition (38ºC) using batch digester in Botany laboratory of Haramaya University. In all treatments, total solids (TS), volatile solid (VS), organic carbon, and pH were measured before and after digestion. while, Organic carbon to Nitrogen ratio was measured before anaerobic digestion, and found in the range of optimal nutrient requirement for anaerobic microorganisms. The daily biogas production was subsequently measured by water displacement method for 30 days. All measured physico-chemical parameters of each substrate significantly varied between before and after anaerobic digestion (AD). Gas production was noticed in all of the substrate variation from the first day of digestion experiment and became almost zero at the 30th day in all substrates. The highest total biogas yield was obtained from 75% GS + 25%CD mix ratio (1168ml) while the lowest yield was obtained from CD alone (744ml). Assessment of cumulative biogas production revealed that substrate in a mix ratio of 75% GS and 25% CD (1168ml) showed the highest production, suggesting this mix ratio of the two substrates is an optimal mix to yield maximum amount of biogas. In general, results show that the increment of biogas yield, and VS and TS reduction can be significantly enhanced when GS and CD are co-digested. However, in order to get more biogas production from the substrate mixes with maximum biogas production (75% GS+25%CD) factors affecting the production such as temperature and pH should be evaluated at certain time intervals during anaerobic digestion (AD).

Key Words: Biogas, co-digestion, Cow dung, *Guizotia scabra*, Total solid, Volatile solid.

1. INTRODUCTION

The world's economy primarily depends on non-renewable natural fossil fuels like natural gas, petroleum and coal as a source of energy. Consumption of fossil fuels is increasing with an increase in human population, transportation and technological advances and thereby resulting in decrease in these natural resources. Furthermore, fossil fuels are sources of greenhouse gases (GHGs), which in turn contribute to air pollution and change climate. The daily increase in the world's energy demand, global warming, depletion of fossil fuels and high prices of petroleum-based fuels have forced us to search for alternate energy sources that are sustainable, efficient, renewable, and cost effective with less negative impacts or less emission of greenhouse gases (Nigam and Singh, 2011).

Plant biomass can be considered as an excellent alternative source of bio-fuels such as biogas and bioethanol. Production and use of bio-fuels help in reducing emissions of greenhouse gases and reduce the demand for petroleum-based fuels. Bio-fuels include bio-methanol, bio-ethanol, biodiesel, biogas, bio-hydrogen, etc. (Balat, 2008). Among all these, biogas is one of the most promising energy source for developing countries such as Ethiopia (Tamburini *et al*., 2011).

Biogas is a clean, environmentally friendly and renewable form of energy generated when microorganisms degrade organic materials in an oxygen free environment. The formation of biogas can occur either in natural environment or controlled conditions in constructed biogas plants, so called anaerobic digesters. The potential feedstock for the production of biogas include; municipal solid waste, industrial organic waste, garden waste, agricultural waste (manure and crop residue), energy crops, cellulose rich biomass, algae and seaweed (water based), by-products of ethanol and bio diesel production (Lantz *et al* ., 2007; Demetriades, 2008; Börjesson and Mattiasson, 2007).

Biogas is produced through anaerobic digestion in a multi-step biological processes in which different anaerobic microbes are involved. It occurs in four stages including hydrolysis/liquefaction, acidogenesis, acetogenesis and methanogenesis (Kangle *et al*., 2012). Therefore, in biogas production anaerobic digestion is the consequence of a series of

metabolic interactions among various groups of microorganisms. Such process towards producing methane, a biogenic volatile organic compound, can naturally occur in an oxygen-free environment in swamps and spontaneously in landfills containing organic wastes. It can also be induced artificially in digestion tanks to treat sludge, industrial organic wastes and agro-wastes (Igoni *et al*., 2008).

The content of biogas varies with the material being decomposed and the environmental conditions involved. Potentially, all organic waste materials contain adequate quantities of the nutrients essential for the growth and metabolism of the anaerobic bacteria in biogas production (Khanal, 2008). Generally, biogas consists of methane and carbon dioxide with varying amounts of water, hydrogen sulphide (H_2S) and other compounds in small amounts (Keefe and Chynowet, 2000; Madu and Sodeinde, 2001). It is a renewable energy source and in many cases exerts a very small carbon foot print (Corral, 2007). The gas produced has high energy content and can be used in many applications such as heating, cooking, power generation, and lighting and as a biofuel that can be injected into the gas network of city (Murat, 1981).

The largest segment of the population in Ethiopia is located in rural areas and their main source of income comes from agriculture (World Bank, 2014). This same segment of population represents the main energy consumer in Ethiopia and satisfies most of their energy needs with woody biomass (Wolde-Ghiorgis, 2002; Gebreegziabher, 2007). The extensive demand for firewood has caused energy and environmental crisis since most of the forest coverage has been depleted over the last 35 years. This undermines firewood availability, soil fertility and the preservation of aquifers. Ultimately, Ethiopian farmers have to spend more resources (e.g. time or money) to have access to fuel wood. Meanwhile, their agricultural yield is reduced due to the lack of nutrients in the soil and the shortage of water (Boers *et al*., 2008). According to the environmental protection authority of Ethiopia some two million hectares of land in the country has now become irreversibly barren as a result of the extensive deforestation (WIC, 2002). In order to curb such environmental problems caused by deforestation to fulfill their energy demands, alternative environmentally friendly energy source such as biogas is a must.

Biogas has high economical benefits compared to other fuel sources. This is because; it requires limited capital in construction and maintenance. Its raw materials are easily available in villages and towns. It has also less impact on the environment. Therefore, use of biogas energy has to be given priority in Ethiopia to reduce deforestation, land degradation, and improve the living condition of the society (i.e., health and socio economic situation of the households, including gender issues). Furthermore, it reduces greenhouse gasses (GHG) that contributes to climate change and keeps the quality and sanitation of cities and towns by removing the waste using biogas technology (Muller *et al.,* 2007). Well-functioning biogas systems can yield a whole range of benefits for the users, the society and environment in general (Bekele, 2011).

Through co-digestion of different substrates biogas can be produced. The most common approach to biogas production is the use of such basic substrates as manure or sewage sludge mixed with one or more other agro-waste substrates such as fruit peels and crop straws (Braun, 2002). The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates (Mata-Alvarez *et al*., 2000).

Co-digestion of different feed stocks have many advantages in balancing nutrients, C/N ratio equilibrium and the minerals and metals required for equilibrium, and increase buffering capacity of the system. Therefore, co-digestion enhances process stability and performance of organic matter biodegradation, optimizing the biogas and methane yield (Huang *et al*., 2016; Zhang *et al*., 2014). With this regard, there have been a lot of research already done on biogas production using co-digestions of animal manure and lignocellulosic materials such as Khat (*Catha edulis*) left over co-digested with poultry manure (Gizachew, 2015), Parthenium weed co-digested with poultry manure (Getu, 2016) and *Lantana camara* co-digested with cow dung (Bizunesh, 2016). However, no work has evaluated the biogas production potential of *Guizoita scabra*, a herbaceous plant abundantly found in different parts of Ethiopia. This study was, therefore, designed to evaluate biogas production potential of *Guizoita scabra* leaves co-digested with cow dung with the following objectives.

General objective:

The general objective of this study was to evaluate production of biogas from *Guizoita scabra* leaves co-digestion with Cow dung through anaerobic digestion.

Specific objectives:

- To determine the physico-chemical features (TS, VS, pH, Organic carbon, Nitrogen and carbon to nitrogen ratio) of *Guizotia scabra* leaves and cow dung.
- To evaluate the average daily and cumulative biogas production from mixture of *Guizotia scabra* leaves and cow dung combined in different proportions;
- To compare the biogas yield from batch fermentation of solo and mixed substrates of *Guizotia scabra* leaves and cow dung.

2. LITERATURE REVIEW

2.1. Bio-fuels

Bio-fuels are solid, liquid or gaseous fuels that are produced from biomass. The biomass or organic matter that is converted to bio-fuels may include food crops, dedicated bio-energy crops (e.g switch-grass or prairie perennials), agricultural residues, wood/forestry waste and by-products, animal manure and algae (Giampietro *et al*., 1997; IEA, 2011). Bio-fuels are renewable since they are produced from biomass organic matter, such as plants. They generate about the same amount of carbon dioxide (a greenhouse gas) from the tailpipe as fossil fuels, but the plants that are grown to produce the bio-fuels actually remove carbon dioxide from the atmosphere. Therefore, the net emission of carbon dioxide will be close to zero (Dominik and Rainner (2007).

2.2. Historical Background of Biogas

Biogas is a mixture of gases, mainly methane and carbon dioxide, resulting from anaerobic fermentation of organic matter. In 1630 Van Helmont, a Belgian national, noted that the gas emanating from decaying matter is different from the constituents of air. It was Volta, an Italian national, who introduced biogas in a scientific setting. In 1776 he concluded that the amount of gas released is a function of the amount of decaying vegetation and that upon mixing with a certain proportion of air it becomes explosive. In 1808, Sir Humphrey Davy demonstrated the production of methane by the anaerobic digestion of cattle manure. Anaerobic digestion is a biological process that happens naturally when bacteria break down organic matter in the absence of oxygen. Then Beschamp, a student of Pasteur, discovered that biogas production was connected with microbial activity. In 1886 he discovered methanogens (Verma, 2002).

Following the discovery of methane emissions from natural anaerobic habitats by Volta in 1776, people started using biogas as a fuel, basically for lighting. However, it took until the end of the 19th century to apply anaerobic digestion for the treatment of wastewater and solid wastes. The first digestion plant was reported to have been built at a leper colony in Bombay, India, in 1859. Anaerobic digestion reached England in 1895, when biogas was recovered

from a sewage treatment facility to fuel street lamps in Exeter. The main purpose of anaerobic digestion is to reduce and stabilize solid wastes (Nayono, 2010).

2.3. Biogas technology in Ethiopia

Biogas technology was introduced in Ethiopia in 1979, when the first batch type digester was constructed at the Ambo Agricultural College. Even if biogas technology has multitude of advantages to rural households society and for forming sustainable environment, the wider dissemination of the technology is limited until the National Biogas Program (NBP) is launched in 2008. In the last two and half decades around 1000 biogas plants, ranging in size from 2.5 m^3 to 200 m^3 were constructed in households, community and governmental institutions in various parts of the country. However, Approximately 40% of these plants are not operational due to lack of effective management and follow-up, technical problems, loss of interest, evacuation of ownership and water problems and biomass problem. Other reasons for the limited success of the technology in Ethiopia include the adoption of a project-based stand-alone approach without follow-up structure in place, variations in design, and the absence of a standardized biogas technology (NBP, 2007). To implement the technology widely, it needs encouraging the households because in lacking technical and financial support to rural households who are more or less unaware of the technology difficult to use it consistently (Getachew *et al*., 2006).

2.4. Biogas and its Composition

Biogas is combustible mix of gases and eco-friendly renewable form of energy produced by anaerobic microbes when organic materials are fermented in a certain range of temperatures, moisture and pH under air-tight conditions (Adelekan and Bamgboye, 2009; Ilaboya *et al*., 2010). Anaerobic digestion of organic materials mainly consists of four main stages which are interdependent in such a way that the product from one stage is a precursor for the next stages. Each stage involves different types of microorganisms (Sagagi *et al*., 2009).

Biogas is odorless and colorless gas that burns with 60% efficiency in conventional biogas stove. This gas is useful to substitute firewood, cow-dung, petrol, diesel and electricity depending on the nature of the task and local supply conditions and constraints. Biogas

digester system provides a residue organic waste after its anaerobic digestion that has superior nutrient quality over normal organic fertilizer, as it is in the form of ammonia and can be used as manure. Anaerobic biogas digester also functions as waste disposal systems, particularly for human wastes and can therefore, prevent potential contamination and the spread of pathogens and disease causing bacteria. Biogas technology is particularly valuable in agriculture residual treatment of animal excreta and kitchen refuse (Amye *et al*., 2014).

Biogas is a well-established fuel that can supplement or even replace wood as an energy source for cooking and lighting in developing countries. Currently, as the fossil-based fuels become scarce and more expensive, the economics of biogas production is turning out to be more favorable. Biogas is a readily available energy resource that significantly reduces greenhouse gas emission compared to the emission of landfill gas to the atmosphere (Nabuuna and Okure, 2005).

The composition of biogas largely depends on the type of substrate used for its formation. Generally, biogas consisted of methane (50- 70%), carbon dioxide (30-40%) and traces of gasses hydrogen, water vapor and hydrogen sulphide (Rahmat *et al.,* 2014).

Table 1. Summarizes a typical approximate composition of biogas (Bilhat, 2009)

Substances	Percentages
Methane	50-70
Carbon dioxide	30-40
Hydrogen	5-10
Nitrogen	1-2
Water vapor	0.3
Hydrogen sulphide	Traces

2.5. Feed-stocks (Substrates) for Biogas Production

Raw materials for biogas production such as cow or pig dung, poultry waste, water hyacinth, straws, weeds, leaf, human and animal excreta, domestic rubbish and industrial solid and liquid wastes are available in Ethiopia. Potentially, all organic waste materials contain adequate quantities of the nutrients essential for the growth and metabolism of the anaerobic bacteria in biogas production. However, the potential biogas yield and composition may vary depending on the nature of the substrate. This needs evaluation of different substrates in different combinations so as to balance the macro- and micro-nutrients required by microbes for efficient and quality biogas production. Literally the agro-wastes such as crop residues and straws are left overs and may spoil the environment if not decomposed well. The use of these bio-wastes in biogas production offers many advantages including: reduction of greenhouse gas emissions, reduction of odor, betterment of fertilizer, energy for heat and power (Khan, *et al*., 2013).

2.5.1. *Guizotia scabra*

The genus *Guizotia* is a small endemic genus that belongs to the family Asteraceae, tribe Heliantheae. This genus is native to tropical Africa with the highest concentration of species in Ethiopia (Baagøe, 1974). *Guizotia* species are probably the most important of all broad leaved weeds in Ethiopia, almost universally distributed at middle and higher elevations. *Guizotia scabra is* commonly known as Tufo. It contains two sub-species, sub-species *scabra* and *schimperi*. *Guizotia scabra* sub-species *scabra i*s a perennial, course, densely scabrous plant with stiff leaves and found in a wide variety of soils. It has been encountered in swampy areas, wet and dry grassland, on stony hill slopes and as a ruderal. It is an erect, fast-growing to 1-2m. In Ethiopia *Guizotia scabra* is most common at 2500–3500m altitude and distributed, as part of natural vegetations, in East Africa, Cameron and Nigerian highlands, commonly with in an altitudinal range of 1100-2700 m (Baagøe, 1974; Hiremath and Murthy, 1986).

2.5.2. Cow dung

Anaerobic digestion has been considered as waste-to-energy technology, and is widely used in the treatment of different organic wastes, for example: organic fraction of municipal solid

waste, sewage sludge, food waste and cow dung (Li *et al*., 2009). Cow dung has high nitrogen content and due to pre-fermentation in the stomach of ruminant, and has been observed to be most suitable material for high yield of biogas through the study made over the years (Chonkor, 1983).

Mixing cow dung with organic wastes from industry and households has been successfully applied for biogas production. Co-fermentation offers economic and environmental benefits as it entails processing waste streams in single facility. There are three main advantages of using cattle dung for co-fermentation. Firstly, it is a good source for nutrients such as Nitrogen and Phosphorous, vitamins and other compounds necessary for microbial growth. Secondly, it plays a role in neutralizing pH and improving buffering capacity. Thirdly, the high water content in dung helps dilute the concentrated organic wastes, which would be inhibitory and difficult to treat separately. Moreover, a high buffering capacity in manure makes the process more resistance to the effect of volatile fatty acids (VFAs) accumulation and thus avoids inhibition process. Several studies have reported that biogas process could be improved and stabilized by applying co-digestion strategy (Fang, 2010).

2.6. Anaerobic Digestion

Anaerobic digestion is a biochemical process accomplished by the combined action of a group of several types of microorganisms, which metabolize the organic compounds into a gaseous mixture consisting of mainly methane and carbon dioxide (biogas) in anaerobic conditions. It is a widely used technology in order to cost-effectively reduce the volume of the biomass such as sewage sludge, organic fraction of municipal solid waste, manure, and lignocellulosic biomass, and to capture energy in the form of methane (Weiland, 2010). It is also consisting of mixed biological systems in which organic materials such as carbohydrate, lipids, and proteins are utilized by microorganism to produce methane and carbon dioxide-rich in their normal metabolic activities. The anaerobic digestion is carried naturally in the anaerobic environments such as the bottom of ponds and marshes (Rouse, 2008). Digestion occurs under certain conditions (psychrophilic, mesophilic, and thermophilic), which differ mainly based on bacterial affinity for specific temperatures (EPA, 2002).

2.7. Biological Processes in Biogas Production

Conceptually, the microbial processes of AD can be described by the sequential steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis as follows (Bitton, 2005).

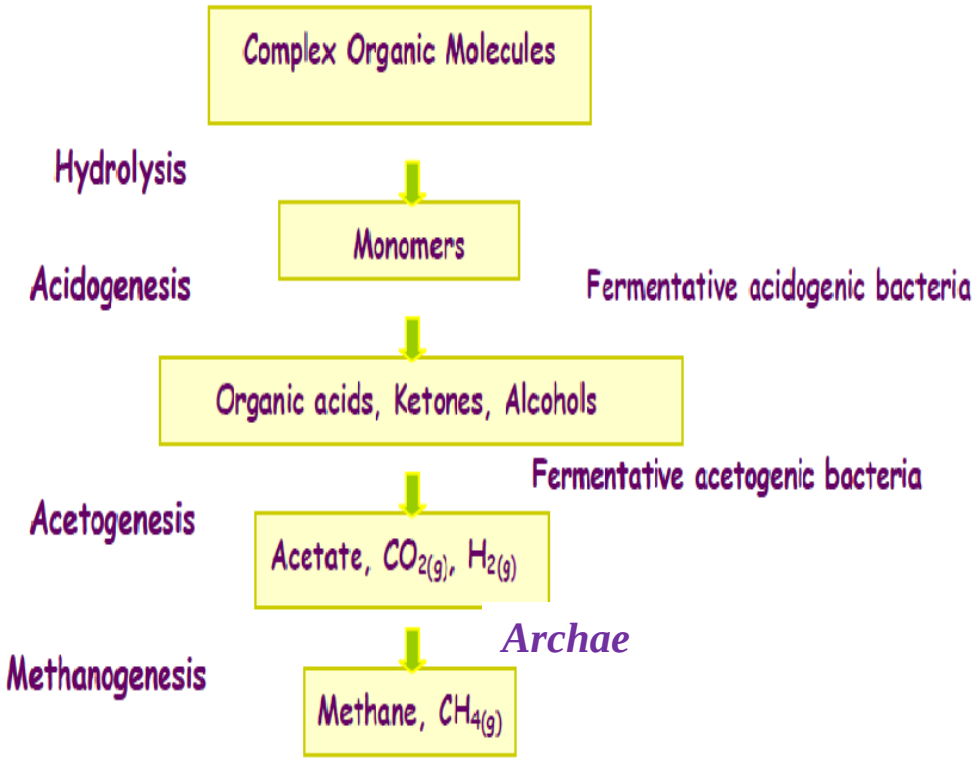

Figure 1. Schematic diagram of anaerobic digestion process for Biogas production (Awosolu, 2007).

2.7.1. Hydrolysis

In most cases biomass is made up of large organic polymers. To provide energy to bacteria from such materials, the chains must first be broken down in to their relative monomer units (e.g. cellulose, fat and proteins) into smaller, soluble molecules (e.g. sugars, fatty acids and amino acids). The process of breaking these chains and dissolving the smaller molecules into solution is called hydrolysis. The hydrolysis reactions are catalyzed by extracellular enzymes (cellulases, lipases, proteases, amylases, etc.) secreted by the microbial bacteria. Hydrolysis rate depends on temperature, biodegradable organic matter, biomass nature, and pH and particles size. These constituent parts or monomers are readily available by other bacteria.

Therefore hydrolysis of these high molecular weight polymeric components is the necessary first step in anaerobic digestion (Sleat and Mah, 2006). A complex group of microorganisms participates in the hydrolysis and fermentation of organic material (Rojas *et al*., 2010). This first step is inhibited by lignocelluloses containing materials, which are degraded only very slowly or incompletely (Rilling, 2005).

2.7.2. Acidogenesis

In the acidogenesis phase, the monomers produced in the hydrolysis phase are utilized by fermentative bacteria or anaerobic oxidizers (Garcia-Heras, 2003). These microorganisms are both obligate and facultative anaerobes. In a stable anaerobic digester, the main degradation path way results in acetate, carbon dioxide and hydrogen. The intermediates, such as volatile fatty acids and alcohols, play a minor role. This degradation path way gives higher energy yield for the microorganisms and the products can be utilized directly by methanogenic microorganisms (Schink, 1997). However, when the concentration of hydrogen is high, the fermentative bacteria will shift the path way to produce more reduced metabolites. The products from acidogenesis step consist of approximately 51% acetate, 19% H_2/CO_2, and 30% reduced products, such as higher VFA, alcohols or lactate (Angelidaki *et al.*, 2002).

2.7.3. Acetogenesis

In the third step, acetogenesis, the products of the acidification are converted into acetic acids, hydrogen, and carbon dioxide by acetogenic bacteria. Acetogenic bacteria such as *Syntrobacter wolini* and *Syntrphomonas wolfei* convert volatile fatty acids (e.g. propionic acid and butyric acid) and alcohol into acetate, hydrogen, and carbon dioxide, which are used in methanogenesis (Zaher *et al*., 2007).

2.7.4. Methanogenesis.

The final step in bio-methane production, methanogenesis, is performed by a specialized group of microorganisms belonging to the methanogens. There are three known types of methanogens acetoclastic, hydrogenotrophic, and methyl-trophic. Acetoclastic methanogens convert acetate to CH_4 and CO_2, hydrogenotrophic methanogens use H_2 or formate to reduce CO_2 to CH_4, and methylotrophic methanogens produce CH_4 from methyl compounds, such as

methanol, methylamines, and methyl-sulfides (Liu and Whitman, 2008). Methanogenesis municipal AD, about 70% of the CH_4 is produced from acetate, and the rest from H_2 and CO_2. Only a minimal amount of CH_4 is produced via methyl-trophic methanogenesis (Ferry, 1993).

2.8. Factors that Affect the Process of Biogas Production

The operating parameters of the digester must be controlled so as to enhance the microbial activity and thus increase the anaerobic degradation efficiency of the system. The production of biogas is factored by many operational parameters. Some parameters that affect the production of biogas include temperature, pH, particle size, agitation, rate of organic load, retention time etc. Any rapid change in these parameters can adversely affect the production of biogas (Yadvika *et al.,* 2004).

2.8.1. Nature of the substrate

Substrate is material and energy source for the microorganism. Substrate will be consumed by microorganism and converted to methane as well as the use for growth. Types of substrate determine the rate of the digestion process, and lack of substrate ends the metabolism of the microorganism. It also determines the time of digestion, since more complex substrate will take longer time for degradation by microorganism (Gerardi, 2003).

During the digestion process, microorganism produces intermediate products. Intermediate products usually are short lived and do not accumulate in the reactor. However, the production rate of intermediate products depends on the composition of the substrate and can lead to the accumulation of intermediate products. The change of operational conditions likes pH or temperature can also induce the accumulation of intermediate products. The accumulation of intermediate products can inhibit digestion process. For instance substrate containing high fats can give high production of the fatty acids and induce to decrease pH, which will inhibit the microorganism activity further (Deublein, 2008).

2.8.2. Carbon to nitrogen ratio

The relationship between the amount of carbon and nitrogen present in organic materials is represented by the C/N ratio. Optimum C/N ratios in anaerobic digesters are between 20 – 30

in order to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present in the wastes (Fricke *et al.,* 2005). Hartmann and Ahring (2006) stated that a solid waste substrate with a high C/N ratio is an indication of rapid consumption of nitrogen by methanogenesis and results in lower gas production. On the other hand, a lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria. Optimum C/N ratios of the digester materials can be achieved by mixing materials of high and low C/N ratios, such as organic solid waste mixed with sewage or animal manure (Zaher *et al.,* 2007).

2.8.3. pH value

The pH value of the digester content is an important indicator of the performance and the stability of an anaerobic digester. An optimum biogas production is achieved when the pH value of input mixture in the digester is between 6.25 and 7.50 (Mahanta *et al*., 2004). The pH value in a biogas digester is also a function of the retention time. Most anaerobic bacteria including methane forming bacteria function in a pH range of 5.5 to 8.5, (Fang, 2010) but, optimally at a pH of 6.8 to 7.6, and the rate of methane production may decrease if the pH is lower than 6.3 or higher than 7.8 (Gerardi, 2003).

Significant changes in alkalinity or pH are introduced in an anaerobic digester by substrate feed or the production of acidic and alkali compounds, such as organic acids and ammonium ions, respectively, during the degradation of organic compounds in the digester. Alkalinity in an anaerobic digester is also derived from the degradation of organic nitrogen compounds, such as amino acids and proteins, and the production of carbon dioxide from the degradation of organic compounds. When amino acids and proteins are degraded, amino groups (and hence ammonia) are released and alkalinity is produced. The pH value depends on the ratio of acidity and alkalinity and the carbon dioxide content in the digester, the determining factor being the density of the acids. Cow dung is also used to facilitate the bacterial growth in the digester and thus hasten the biogas generation. It plays a role in neutralizing pH and improving buffering capacity by making the process more resistant to the effect of VFA accumulation and thus avoids inhibition processes. It also helps to maintain a stable and reliable digestion performance and yields good quality fertilizer (Fang, 2010).

2.8.4. Temperature

Temperature is one of the most important factors affecting microbial activity within an anaerobic digester and methane production is strongly temperature dependent. Fluctuations in temperature affect the activity of methane forming bacteria to a greater extent than the operating temperature (Sibisi and Green, 2005). The anaerobic process temperature of the reactor has influence to the physical and chemical properties of the substrate, which in turn affects the thermodynamic and kinetic reaction of the biological processes. There are several advantages with increasing temperatures (Abdelgadir *et al.*, 2014; Van Lier *et al*., 1996).

Moreover, increased temperature also increases death rate of pathogenic bacteria, reducing time required for pathogen destruction in AD process (Bendixen, 1994; Smith *et al*., 2005). However, high temperature (thermophilic) can have negative effects as well. Increasing temperature increases the fraction of free ammonia (NH_3) that is inhibitory to microorganisms. Ammonia inhibition could result process disturbance in thermophilic process. The stability of the mesophilic process makes it more acceptable in current AD facilities, but achieved at longer retention times (Ostrem *et al*., 2004).

2.8.5. Retention time (RT) Error! Bookmark not defined.

The number of days the organic material stays in the digester is called the retention time. There are two significant retention times in an anaerobic digester: solids retention time (SRT) and hydraulic retention time (HRT). The SRT is the average time the solids are in the anaerobic digester. The HRT is the time the liquid is in the anaerobic digester (Demetrides, 2008). Theoretically, long retention times will lead to low efficiency of the process. In anaerobic conditions, the decomposition of organic substances is slow and this will take some time for digestion to complete. Types of microbial and its temperature range are one of the reasons that will affect the retention time. Thermophilic temperature system in anaerobic digestion will have shorter retention time comparable to mesophilic temperature system. At high temperature, the particles kinetics rate increases so is the reaction rate. Thus, conversion processes takes place faster and lessen the retention time. At the same time, shorter retention time subjected the active microbial colony to washout while longer retention time means larger digester volume and increases the operational cost (Yadvika *et al*., 2004). It is almost

critical to determine the suitable HRT for anaerobic digestion process as to ensure a stable condition inside digester. A condition where the number of removed microorganism with the digestate should not be greater than the number those produced by duplication (Dobre *et al*., 2014).

2.8.6. Organic loading rate (OLR)

The rate at which substrate is supplied to the digester is referred to as organic loading rate and is usually expressed in terms of Kg volatile solids per m^3 and day. The gas production rate in the digester is highly dependent on the organic loading rate (Yadvika *et al*., 2004). The organic loading rate (OLR) determines the volatile solids input to the digester. This parameter has a significant influence on the process performance. It is expressed as the amount of organic matter (as volatile solids) per reactor volume. A higher OLR will demand more of the bacteria, which may cause the system to crash if it is not prepared. Under feeding the reactor could also lead to reduction in the digester performance due to insufficient nutrients for microbial growth. Organic loading rate (OLR) is a measure of the biological conversion capacity of the AD system. Feeding the system above its sustainable OLR, results in low biogas yield due to accumulation of inhibiting substances such as fatty acids in the digester slurry (Vandevivere *et al.*, 1999). In such a case, the feeding rate to the system must be reduced. OLR is a particularly important control parameter in continuous systems.

2.8.7. Particle size

The size of the feedstock must not be too large otherwise it will result in the obstruction of the digester and also it would be difficult for microorganisms to carry out its digestions, Smaller particles on the other hand will deliver large surface area for adsorbing the substrate that would result in increased microbial activity and hence increased gas production (Sharma *et al*., 1988). Mechanical pretreatment such as crushing might significantly reduce the volume of digester required, without decreasing biogas production (Yadvika *et al*., 2004).

2.8.8. Agitation

Agitation or mixing of digester contents significantly helps to ensure intimate contact between micro-organisms, which leads to improved fermentation efficiency. Varying degrees of mixing of digester contents improves biogas production (Prasad *et al*., 2008). The agitation of the digester contents has a number of benefits. It helps to mix up material, evening out any localized concentrations. It also helps to stop the formation of 'dead zones' or scum. In addition, it increases the waste's availability to the bacteria, helps remove and disperse metabolic products and also acts to ensure a more uniform temperature within the digester. There have been some suggestions that efficient mixing enhances methane production, but the evidence is inconclusive, so it seems likely that this may only be of noticeable benefit for some systems or operational regimes. Mixing also promotes heat transfer, particle size reduction as digestion progresses and release of produced gas from the digester contents (Rojas *et al*., 2010).

2.8.9. Ammonia

Nitrogen in the form of NH_4-N is required by bacteria for their cell mass synthesis. The major nitrogen compound is obtained from nitrogenous materials available in organic matter usually proteins and urea. Ammonia is produced during hydrolysis of proteins and urea. Urea is readily hydrolyzed to ammonia and carbon dioxide by the enzyme urease present in organic matter (Arceivala and Asolekar, 2007).

2.8.10. Seeding

To start up a new anaerobic process, it is critical to use inoculums of microorganisms to commence the fermentation process. The common seeding materials include digested sludge from a running biogas plant or material from well-rotted manure pit or cow manure slurry (Yadvika *et al*., 2004). Sunarso *et al.* (2012) states that rumen fluid inoculums caused biogas production rate and efficiency increase more than two times in compare to manure substrate without rumen fluid inoculums. Rojas *et al.* (2010) states that the addition of manure slurry to the batch reactor as part of the starter improved the biogas production. Normally, the volume of the inoculums should be above 10% of the total working volume of the digester, and 20–

30% supplementation of inoculums would favor the smooth start-up of AD digesters (Neves *et al*., 2006).

2.8.11. Toxicity

Mineral ions, heavy metals and detergents are some toxic materials that inhibit the normal growth of pathogens in the digester. Small quantity of mineral ions (e.g., sodium, potassium, calcium, magnesium, ammonium and sulphur) also 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, heavy metals such as copper, nickel, chromium, zinc, lead etc., in small quantities are essential for the growth of bacteria but their higher concentration has toxic effects. 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 (Mahanta *et al.*, 2005).

2.8.12. Water content

Water is the vital element for micro-organisms life and their activity. The movement of bacteria and activity of extra cellular enzyme are highly determined by the water content in the digester (Nijaguna, 2002). 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. In both cases, gas production will be less than optimal. Furthermore, most biogas digesters are designed for a total solids content of about 8%. A change of this ratio will have an impact on the retention time and the hydraulic functioning of the digestion process (Jan and Felix, 2010).

Optimum moisture content has to be maintained in the digester and the water content should be kept in the range of 60-95 % (Demetriades, 2008). However, the optimum water content is

likely to differ with different input materials depending up on the substrates chemical characteristics and bio-degradation rate (Nijaguna, 2002).

2.8.13. Co-digestion Process

Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. Substrates such as food wastes, sewage waste, cattle manure, certain energy crops and algae are good bases to obtain processes with good nutrient and trace element balances. These kinds of substrates can often be implemented for "mono-substrate" digestion, while substrates dominated by carbohydrates or fats needs to be co-digested or digested in processes modified by e.g. nutrient and trace element additions, sludge recirculation, etc. The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates (Biogas Research Center, 2014).

Lignocellulosic materials are characterised as carbon-rich, poor in buffering capacity and deficient in nutrients (Mata-Alvarez *et al.*, 2014). Mono-digestion of lignocellulosic materials often results in a slow process and low methane yield (Sawatdeenarunat *et al.*, 2015). This limitation can be overcome by using a co-substrates, such as animal manure, can be used together with lignocellulosic biomass to supplement it with macro- and micronutrients and buffering capacity (Mata-Alvarez *et al.*, 2014). In a co-digestion process organic wastes rich in proteins can provide the buffering capacity and a wide range of nutrients, while substrate with a high carbon content can balance the C/N ratio for all substrates characterized by a low C/N ratio, decreasing the risk of ammonia inhibition (Hills and Roberts, 1981; Hashimoto, 1986).

Traditionally, anaerobic digestion was a single substrate, single purpose treatment. It has then realized that AD as such became more stable when the variety of substrates applied at the same time is increased. The most common situation is when a major amount of a main basic substrate (e.g. manure or sewage sludge) is mixed and digested together with minor amounts of a single, or a variety of additional substrate (Braun, 2002).

2.9. Benefits of Biogas Technology

Biogas energy, a clean, renewable form of energy, could augment conventional energy sources because of its environment friendliness allowing for efficient waste utilization and nutrient recycling (Bhat *et al*., 2001). Generally, biogas digesters have come to symbolize access to modern energy services in rural areas and are slated to considerably improve health and sanitation, and to yield significant socioeconomic and environmental benefits (Srinivasan, 2008).

2.9.1. Economic Benefits

Two of the most important outputs of biogas technology are energy and bio-slurry. Biogas energy is utilized commonly for cooking, lighting, refrigeration, and running internal combustion engine (FAO, 1996). Biogas burns more efficiently as compared to fuelwood and dung. It burns at an efficiency of about 60 % whereas fuelwood burns at 5 % to 8 % efficiency in open fire place and dung burns at 60 % of that of fuelwood (FAO, 1997). Unlike the use of traditional biomass fuels, cooking with biogas is much easier because there is no need to keep the fire burning (Arthur *et al.*, 2011).

Biogas installations can generate electricity and offer transportation fuel. Electricity generated from biogas could be useful for local pumping, lighting, communication, refrigeration, etc. When methane, the combustible component of biogas, is enriched, it can be used as transportation fuel (Larson and Kartha, 2000; Murphy *et al*., 2004). With regard to the role of biogas as transportation fuel, Kapdi *et al.* (2005) stated that after removing carbon dioxide, biogas enriched in methane becomes equivalent to natural gas. Thus, methane enriched biogas can be useful for all applications that natural gas can do.

The bio-slurry from biogas digesters has been attested to be the best organic fertilizer which will lead to increased crop productivity. It can substitute chemical fertilizer and thus reduces the importation of chemical fertilizer and saves foreign currencies (Arthur *et al*., 2011). As stated by Breinholt (1992), the ammonia content of bio-slurry from biogas digester is about 10 % higher than the fresh manure. Moreover, bio-slurry is easier to dose and apply on crop fields

than the fresh manure as it is less viscous and lumpy manure. Biogas effluents are also rich in phosphorus (the most expensive fertilizer) and potassium.

Biogas technology generates employment opportunities for both skilled and unskilled labour. Specifically, in a well organized biogas development sector, biogas technology expansion opens employment opportunities for masons, plumbers, civil engineers, and agronomists. They are usually key promoters of the technology. Building of biogas installations, design and production of appliances and construction equipments are crucial areas of employment opportunity. Researchers may engage themselves in the area of improving the biogas system (Lam *et al.,* 2009; Arthur *et al.,* 2011). Ghimire (2008) enumerated the economic roles of biogas technology as follows. It saves expenditures on fuel sources; saves time to utilize in other income generation activities; increases soil fertility and reduces the required quantity of chemical fertilizer due to the use of bio-slurry; reduces health expenditures due to a decrease in smoke-borne diseases; and creates employment opportunities.

2.9.2. Health , Social and Environmental Benefits of Biogas

The use of biogas technology has numerous health, social benefits and environmental benefits The health benefits encompass: reduction in smoke borne diseases such as headache, eye-burning, eye-infection, and respiratory organ infection; improvement in household sanitation via toilet connection with bio- digesters and absence of sooth and ashes in the kitchen; and reduction in burning accidents (Ghimire, 2008). Similarly, Bajgain and Shakya (2005) revealed that utilization of biogas greatly ameliorates the quality of indoor air. It burns cleanly so that its use minimizes eye illnesses which results from burning of traditional biomass fuels. Besides, it assists maintaining sanitation of areas surrounding households via dung management and hygienic toilets connected to biogas digesters. Thus, it lowers the probability of expansion of contagious diseases. In other words, as stated by Aggarangsi *et al.* (2013), biogas technology provides health benefits not only to its users but also to the whole community in its environs.

Globally, around two million deaths a year from pneumonia, chronic lung disease, and lung cancer are linked to indoor air pollution from the use of solid fuels. In least developed and Sub- Saharan Africa countries, more than half of all the deaths from these three diseases are

associated with solid fuel use while it is merely about 38 % for the overall developing countries (Legros *et al.*, 2009). Thus, clean energy interventions such as dissemination of biogas technology in these regions can considerably reduce deaths due to indoor air pollution. Biogas technology has also various social roles. It improves social relations via minimizing bad odors and environmental pollutions of organic wastes which would have been otherwise serve as a source of grievance among neighbours and negatively affect social relations (Aggarangsi *et al.,* 2013). It saves time for social activities; it improves social status in the community; it lessens women and children's work burden; and it offers brighter light that assists quality education and household duties (Ghimire, 2008).

Biogas technology offers a wide range of environmental benefits. It provides sustainable source of energy and soil enriching bio-slurry as a by-product, ii) it gives an opportunity to treat and re- utilize variety of organic wastes, iii) it minimizes the environmental impacts of GHG emissions, and iv) it reduces land use problem associated with disposing organic waste (Aggarangsi *et al*., 2013). Biogas technology is one of the promising solutions to the diverse environmental problems associated with the use of traditional biomass fuels. According to Arthur *et al*. (2011), the rampant exhaustion of wood-fuel supplies, predicted increase in wood-fuel demand in the future and the resulting social and environmental effects urge the need to look for alternative sources of cooking fuel in developing countries. Consequently, biogas technology has been identified as one of the promising options to reverse deforestation and related problems.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted at Botany Laboratory of the Biological Science and Biotechnology, at Haramaya University. The University is found at 525 km away from Addis Ababa to the east. It is located at 9º26'N latitude, 42º03'E longitude and an altitude of 1980m.a.s.l. (FAO, 1990).

3.2.Sample Collection and Preparation of Substrate for Anaerobic Digestion

Two types of substrates viz. *Guizotia scabra* (GS) and fresh cow dung were used as feedstock for anaerobic digestion. *Guizotia scabra* was collected from Bodji Dermeji, west wollega Zone and the fresh cow dung was obtained from dairy farm in Haramaya University. After collection, both GS and CD were first sun dried and crushed separately using all-purpose high speed smashing machine to break them into smaller particles. Prior to subjecting to AD, the total solid of both substrates was determined. To help microbial seeding, Fresh rumen fluid was collected and filtered to remove large solid particle in the slurry and afterwards stored for a week time by incubating at 38ºC in incubator so that easily degradable volatile solid (VS) will be removed (Sunarso *et al.*, 2012).

3.3. Design of the Experiment

The experiments were arranged in a completely randomized design with three replications. Anaerobic digestion was conducted in batch mode in 0.5L digester using the two substrates, i.e., GS and CD in five proportions to have five substrate treatments. The five substrate treatments were 100% GS; 100% CD; 75% : 25% mix of GS:CD; 25%:75% mix of GS:CD; 50%:50% mix of GS:CD. To have 8% TS in fermenting slurry, appropriate amount of distilled water and rumen fluid (100 ml) were mixed (Techobanoglous *et al*., 1993). The temperature of bio-digester was maintained at 38°C by keeping in oven, which represents mesophilic condition (Knottier, 2003). The pH of the slurry was maintained within the pH range for optimal biogas production, i.e. about neutral (Thy *et al*., 2003; Yadvika *et al*., 2004).

3.4. Digester Configuration and Set up of Experiment for Biogas production

The experimental setup for this study was a batch digester constructed from a 0.5L capacity plastic bottle. Three plastic bottles were arranged in order with the first bottle containing slurry, the middle contained acidified brine solution and the last in order was empty for collecting the brine solution that was expelled out from the second container. The acidified brine solution was prepared by dissolving NaCl in distilled water with few drops of sulphuric acid until a supersaturated solution is formed to prevent the dissolution of biogas in the water. All the three containers were interconnected with plastic tubes having a diameter of 1 cm. The tube connecting the first bottle to the second was fitted just above the slurry in the first bottle to help gas collection. Thus, the biogas produced by fermentation of the slurry was driven from the first bottle to the second bottle that contained a brine solution to displace a volume of the brine solution equivalent to the volume of biogas produced. The lids of all digester were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas (Teame *et al.*, 2014)

3.5. Determination of the Physico-chemical Properties of the Substrates

Both GS and CD and their combinations in different ratios were analyzed for total solids (TS), volatile solids (VS), fixed solids, and pH before and after AD process based on procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA, 1999) as follows.

3.5.1 Total solids

First a clean evaporating dish was oven-dried (at 105 ^{0}C for 1hour), cooled in desiccators and weighed immediately before use. Feedstock (10g) was placed on the evaporating dish and put in an oven (105°C) using a crucible to evaporate for 24 hours. After 24 hours the crucible was taken out from the oven, cooled in desiccators and weighed. Then, the percentage of TS was calculated using the following formula (APHA 2540 B, 1999).

$$\%\text{TS} = \frac{mDS}{mFS} \times 100$$

Where,

%TS = percentage of total solids

mDS = mass of dry sample (final weight) in gram

mFS = mass of fresh sample in gram

3.5.2. Volatile and fixed solids

Once the TS was determined, the oven dried substrate was ignited at 550°C in a muffle furnace for 3 hours to determine the volatile and fixed solid content of the substrate. The following formula was employed to calculate the percentage of volatile solids content of the TS (APHA 2540 E, 1999).

$$\%\text{VS} = \frac{mDS - m(ash)}{mDS} \times 100$$

Where,

% VS = percentage of volatile solids

mDS= mass of dry solids in gram

m (ash) = remaining mass after ignition =fixed solid in gram.

3.5.3. C: N Ratio

3.5.3.1. Organic Carbon

For the determination of C: N ratio, first the amount of organic carbon and total nitrogen were determined. Organic carbon was determined using Walkley-Black method (Walkley – Black, 1934). For this 1g of dried organic substrate was weighed and transferred to a 500-mL Erlenmeyer flask. About 10ml of 0.167M $K_2Cr_2O_7$ was added by means of a pipette and 20 ml of concentrated H_2SO_4 was added by means of a dispenser and the mixture was swirled gently to mix thoroughly and the mixture was allowed to stand for 30 minutes. The flasks were placed on an insulation pad during this time to avoid rapid heat loss. The suspension was

diluted with 200mL of water to provide a clearer suspension for viewing the endpoint. Then 10ml of 85% H_3PO_4 and 0.2g of NaF were added using a suitable dispenser, (The H_3PO_4 and NaF were added to complex Fe^{3+} which was interfere with the titration end point). Finally, 10 drops of ferroin indicator was added. (The indicator was added prior to titration to avoid deactivation by adsorption). The mixture was then titrated with 0.5 M $FeSO_4$ to a burgundy end point. The color of the solution at the beginning was yellow-orange but turned to dark green at the endpoint (the change in color depends on the amount of un-reacted $Cr_2O_7^{-2}$ remaining, which shifts to a turbid grey before the endpoint and then changes sharply to a wine red at the end point). Use of a magnetic stirrer with an incandescent light made the endpoint easier to see in the turbid system (fluorescent lighting gives a different endpoint color).

Calculation

$$\%C = \frac{(\mathrm{B} - \mathrm{S}) \times \mathrm{N} \times 0.39 \times \mathrm{mcf}}{\mathrm{Wo}}$$

Where:

B = ml of $FeSO_4$ solution used to titrate blank

S = ml of $FeSO_4$ solution used to titrate sample

N= Normality of $FeSO_4$ (0.5N)

0.39= mill equivalent weight of C in g

mcf= moisture correction factor

Wo= dry sample weight in g

3.5.3.2 Total Nitrogen

The total nitrogen in the sample was determined using the Kjeldahl method. This method has three main steps. These were digestion, distillation and titration. One gram of sample and 6 ml of concentrated H_2SO_4 were added into a test tube and mixed carefully. Then 3.5 ml of H_2O_2 was added step by step and violet color due to reaction was observed. As soon as the violent reaction was ceased the tube was shaken by hand. After adding 3g catalyst mixture the sample was allowed to stand for 15 minutes in the test tube rack before digestion. Then the digester was allowed to wait until its temperature reached 370°C. When the digester's temperature reached 370°C digestion continued for about 4 hours until a clear

solution was observed. After the digestion process, tube was transferred to the fume hood for cooling. About 50 ml of distilled water was added and shaken by hand to avoid sulphate precipitation in the solution. At this time 25 ml of 40% NaOH solution was added into the digested and diluted solution. Then 250 ml of conical flask containing 25 ml of boric acid, 25 ml of distilled water and an indicator solution was placed under the condenser of the distiller with its tip immersed into the solution and the distillation continued for about 8 minutes until the total volume became between 200 ml to 250 ml. Finally the solution was titrated using 0.1N H_2SO_4 to a reddish color and %Nitrogen was calculated using the following formula:

$$\%\mathrm{N} = \frac{\mathrm{V} \times \mathrm{N} \times 0.014 \times 100 \times \mathrm{mcf}}{\mathrm{Wo}}$$

Where,

V = Volume H_2SO_4 in ml consumed during titration
N= Normality H_2SO_4 (0.1N).
0.014 = mill equivalent weight of nitrogen in g
mcf = Moisture correction factor
Wo = Sample weight on dry matter in g

Finally, C/N ratio was calculated by, $\frac{\%C}{\%N} = C:N$

3.5.4. pH Value Determinations

The pH value was determined using digital pH meter (HANNA HI 8314) before and after AD. In the case of before AD, an electrode was inserted into samples of substrate that was diluted using distilled water before inoculation of rumen fluid. The pH measurement after AD was done using pH electrode which was inserted into samples of substrate that was digested for about 30 days in AD process.

3.6. Evaluation of the Amount of Biogas

Biogas was collected by water displacement method. In order to prevent the dissolution of biogas in the water, acidified brine solution was prepared following the method suggested by Elijah *et al*. (2009). As biogas production commenced in the fermentation chamber, it was delivered to the second chamber, which contained the acidified brine solution. Since the biogas was insoluble in this solution, a pressure was built-up to provide the driving force for displacement of the solution. Thus, the displaced brine solution was measured to represent the amount of biogas produced daily starting from first day of incubation.

3.7. Data Analysis

After the completion of the whole laboratory process the data were subjected to analysis of variance (one-way ANOVA) using SPSS version 20. Paired samples t-test was used to investigate statistical significance within a bio-digester i.e between before and after anaerobic digestion. Difference between means was considered statistically significant at $P<0.05$.

4. RESULTS AND DISCUSSION

4.1. Physico-chemical Properties of the Substrates Used in Co-digestion.

4.1.1. Analysis of pH and %C Before and After Anaerobic Digestion.

Reports show that the pH value of the digester content is an important indicator of the performance and the stability of an anaerobic digester. For example, according to Mahanta *et al*. (2004) an optimum biogas production is achieved when the pH value of input mixture in the digester is between 6.25 and 7.50. Fang (2010) also reported that most anaerobic bacteria including methane forming bacteria function in a pH range of 5.5 to 8.5, but more optimal is pH of 6.8 to 7.6. Therefore, before, AD the pH of all substrates was adjusted to about neutral, which is suggested as optimal. Therefore, there was no difference between treatments in terms of pH before AD (Table 2). Comparison of pH values between before and after AD showed that pH values were significantly increased for all treatments after AD ($P<0.05$). Maximum pH value (8.55±0.06) was observed in 100% *Guizotia scabra*. Whereas the minimum pH value (7.96 ±0.01) was observed in 100%CD. Although the pH values increased after AD, the difference between treatments, however, was not significant (Table 2). According to Gerardi (2003) the rise in pH value after AD may attributed to ammonia accumulation that results from protein degradation.

Eventhough percent organic carbon appeared to slightly vary between treatments before AD, the difference between treatments was not statistically siginificant ($P<0.05$) (Table 2). However, there was a significant difference between treatments after AD, and between before and after AD (Table 2). Percent organic carbon of all treatments significantly ($P<0.05$) decreased after AD. It is obvious that reduction in organic carbon after 30 days of incubation is due to decomposition of organic matter by microorganisms to different products including methane (CH_4). However, the amount of organic carbon degradation varied between the different treatments with a substrate composed of 75% GS and 25% CD more reduced after AD, which could be also explained by the highest cumulative biogas yield observed as shown in Figure 5. The decrease in Carbon reflects the substrates degradation process during anaerobic digestion (Devlin *et al*., 2011).

Table 2. pH and and % organic carbon of the substrates both before and after AD. Values are mean ± SE, n=3.

	Parameters			
Treatments	pH Before AD	pH After AD	%C Before AD	%C After AD
T1	7.00 ± 0.01^{Ab}	8.55 ± 0.06^{Aa}	46.76 ± 0.35^{Aa}	38.22 ± 0.64^{Ab}
T2	7.22 ± 0.02^{Ab}	7.96 ± 0.01^{Aa}	44.14 ± 0.06^{Aa}	37.98 ± 0.16^{Ab}
T3	7.00 ± 0.04^{Ab}	8.38 ± 0.00^{Aa}	46.09 ± 0.64^{Aa}	30.85 ± 0.23^{Cb}
T4	7.07 ± 0.01^{Ab}	8.05 ± 0.02^{Aa}	44.59 ± 0.63^{Aa}	34.67 ± 0.11^{Bb}
T5	6.93 ± 0.02^{Ab}	8.25 ± 0.03^{Aa}	45.67 ± 0.17^{Aa}	31.91 ± 0.29^{Cb}

Means followed by different small letters in row show significant at P<0.05 for paired samples T-test within treatment. Means followed by different capital letters in column show significant difference at P<0.05 between treatments. T1=100%GS, T2=100%CD, T3=75%GS+ 25%CD, T4=25%GS + 75%CD, T5=50%GS + 50%CD. Note: GS=*Guizotia scabra*, CD=cow dung.

4.1.2. Analysis of TS and VS values of Substrate Co-digestion before and after AD

When analyzed before AD, the TS showed significant (P<0.05) difference between treatments with 100% GS substrates having the highest value followed by decrement in TS value with decreasing proportion of GS in the mix (Fig.2). This may show that GS contains more biodegradable substrates for biogas production. Similar trend was also observed after AD. However, when substrates were analyzed for TS after 30 days of incubation, values of TS in all treatments decreased significantly as compared to before AD (Fig. 2). The maximum reduction in TS was recorded from mix ratio of 75%GS and 25% CD that suggests high digestion of organic matter by microbes including methanogenic bacteria to result in more biogas yield, which actually is evidenced from cumulative biogas yield indicated in Fig. 5 below.

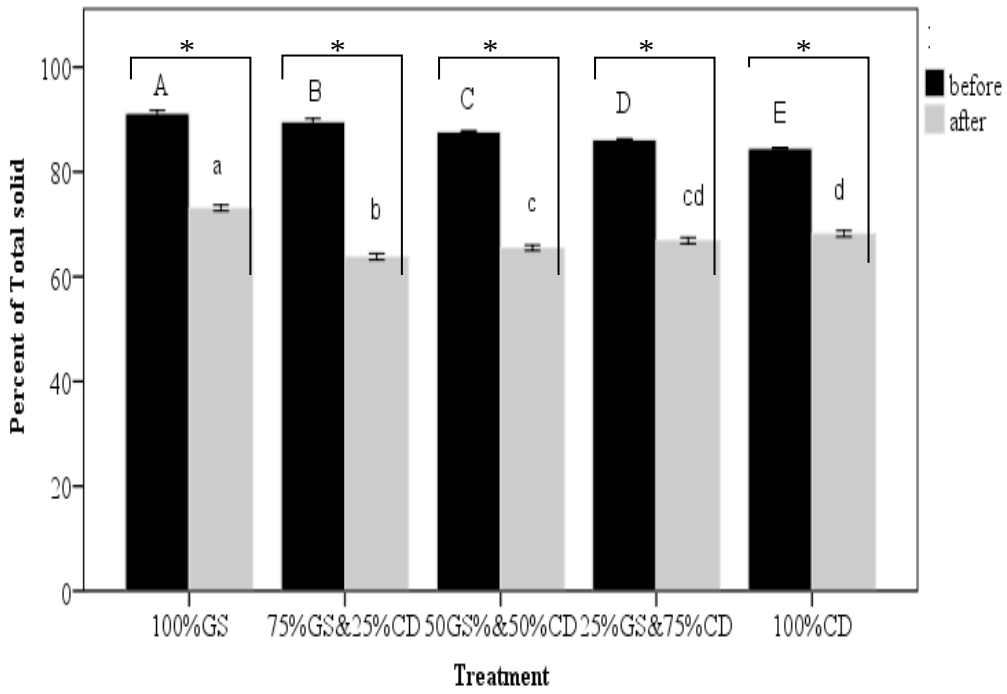

Figure 2. Values of TS for substrates before and after anaerobic digestion. Capital letters represent differences between %TS of the various substrates before digestion while small letters represent that of after digestion. Bar graphs with different capital or small letters are significantly different. Asterisk (*) shows there was significant difference in %TS within treatments between before and after digestion at $P<0.05$. Note: GS=*Guizotia scabra*, CD=cow dung.

Values of VS showed no statistically significant difference between treatments before AD although there was slight variation. However, they significantly varied after AD between treatments. Compared to before AD, VS significantly lower after AD with higher reduction seen in 75 % GS + 25 % CD mix substrates (Fig. 3). The outcome of this is the same as that of TS reduction which goes in par with the observed biogas yield. Overall, in this study, the maximum decrement of TS and VS after digestion has been directly related to maximum biogas productions. That is in bio-digester T3 (75%GS+25% CD) maximum reduction in TS and VS was noticed and highest biogas yield was also measured in this digester. Therefore, total solids and volatile solids destruction is a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012) and it is a good indicator of biogas production (Anonymous, 1981).

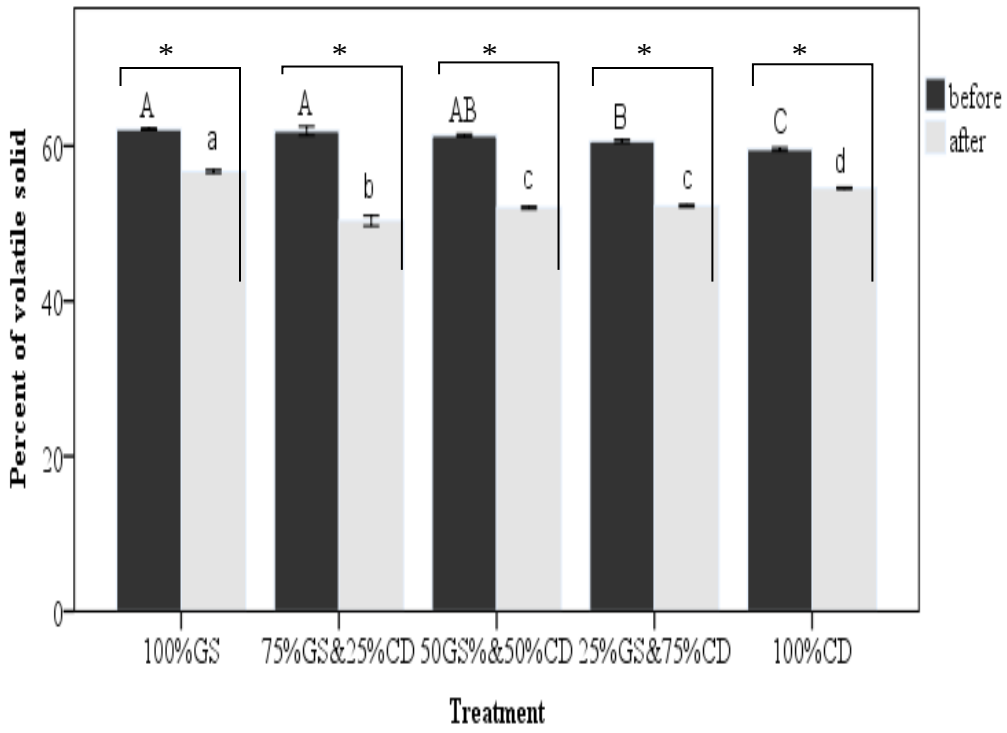

Figure 3. Values of VS for substrates before and after digestion. Capital letters represent differences between % VS of the various substrates before digestion while small letters represent that of after digestion. Bar graphs with different capital or small letters are significantly different where as those with same capital or small letters are not significantly different. Asterisk (*) shows there was significant difference in %VS between before and after digestion at $P<0.05$. Note: GS=*Guizotia scabra*, CD=cow dung.

4.1.3. Carbon to Nitrogen ratio before AD

Carbon to nitrogen ratio is a major factor affecting the anaerobic process which in turn affects methane yield and production rates. Therefore, the balance of carbon and nitrogen in a feed material is important. A suitable C: N ratio plays an important role for proper proliferation of the bacteria in the degradation process. It is generally known that during digestion, micro-organisms utilize carbon 25 to 30 times faster than nitrogen, i.e., carbon content in feedstock should be 25 to 30 times of the nitrogen content. To meet this requirement, constituents of feedstock should have C: N ratio of 20:1 to 30:1 (Marchaim, 1992; Fulford, 1988). Therefore,

before incubation C: N ratio of the substrates used in this experiment was determined from the initial % organic carbon and total nitrogen values. Results showed that values of carbon to nitrogen ratio of all the treatments ranged from 22.75:1 to 25.01:1 which is in agreement with the optimum C: N ratio 20 to 30 as stated by Fricke *et al.* (2005). Therefore the nutritional balance of all substrate types were suitable for methane production.

Depending upon the relative richness in carbon and nitrogen content, feed material can be classified as nitrogen or carbon-rich. If the C:N ratio is very high, the nitrogen will be consumed rapidly by methanogens to meet their protein requirements and will no longer act on the left over carbon content of the material. As a result, gas production will be low. On the other hand, if the C: N ratio is very low; nitrogen will be liberated and accumulated in the form of ammonia (NH3). The increased concentration of NH3 will increase the pH value of the slurry in the digester and ultimately lead to the inhibition of the growth of bacteria (Braun, 1982). The digestion of plant waste containing high nitrogen to carbon ratio is more likely to result in toxic conditions for bacteria arising from the concentration of free ammonia (Arogo *et al.*, 2009). As a result, gas production will be low.

Table 3. Carbon to nitrogen ratio of substrates before anaerobic digestion. (Values are mean ± SE, n= 3)

Treatments	%C Before AD	%N After AD	C:N Before AD
T1	46.76±0.35^{A}	1.87±0.06^{A}	25.01:1
T2	44.14±0.06^{A}	1.94±0.05^{A}	22.75:1
T3	46.09±0.64^{A}	1.88±0.05^{A}	24.51:1
T4	44.59±0.63^{A}	1.92±0.04^{A}	23.22:1
T5	45.67±0.17^{A}	1.90±0.04^{A}	24.03:1

Means followed by capital letters in column show no significant difference between treatments at $P<0.05$. T1=100% GS, T2=100% CD, T3=75%GS + 25% CD, T4=25% GS + 75% CD, T5=50% GS+ 50% CD. %C= percentage of organic carbons, %N = percentage of nitrogen.

4. 2. Determination of Average Daily and Cumulative Biogas Production from Solo and Co-Digestion of the Selected Substrates

Determination of average daily and cumulative biogas production from individual and co-digestion of different mixed ratio were analyzed and the results were indicated in Figures 4 and 5, respectively. Biogas production was monitored for 30 days of fermentation for all substrate types and production was noticed from day one of the experiment in all substrate types in a flactuating manner with gradual increase to peak on the 6th or 7th day of incubation and subsequent fall therefater to 0 on the 30th day of incubation**.** The fact that gas production occurred on the first day of the experiment suggests the existence of microbes in the added rumen fluid inoculum to act on readily degradable materials of the substrates (Teame, 2014; Kamthunzi, 2008). Peak production of biogas at about a week time of incubation suggests high proliferation of bacteria and decomposition of the available substrates to biogas, but reduction afterwards may show depletion of substrates or change of the digester's environment that actually needs detailed analysis of nutrient balance and accumulation of some other toxic substances.

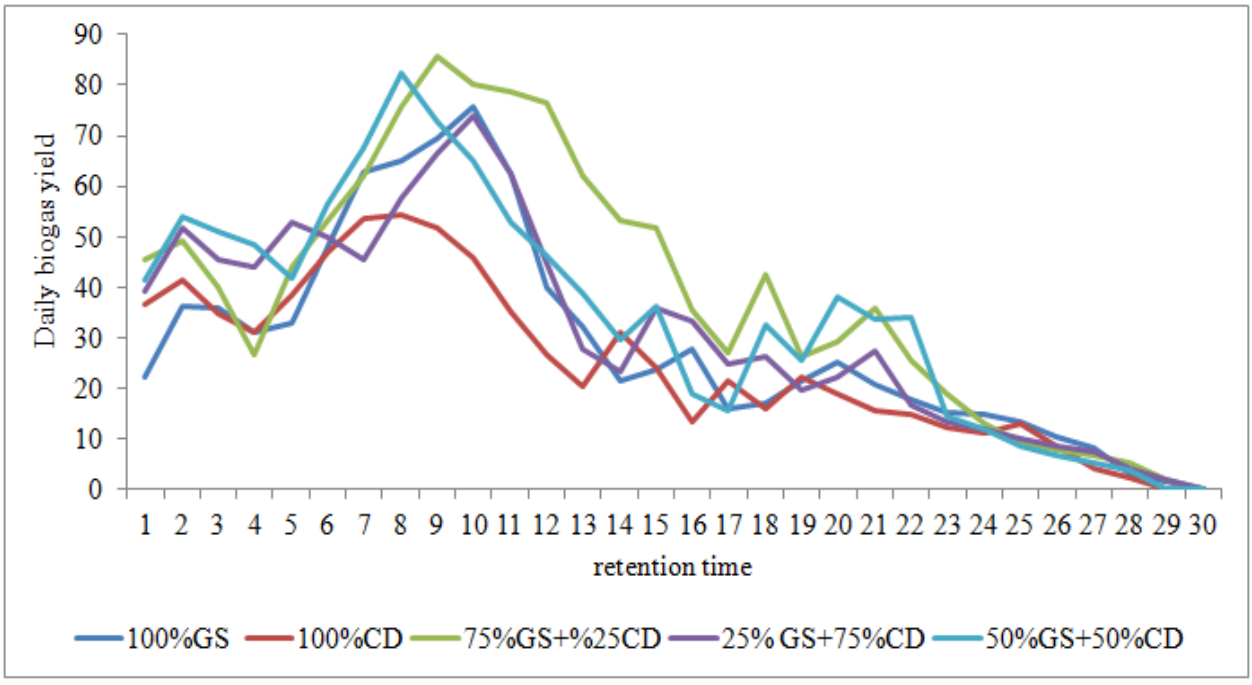

Figure 4. Daily mean Biogas yield in (ml) of *Guizotia scabra leaves* co-digested with cow dung in different mix ratios.

There was a significant difference between the substrates in an overall biogas yield ($p<0.05$) (Figure 5). Cumulative biogas yield of the three mixtures of GS and CD were significantly higher than CD substrate alone (Figure 5). That means, compared to 100% CD all substrate types resulted in higher cumulative biogas yield Similar results were reported by Tamrat Aragaw *et al*. (2013) from the co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. The maximum biogas production was measured in 75% GS+25% CD, whereas the minimum biogas production was measured from 100% CD. The %VS of 100% GS was higher but, it did not result more biogas than the combination with 75% GS+25% CD. This might be due to the less favorable situation of 100% GS to microorganisms as compared to 75% GS+25% CD. As the proportion of GS in the mix ratio increased with in 75% GS+25% CD, the cumulative biogas yield increased, suggesting that high favorable situation with increasing GS proportion from that of 75%. This observation was in accordance with the results of an experiment done by Callaghan *et al.* (1999). On the other hand, as the proportion of CD in the mix ratio increased from 25% to 75%, the cumulative biogas yield decreased, suggesting less favorable situation with increasing CD except for 100% GS which is without the combination of CD. Thus, it can be concluded that co-digestion of GS and CD is more productive with CD proportion not exceeding 25%.

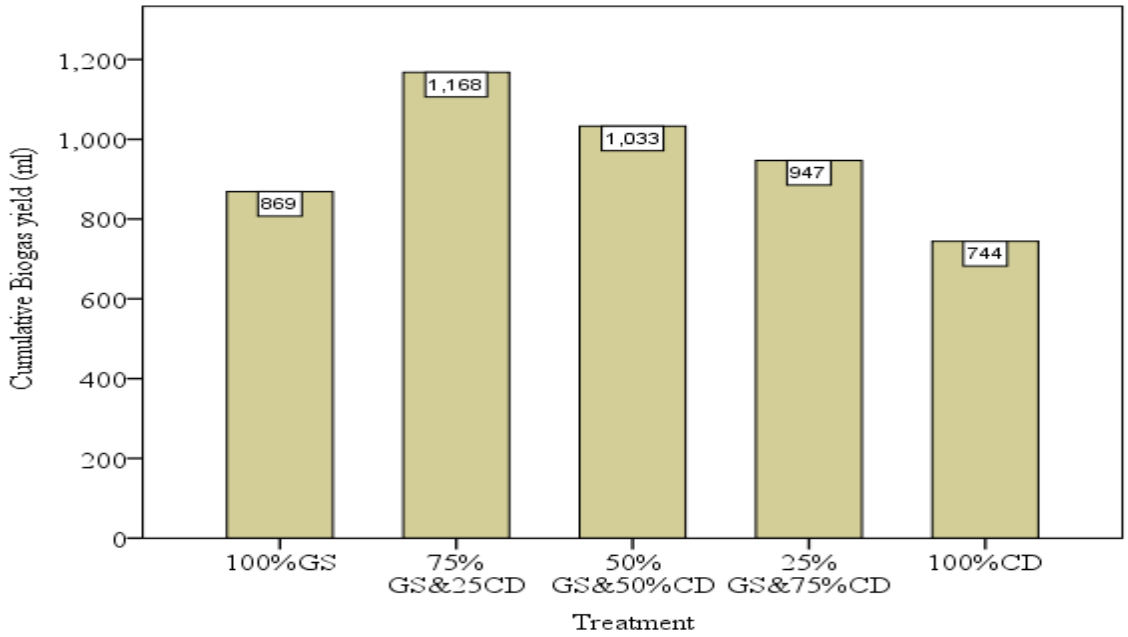

Figure 5. Cumulative biogas yield of the different substrate combinations. (Values are mean ± SE, n=3).

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

World energy consumption has increased steadily over the last century with increase in human population, transportation and technological advances. Biogas technology is a modern and eco-friendly technology based on the decomposition of organic materials in anaerobic environment at suitable and stable temperature by anaerobic bacteria. Biogas production from lignocellulosic materials such as agricultural plant residues can contribute to sustainable development of energy supply. Therefore, in this research, evaluation of biogas production from *Guizotia scabra* (GS) co-digestion with cow dung (CD) in five mix ratios viz. 100% GS, 100% CD ,75% GS + 25% CD, 25% GS +75% CD, 50% GS + 50% CD were carried out under mesophilic conditions (38ºC). In all treatments, TS, VS, organic carbon, and pH were analyzed before and after digestion.

In this study, C: N ratio of all treatments was found in between 20:1-30:1 which was a suitable condition for methanogenic bacteria to reproduce and produce optimum biogas. The comparison of pH values between before and after AD showed that pH values are significantly increased for all treatments after AD. Comparison of initial and final % organic carbon, TS and VS showed that all of them were significantly decreased after AD in all substrate types. However, maximum reduction in these values was observed in 75% GS and 25% CD after anaerobic digestion, which was in a par with the amount of biogas produced.

5.2. Conclusion

The general outcome of this study suggested that *Guizotia scabra* co-digested with cow dung improved the biogas production potential compared to mono-digestion of pure cow dung and *Guizotia scabra* alone. The experimental data showed the highest biogas production was obtained from mix ratio of 75% GS and 25% CD suggesting that, this mix ratio is optimal mix to yield maximum amount of biogas as compared to other mix ratio used in this study.

Therefore, it can be concluded that, this mix ratio enhances the rate as well as amount of biogas yield.

5.3. Recommendations

Based on the findings of this study, the following recommendations are forwarded:

- The co-digestion of *Guizotia scabra* in different proportions with other feed stock, especially the feed stock those have less C:N ratio need to be studied.
- The fertilizing value (mineral and micro-nutrient content) of *Guizotia scabra* slurry should be studied.
- Biogas production from *Guizotia scabra* need additional studies to improve the gas yield through different pretreatments.
- Awareness and skill development training on the sustainable use of as *Guizotia scabra* additonal substrate for biogas production for users and organizations is essential.
- This investigation was also done at mesophilic temperature of 38^0C, but it should also be carried out at room temperature (20^0C) and at thermophilic conditions.
- Efforts should also be made to determine quality of biogas produced from *Guizotia scabra* of the different combinations by Gas Chromatography.

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7. APPENDICES

7.1. Appendix I. Tables

Table 1. The proportion of different substrates added in the five digesters in three replicates.

Digester	Main content of digester								
	Mix ratio		TS in (g)		Amount of dry substrate in (g)		amount of water added (ml)	amount of inoculum added (ml)	Total volume (ml)
	%GS	%CD	GS	CD	GS	CD			
A	100	0	24	0	26.32	0	173.68	100	300
B	0	100	0	24	0	28.40	171.60	100	300
C	75	25	18	6	19.74	7.1	173.16	100	300
D	25	75	6	18	6.58	21.30	172.12	100	300
E	50	50	12	12	13.16	14.20	172.64	100	300

GS=*Guizotia scabra* , CD=cow dung , TS=Total solids

2. Percent (%) of TS and VS of substrates before and after AD (Values are mean ± SE, n=3).

Treatments	Parameters			
	%TS Before AD	**%TS After AD**	**%VS Before AD**	**%VS After AD**
A	91.20 ± 0.55^{Aa}	73.10 ± 0.57^{Ab}	62.17 ± 0.09^{Aa}	56.71 ± 0.20^{Ab}
B	84.50 ± 0.06^{Ea}	68.20 ± 0.59^{Bb}	59.55 ± 0.23^{Ca}	54.55 ± 0.10^{Bb}
C	89.62 ± 0.56^{Ba}	63.80 ± 0.60^{Db}	61.94 ± 0.17^{Aa}	50.35 ± 0.66^{Db}
D	86.20 ± 0.06^{Da}	66.90 ± 0.58^{Cb}	60.62 ± 0.19^{Ba}	52.27 ± 0.14^{Cb}
E	87.71 ± 0.12^{Ca}	65.49 ± 0.52^{Cb}	61.35 ± 0.17^{Ba}	52.04 ± 0.15^{Cb}

Means followed by different small letters in row were significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column were significantly at $P< 0.05\%$ level of significance between treatments.

A=100%GS, B=100%CD, C=75%GS&25%CD, D=25% GS&75% CD, E=50% GS& 50%CD

Table 3. Daily mean biogas yields from sole alone and co-digestion of GS and CD ±SE (ml) (n=3)

No. days	**A**	**B**	**C**	**D**	**E**
1	22.00±1.15	36.67±1.15	45.33±0.89	39.33±0.88	41.33±0.33
2	36.33±0.88	41.33±0.88	49.00±0.57	51.67±1.20	54.00±0.58
3	35.67±1.20	34.67±0.89	40.00±1.00	45.33±0.89	51.00±0.57
4	31.00±0.57	31.00±0.57	26.67±1.20	44.00±0.58	48.33±0.88
5	33.00±1.52	38.33±2.66	44.00±1.15	52.67±0.33	41.67±0.89
6	48.00±1.15	47.00±1.15	53.33±0.88	50.00±0.57	56.67±1.45
7	62.67±1.45	53.67±1.20	62.00±1.15	45.33±1.45	67.67±0.80
8	65.00±1.15	54.33±1.20	75.67±1.20	57.67±0.89	82.33±0.89
9	69.33±0.66	51.67±0.88	85.67±0.66	66.33±2.18	72.67±1.20
10	75.67±1.45	45.67±1.20	80.33±2.02	74.00±0.57	65.00±1.15
11	62.33±0.88	35.00±0.57	78.67±0.33	62.33±0.88	53.00±1.15
12	40.00±1.15	26.67±1.20	76.33±1.45	44.67±1.20	46.33±1.20
13	32.00±1.00	20.33±0.88	62.00±0.57	27.67±0.89	38.67±1.20
14	21.33±0.88	31.00±0.57	53.33±0.88	23.33±1.20	29.67±0.88
15	23.67±2.02	24.00±0.58	51.67±0.66	36.00±1.15	36.33±1.20
16	27.67±0.88	13.33±0.88	35.33±2.02	33.33±0.88	19.00±0.57
17	16.00±1.00	21.33±0.89	27.00±1.73	24.67±1.20	15.67±1.20
18	17.00±1.00	16.00±0.57	42.33±1.85	26.33±0.89	32.67±1.20
19	21.33±0.89	22.00±0.57	26.33±0.88	19.67±2.33	25.33±0.88
20	25.00±0.57	18.67±0.33	29.00±1.73	22.00±1.52	38.00±0.57
21	20.67±1.20	15.33±0.88	36.00±1.15	27.33±1.45	33.67±1.20
22	17.67±1.45	14.67±0.66	25.33±2.02	16.67±1.20	34.00±2.30
23	15.00±1.73	12.33±0.67	18.67±0.90	13.33±0.88	14.33±1.20
24	14.67±0.66	11.00±0.57	13.00±0.57	11.67±1.20	12.00±0.57
25	13.33±0.33	13.00±0.58	9.33±0.88	10.00±1.15	8.33±1.20
26	10.33±0.88	8.67±0.33	7.67±0.89	8.67±0.88	6.67±1.45
27	8.00±0.57	4.00±0.57	6.67±0.89	7.33±0.89	5.00±1.15
28	3.00±0.57	2.33±0.33	5.33±0.90	4.00±0.57	3.67±0.66
29	1.33±0.33	0.00±00	2.00±0.88	1.67±0.33	0.00±00
30	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00
Total	869	744	1168	947	1033

A=100% GS, B=100% CD, C=75% GS & 25% CD, D=25% GS &75% CD, E=50% GS & 50% CD

7.2. Appendix II. Figures

Figure 1. *Guizotia scabra* sub species *scabra*

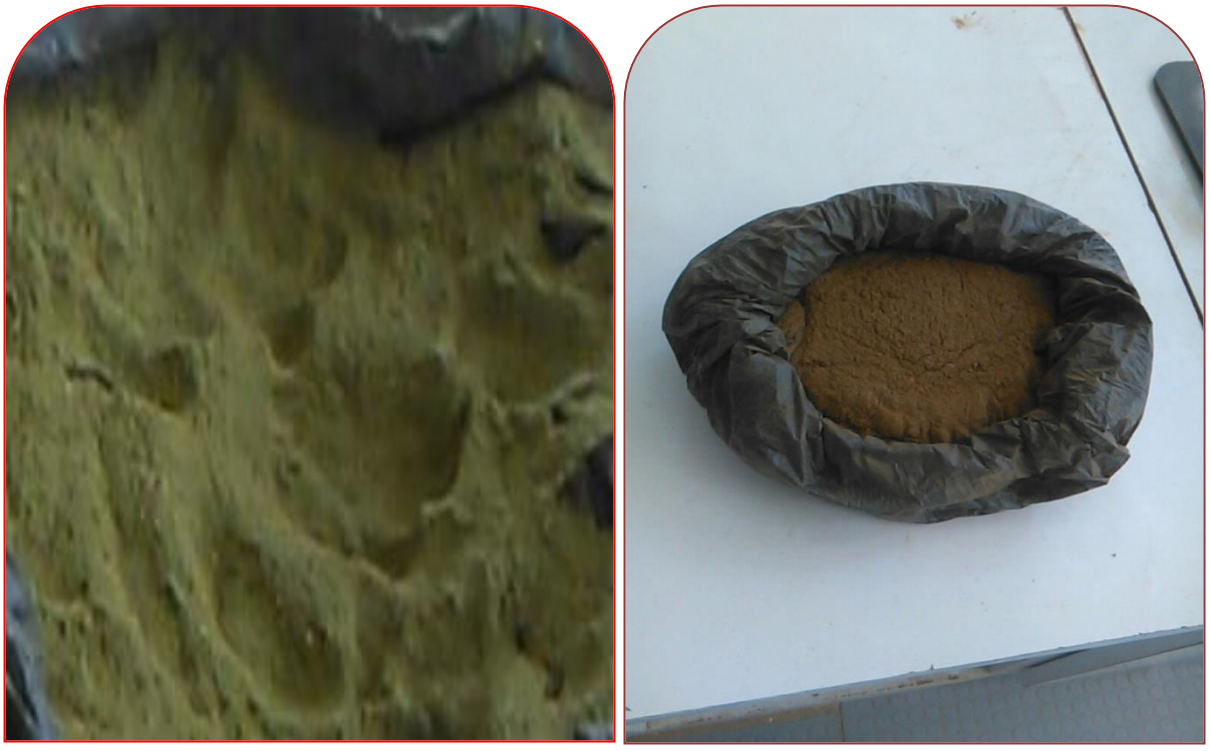

Figure 2. Dried powdered *Guizotia scabra* leaves and cow dung

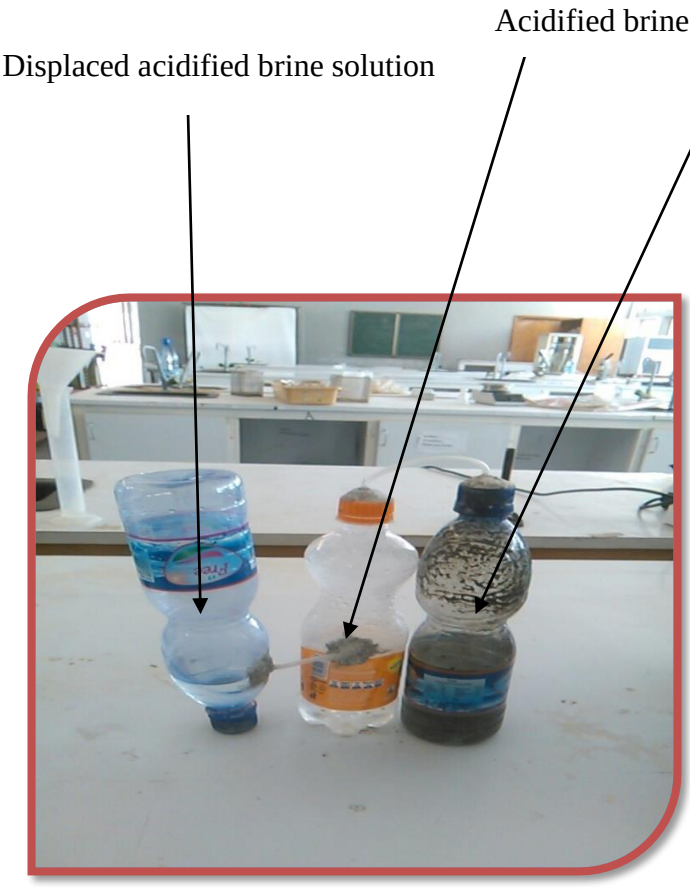

Figure 3. Batch form of experimental setup

Figure 4. Digesters in the oven at 38^0C

Figure 5. digesters arrangement

Figure 6. Rumen fluid used as inoculums.