EVALUATION OF HEAT PRE-TREATMENT ON BIOGAS PRODUCTION FROM CORN STOVER AND GOAT MANURE UNDER ANAEROBIC CONDITION

M.Sc. THESIS

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DECEMBER, 2020

HARAMAYA UNIVERSITY, HARAMAYA

Evaluation of Heat Pre-Treatment on Biogas Production from Corn Stover and Goat Manure under Anaerobic Condition

A Thesis Submitted to the College of Natural and Computational Sciences, School of Biological Science and Biotechnology

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

By

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December, 2020

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DEDICATION

This study is heartedly dedicated to my beloved Families, who have been my source of inspiration and strength to accomplish my thesis work.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

The author was born on January 5, 1992 from his father Alemu Innabo and his mother Shega Mekiso at Misha woreda, Hadiya Zone in Southern Nations, Nationalities and Peoples' Regional State (SNNPRS). He attended his elementary and junior education at Shiro primary and junior school, and secondary and preparatory school at Morsito senior secondary and preparatory school from 2008-2011 at Morsito town. Then, he joined Wolaita Sodo University in 2012 and graduated with B.Sc. degree in Applied Biology in June, 2014. After graduation the author joined Hawassa University for Post Graduate in Diploma Training (PGDT) in 2015 and completed in August, 2015. Then, after serving as a primary school teacher for two years in Tinika primary school and women's and children's affair for one year in Haddiya Zone, Misha Woreda, he joined Haramaya University in 2019 to pursue his M.Sc. degree in Biotechnology.

ACKNOWLEDGEMENTS

First of all, I would like to thank the Almighty God for being with me in all my difficulties and for his blessing and inspiration leading to the successful completion of this thesis work.

My deepest gratitude and appreciation goes to my major advisor Dr Meseret Chimdessa (PhD) for his excellent guidance, constant encouragement, and care during the entire course of my thesis work. I also extend my deepest gratitude to my co-advisor Dr Manikandan Muthuswamy (PhD) for the proper guidance during the research work. I feel very fortunate to have an opportunity to work under his supervision.

I would also like to acknowledge Haramaya University Botanical laboratory technicians especially Mr Yimasilali Atinafu, Central Laboratory technicians and general laboratory technicians who provided me with some required facilities. My appreciation goes to Haramaya University Abattoir house workers; who provided me rumen fluid (inoculum) and animal farm workers for goat manure sample which was used in the experimental investigation of this thesis work.

Lastly but not the least I would like to express my deepest gratitude to all my family members for their moral encouragement which put me on the right track to achieve the long way to destiny.

LIST OF ACRONOMYS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
C/N	Carbon to nitrogen ratio
CAFOs	Confined Animal Feeding Operations
CS	Corn Stover
GHG	Green House Gas
GM	Goat Manure
HRT	Hydraulic Retention Time
mcf	Moisture correction factor
mDS	Mass of dry sample
OLR	Organic Loading Rate
SPSS	Statistical package for social science
TS	Total Solid
VFA	Volatile Fatty Acid
VS	Volatile Solid

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Evaluation of Heat Pre-Treatment on Biogas Production from Corn Stover and Goat Manure under Anaerobic Condition

ABSTRACT

Biogas technology, which is meant to produce methane rich gas through anaerobic digestion of biological materials, is an environmentally friendly technology that decreases environmental pollution through decomposing organic wastes and positively impacts the socio-economy of the society. Therefore, the research was conducted to investigate the production of biogas from corn Stover co-digested with goat manure through anaerobic digestion. In this study, five different proportions of Corn Stover and Goat manure (100%Corn Stover, 75%Corn stover+25%Goat manure, 50%Corn Stover+50%Goat manure, 25%Corn Stover+75%Goat manure, 100%Goat manure) were used to obtain the suitable mix ratio by incubating at 38ºC using batch fermentation after the substrates were thermally treated at a temperature of 80°C and untreated separately. Having determined the optimum mix ratio, thermally treated substrates at 80ºC were applied to compare the results with those obtained with untreated substrates. In all treatments, physico-chemical parameters such as total solid (TS), volatile solid (VS), organic carbon, total nitrogen, carbon to nitrogen ratio and pH were measured before and after anaerobic digestion (AD). Gas production was noticed in all of the digesters from the 1st day of AD. The daily biogas production was subsequently measured by water displacement method for 30 days. Results showed that, in untreated substrates, biogas yield was minimum in the first three days of incubation and peaked at around 7th day of incubation with gradual reduction and close to none on 30th day. For thermally pre-treated substrates, however, biogas yield was even higher on the first day of incubation and peaked earlier (on the 3rd day) than untreated substrates. Out of the five treatments, treatment 4 (T_4=25%CS+75%GM mix ratio) has produced high cumulative amount of biogas in both 80°C thermal pre-treatments and untreated (1426.72 and 1177.65mL), respectively. Better cumulative biogas yield was observed on the all treatments pre-treated under 80°C than untreated. The highest reduction of TS (22.01%) and VS (11.33%) were recorded in T_4 thermally treated at 80°C. The result also revealed that co-digestion enhances the production of biogas and an increment of biogas was observed with the increment of the GM concentration in the co-digestion. Generally, the T_4 was the best mix among all other treatments which produced high cumulative biogas in 80°C thermal pre-treatment (1426.72mL). Overall the results indicated that the biogas yield and VS and TS reduction of the 25%CS+75%GM mix ratio can be enhanced with the use of thermal pre-treatments prior to anaerobic digestion.

Keywords/phrases: *Anaerobic digestion, biogas, corn stover, co-digestion, goat manure, thermal pretreatment,*

1. INTRODUCTION

Biogas technology is growing as a number of countries are accessing up biogas targets as a main approach for treating a variety of organic wastes. Biogas production decreases environmental pollution through decomposing organic wastes and positively impacts the socio-economy of the society (Lawrence, 2012). Today, utilization of biogas as an alternative energy source is steadily increasing. It accounts for up to 20% of renewable energy consumption in the European Union. About 52% of the biogas plants produce biogas from agricultural wastes, and about 36% are utilizing sewage sludge and the remaining 12% are landfill treatment plants. Germany is by far the major biogas producer in the world (Bisypln, 2012). For their economic progress, African countries need sustainable energy supplies. Unreliable energy supply may end up with low level of private investment in African continent. Therefore, improvement in the quality and magnitude of energy services in developing countries is required to meet developmental objectives including the Sustainable Development Goals (SDGs). Although reliable regional energy statistics are not readily available, the existing estimates of energy use in Eastern and Southern Africa indicate that there is a significant and persistent dependence on traditional biomass energy technologies and limited use of modern, sustainable energy technologies (Bartram *et al*.,2018).

Many developing countries resolved their energy and waste problems by developing biogas technology. Ethiopia is one of the countries with strong initiatives to promote biogas technology to reduce the severe energy problem faced especially in the household energy sector. Much of country's energy requirement had been allocated for household energy consumption. As a result, the promotion of biogas technology in the country was started a long time ago (Siltan Abraha, 1989). Biogas is one of the alternative energy sources to others as it is environmentally friendly. Biogas production from renewable biomass reduces fossil fuel dependence, decrease carbon dioxide emission and recovers bio-energy. Biomass 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 fossil-based fuels become scarce and more expensive, the economics of biogas production is turning out to be more favorable (Agarry an d Aremu, 2013).

Biogas is produced through anaerobic digestion (AD) in multi-step biological processes in which different anaerobic microbes take part. In such multi-step biological processes, complex organic substrates are converted to biogas and digestate by microbial action in the absence of oxygen through four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Kangle *et al*., 2012). Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. Traditionally, anaerobic digestion was a single substrate, single purpose treatment. However, recently it has been realized that AD as such became more stable when the variety of substrates applied at the same time. 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 substrates (Braun,2002).

The use of co-substrates with inoculums usually improves the rate and biogas yield from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates (Alvarez and Liden, 2007). Different feedstock's have main 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*., 2011). Moreover, it has been realized that an aerobic digestion is more stable when a variety of substrates are applied at the same time, and results in an increased biogas yield. Biogas production also directly influenced by total solid, volatile solids, loading rate, substrate particle size, digester temperature, pH and carbon to nitrogen ratio of slurry (Adelekan and Bamgboye, 2009).

Biogas is a renewable energy source and in many cases exerts a very small carbon footprint (Corral and Argelia, 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 bio-fuel that can be injected into the gas network of city (Murat, 1981). The gaseous methane can be combusted or oxidized with oxygen to release energy, hence used as a source of fuel energy for heating purpose, lighting and cooking (Sorathia *et al*., 2012).

Alternative candidate substrate, with high availability and low cost for AD systems, is lignocellulosic biomass (Chandra *et al*., 2012). Unfortunately, biodegradability of this biomass is low due to the rigid structure of cellulose, hemicellulose and lignin (Fernandes *et al*., 2009). Lignin is a non-anaerobically degradable and non-water soluble polymer and these two factors are limiting the biomethanation of lignocellulosic substrates. However, hemicellulose can be hydrolyzed to simpler sugars that are assimilable by microorganisms during AD. Due to the complex physical and chemical nature of lignocellulosic substrates, their complete biodegradation cannot be achieved in anaerobic digesters to result in high biogas yield (Rafique *et al*., 2010).

To overcome biodegradability problem, some pre-treatment methods can be employed (Bruni, 2010]. Pre-treatments, for example, biological (Zhong *et al*., 2011), mechanical (Angelidaki and Ahring, 2000), chemical (Devlin *et al*., 2011), thermal (Mladenovska *et al*., 2006), and combination of these treatments have been done to facilitate the biogas production by overcoming the limitation of hydrolysis, which include the solubilization and biodegradation of hemi-cellulosic and lignin parts of the substrates. Thermo-chemical pretreatments have a great impact on biogas production with a maximum enhancement of 78% for biogas and 60% for methane (Rafique *et al*., 2010).

Thermal pretreatment also has effect on biogas production with a maximum enhancement of 28% for biogas and 25% for methane. During these procedures, biomass structure opens up due to thermal expansion, and this is causing a reduction of the particle size and an increase in the pore volume. Moreover, the polysaccharides present in the lignocellulosic materials are hydrolyzed to simple sugars leading to higher degradation rates from the microorganisms of AD. As a result, the efficiency of the whole process can be increased while the retention time required for optimum biogas production is decreased (Theuretzbacher *et al*., 2015). This indicates that pretreatment of substrates urgently needs further investigation. Regarding to this, there have been a lot of researches already done on biogas production using co-digestions of animal manure and other plant species materials with or without pretreatment. However, no work has evaluated the biogas production potential of corn Stover after pretreatment in combination with goat manure. This study was, therefore, designed to evaluate biogas

production potential of corn Stover co-digested with goat manure with the following objectives.

General objective:

- The general objective of this study was to evaluate biogas production using anaerobic co-digestion of heat pre-treated corn Stover with goat manure.

Specific objectives:

- ✓ To determine the physico-chemical features (Total solid, Volatile solid, pH, Organic carbon, Organic nitrogen and Carbon to nitrogen ratio) of both substrates (Corn Stover and goat manure);
- ✓ To evaluate the impact of temperature pre-treatment of the substrates (Corn Stover and goat manure) on biogas yield of the substrates.
- ✓ To evaluate the average daily and cumulative biogas production from solo and mix of corn Stover and goat manure combined in different proportions;

2. LITERATURE REVIEW

2.1. Biogas and Its Characteristics

Biogas is an alternative and renewable energy source produced through anaerobic digestion of organic matter whereby the organic matter is converted into a combustible biogas rich in methane and a liquid effluent by anaerobic microbes. It consists of 55 to 80% methane and 20 to 45% carbon dioxide. However, depending on the source of organic matter and management of the anaerobic digestion process, small amounts of other gases such as ammonia (NH_3), hydrogen sulfide (H_2S), and water vapor may be present (Ogejo *et al*., 2009).

Anaerobic digestion of organic materials primarily consists of 4 main stages that are mutually beneficial in such some way that the product from one stage could be a precursor for the subse quent stages. Each stage involves different types of microorganisms (Sagagi *et al*., 2009). The first process is called hydrolysis in which the particulate organic materials such as cellulose, hemicellulose, pectin, and lignin undergoing hydrolysis by extracellular enzymes to convert polymers into monomers. Then, the soluble organic matter and the products of hydrolysis are converted into organic acids, alcohols, hydrogen and carbon dioxide by the bacteria of acidogenic and the process is called acidogenesis. Thirdly, the acetogenic bacteria convert the acidogen products into acetic acid, hydrogen and carbon dioxide called acetogenesis. Finally, archaea bacteria are accountable for methane production from the aforementioned substances as well as directly from other substrates of which formic acid and methanol are the most important. The process is called methanogenesis (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 (Taddese, 2018).

Biogas is a well-established fuel that can supplement or even replace wood as an energy source for cooking and lighting in developing countries. 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 GHG emission compared to the emission of landfill gas to the atmosphere (Nabuuma and Okure, 2006). Moreover, with the increasing size and regional concentrations of confined animal feeding operations (CAFOs), there is growing public concern over potential impact on environmental quality caused by CAFO generated wastes (Fulford, 1998). In response to this, regulatory agencies are scrutinizing animal waste management practices and revising regulations to reduce its environmental impact. Handling these wastes in compliance with stricter environmental regulations can have a significant economic impact on CAFOs. As a result, CAFO operators are evaluating waste management practices that convert wastes into higher value products. One approach to increasing the value of waste is to use it as an energy resource. Moreover, (Pound *et al*., 1981) observed that biogas production units provide a decentralized fuel supply and waste management system both of which are very attractive particularly in rural areas of developing countries.

2.2. Agro-wastes as 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 over and may spoil the environment if nor 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.

2.3 Compositions of Biogas

The composition of biogas largely depends on the type of substrate used for its formation. In general, biogas consists of 55 to 80% methane and 20 to 45% carbon dioxide (CO_2). However, depending on the source of the organic matter and the management of the anaerobic digestion process, small amounts of other gases such as ammonia (NH_3), hydrogen sulfide (H_2S), and water vapor (H_2O) may also present. It is the methane component of the biogas that will burn or produce energy. Sources of organic matter that have been used to produce biogas include animal manure, sewage sludge, municipal solid waste, food-processing wastes, and industrial wastes. The gas can be used to generate heat, electricity, or both. It can be burned in a conventional gas boiler to produce heat for nearby buildings or to heat the digester, or used in a gas engine to produce electricity (VCE, 2009).

Table 1: Typical approximate composition of biogas (Bilhat, 2009)

Components	Percentage
Methane	50 – 70
Carbon dioxide	25 – 45
Hydrogen	5 – 10
Nitrogen	1 – 2
Water vapor	2 – 7
Hydrogen sulphide	0.002 – 2
Ammonia	<1
Trace gases	<2

2.4 Substrates (Feed Stocks) for Biogas Production

2.4.1 Corn Stover

Corn Stover consists of residues of maize (*Zea mays* L.) plants grown for grain and left in the field following the harvest. Such Stover makes about half of the yield of corn crop and is similar to straw from other cereals grasses. It includes stalks, leaves, husks, and cobs. Because

the amount of maize dry matter left on the field is similar to the amount of dry grain produced, considerable quantities of maize Stover are available. Corn Stover is one of the largest potential annual crop-based biofuel feedstock for several key reasons: the feedstock is relatively uniform, the cost of production is relatively low, and large quantities are concentrated in some regions. Corn Stover is composed about 70% cellulose and hemicellulose, and 15% to 20% lignin. Cellulose and hemicellulose can be converted to biofuel, and lignin burned as a boiler fuel for steam/electricity generation (Glassner., *et al* 1998). Maize is a socioeconomically important crop used in human diets, animal feed and as an industrial resource. Based on the starch composition of the endosperm in the seed, maize varieties are grouped in three; normal corn (or non-waxy corn), waxy corn and sweet corn. The starch in waxy corn consists of amylopectin while normal corn contains 75% amylopectin and 25% amylose. Sweet corn is a variety of maize with high sugar content. Waxy corn or sweet corn is mostly used for human consumption in various foods, while normal corn is used for commercial purposes, including chemical products, vegetative oil and bio-fuel (Burge, and Deusing, 1989).

2.4.2 Goat manure

Combination of goat manure (GM) 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 multiple waste streams in a single facility. There are three main advantages of using goat manure for co-fermentation. Firstly, it is a good source for nutrients such as trace metals, 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 manure 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 resistant to the effect of volatile fatty acids (VFAs) accumulation and thus avoids inhibition processes. Several studies have reported that the biogas process could be improved and stabilized by applying co-digestion strategy (Fang *et al*., 2010).

2.5 Microbiology of Anaerobic Digestion and Its Steps in Biogas Production

Anaerobic digestion (AD) is a naturally occurring process where biodegradable matter is converted into biogas and a semi-solid material. It converts complex organic materials into simpler constituents in a series of metabolic interactions that involve a wide range of microorganisms in the absence of oxygen (Frigon and Guiot, 2010). AD technology for methane production is a more efficient method for energy generation from biomass compared to other biological processes, such as cellulosic ethanol because all biomass like protein, carbohydrate and lipid can be converted into biogas while only carbohydrates take part for ethanol production (Deublein and Steinhauser, 2011). Another benefit of anaerobic digestion meant for energy generation is the reduction of natural methane emissions from the self-decomposition of biomass in landfills or other open environments because the global warming potential of methane is estimated to be 20 times higher than carbon dioxide (Rutz and Janssen, 2008).

The organic fraction of almost any form of biomass, including sewage, sludge, food wastes, animal wastes and industrial effluents can be broken down through anaerobic digestion (Hassan, 2003). The idea of replacing wood fuel and petroleum oils by alternative fuels, such as biogas, is encouraged by governments in various countries to set up biogas programs. The organic dry matter can be divided into proteins, fats and carbohydrates all of which have different degradation characteristics. For example, leftover foods consisting of cooked foods, such as meat, fish, rice, bread, noodle and vegetable are mainly composed of protein, starch, sugar and fat. These food wastes contain highly biodegradable organic matter and thus result in higher methane production (Lin *et al*., 2011). The nutrient-rich solids left after digestion can be used as fertilizer (Ciborowski, 2004).

The conversion of complex organic compounds into methane and carbon dioxide requires different groups of microorganisms and is carried out in a sequence of four stages: hydrolysis, acidogenesis, Acetogenesis and methanogenesis. Hydrolysis occurs as extracellular enzymes, which are produced by hydrolytic microbes, decompose complex organic polymers to simple soluble monomers. Proteins, lipids, and carbohydrates are hydrolyzed to amino acids, long-chain fatty acids, and sugars, respectively. These small molecules are then converted by

fermentative bacteria (acidogens) to a mixture of volatile fatty acids (VFAs) and other minor products such as alcohol. Acetogenic bacteria then convert these fermentation products into acetic acid, CO_2 and H_2. Finally methanogenic bacteria use hydrogen and acetate (most important substrate) and produce methane and carbon dioxide. Conceptually, the microbial processes of AD can be described by the sequential steps of hydrolysis, acidogenesis, Acetogenesis, and methanogenesis as follows (Bitton, 2005).

2.5.1 Hydrolysis

It is a slow process that depends on the nature of the particulate matter and size of organic matter. For complex substrates with a high solid content, hydrolysis is usually the slowest step and hence the rate limiting step in the overall anaerobic digestion process (Lomborg, 2009). Hydrolysis converts complex organic matters such as carbohydrates, proteins and lipids into soluble organic molecules such as sugars, amino acids and fatty acids by the action of extracellular enzyme, i.e. cellulase, amylase, protease and lipase. Hydrolytic bacteria, which hydrolyze the substrate with these extracellular enzymes, are facultative anaerobes (Bilhat, 2009). Proteins are broken down into amino acids, small peptides, ammonia and CO_2. While polysaccharides are generally converted into sugars. There are three main hydrolytic bacteria: the photolytic bacteria produce an enzyme known as protease for the breakdown of proteins and peptides into ammonia and amino acids, the lipolytic ones generate lipase enzyme for the breakdown of saponifiable lipids into fatty acids and glycerol, and cellulolytic bacteria create hydrolase enzymes for the breakdown of polysaccharides into sugars. Most of these microorganisms are obligate anaerobes and few of them are facultative. The degradable polymeric substrates found in solid waste include lignocelluloses, proteins, lipids and starch (Veeken *et al*., 2000).

2.5.2 Acidogenesis

In the acidogenesis step, the soluble organic molecules from hydrolysis are utilized by fermentative bacteria or anaerobic oxidizers (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). In this step, sugars and amino acids are the major substrates. Results of glycerol fermentation are propionate production and biomass generation (Angelidaki *et al*., 2003). Acidogenesis step is usually considered the fastest step in anaerobic digestion of complex organic matter (Lier, 1996), which can lead to accumulation of VFAs and a drop in pH when acid utilization is inhibited or too slow due to organic overload toxicants or rapid temperature change. The pH drop can inhibit or stop methanogenesis completely. However, when the concentration of hydrogen is high, the fermentative bacteria will shift the path way to produce more reduced metabolites (Angelidaki *et al*., 2002).

2.5.3 Acetogenesis

In Acetogenesis step, the acetate forming microorganisms convert alcohols, volatile fatty acids such as butyric acid, propionic acid and valeric acid other than acetic acid to CO_2, hydrogen and acetic acid. In other words, acetogenic organisms are the vital link between hydrolysis/aci dogenesis and the methanogenesis in anaerobic digestion. Acetogenesis provides hydrogen and acetate which are the two main substrates for the last step in the methanogenic conversion of organic material. Both acidogenesis and Acetogenesis produce the methanogenic substrates: acetate and H_2/ CO_2. The products of acidogenesis (that are volatile fatty acids and alcohols) are utilized by hydrogen producing acetogens, using carbon dioxide and hydrogen ions as electron acceptors (e.g. *Syntrophomonas wolfei*). This bioconversion process is not exergonic and thus, a syntrophic relationship with methanogens is mandatory to maintain the H_2 partial pressure low for acetogenic reactions to be energetic favorable (Treu *et al*., 2016).

2.5.4 Methanogenesis.

The last step in anaerobic digestion in biogas production, methanogenesis, is performed by a specialized group of microorganisms belonging to the methanogens. There are three known types of methanogens; acetoclastic, hydrogen-trophic, and methyl-trophic. Acetoclastic methanogens convert acetate to CH_4 and CO_2, hydrogen-trophic methanogens use H_2 or formate to reduce CO_2 to CH_4, and methyl-trophic 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 CH4 is produced from acetate, and the

remaining from H_2 and CO_2 and formate (Angelidaki *et al*., 2011). Only a minimal amount of CH_4 is produced via methyl-tropic methanogenesis (Ferry, 1993). Archaea bacteria are more sensitive to changes in temperature than other organisms present in the digester. This is due to the faster growth rate of the other groups, such as acetogens, which can achieve substantial catabolism even at low temperature (Parawira, 2004). At higher temperatures, the acetate oxidation pathway becomes more favorable (Fang, 2010). Extended methane production can be conducted via the hydrogenotrophic pathway based on process characteristics (that is, temperature, feedstock characteristics) (Wirth *et al*., 2012; Campanaro *et al*., 2016).

The aceticlastic and syntrophic acetate oxidations are the two potential pathways for methanogenesis consuming acetate. In the first pathway, the aceticlastic methanogens consume acetate and produce methane and carbon dioxide (Angelidaki *et al*., 2011). Regarding the syntrophic acetate-oxidation pathway, initially, the syntrophic acetate oxidation bacteria convert acetate into hydrogen and carbon dioxide and subsequently, these products are taken from hydrogenotrophic methanogens and convert them to methane (Zinder and Koch, 1984; Kougias *et al*., 2016). Methanosarcinaceae spp. and Methanosaetaceae spp. are able to perform the aceticlastic methanogenesis (Fotidis *et al*., 2014). Conversely, syntrophic acetate-oxidizing bacteria can perform the reverse Wood Ljungdahl pathway (a set of biochemical reactions used by some bacteria and archaea called acetogens) followed by hydrogenotrophic methanogens (Methanomicrobiales spp., Methanobacteriales spp. and Methanococcales spp.) (Karakashev *et al*., 2006; Campanaro *et al.*, 2016).

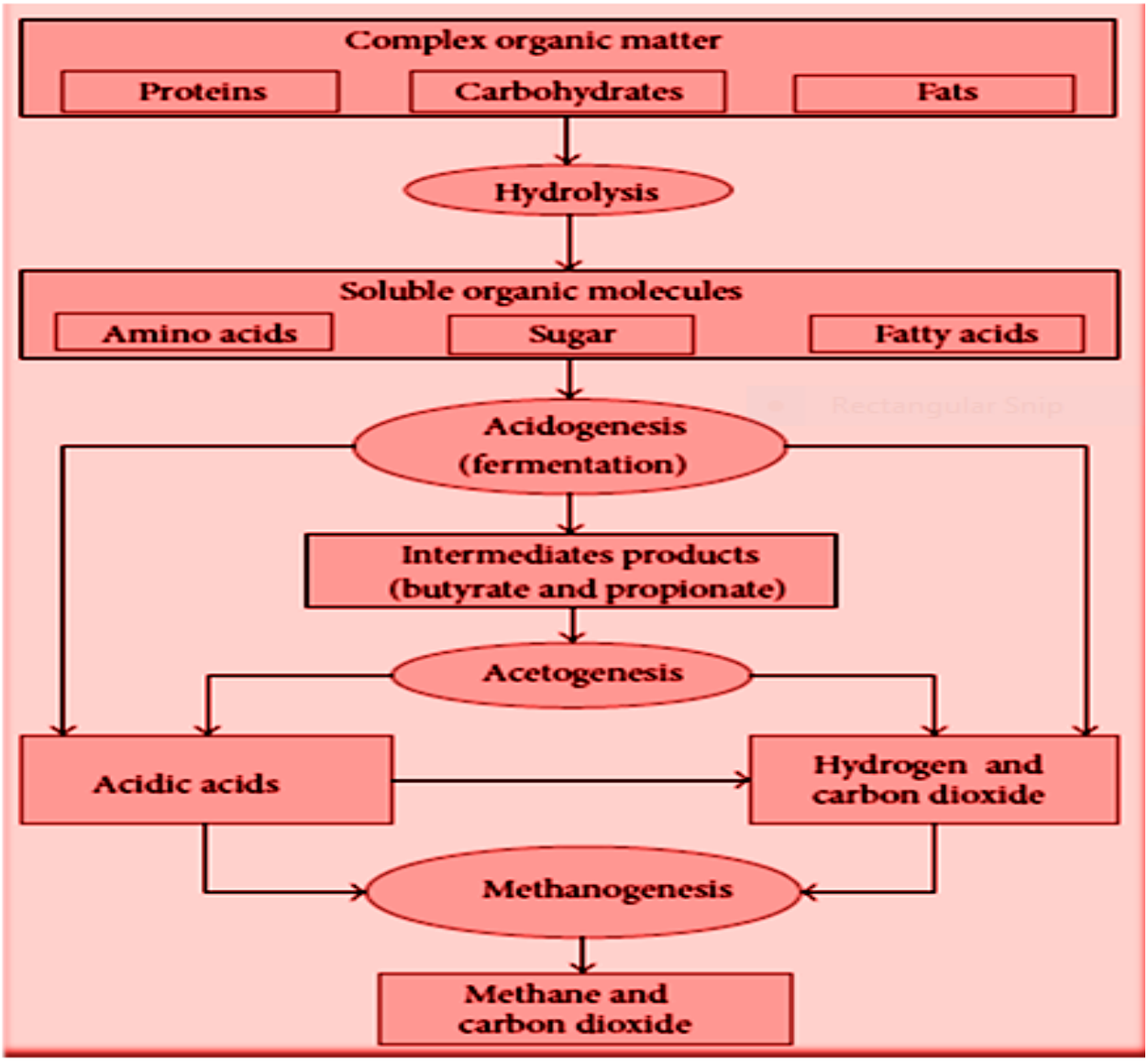

Figure 1: Biogas production from organic substrates (Abdelgadir *et al*., 2014).

2.6. Factors Affecting Biogas Production

The factors affecting the biogas production are mainly caused by the characteristics of the feedstock and operating condition of the process. For anaerobic reactors to perform at their best, they should be operated under steady state conditions. The most important parameters that can often determine the performance of the digester include temperature, pH, toxic materials, total solid, type of substrate, carbon/nitrogen ratio, organic loading rate, hydraulic retention time, ammonia, volatile fatty acids and agitation of the content of digester (Gossa, 2014). These factors can either enhance or inhibit the performance of the anaerobic digestion

by influencing parameters such as specific growth rate, degradation rates, biogas production or substrate utilization.

2.6.1 Temperature

Bacteria have a limited range of temperature in which they are active (Elango *et al*., 2006). Methane production has been documented under a wide range of temperatures, but bacteria are most productive in either mesophilic conditions, or in the thermophilic range. 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).

2.6.2 pH

The pH value of the AD substrate influences the growth of methanogenic microorganisms and affects the dissociation of some compounds of importance for the AD process (ammonia, sulphide, organic acids).The pH-value is the measure of acidity or alkalinity of a solution (respectively of substrate mixture, in the case of AD) and is expressed in parts per million (ppm). 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 *et al*., 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. GM is also used to facilitate the bacterial growth in the digester and thus speed up 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 *et al*., 2010).

2.6.3 Hydraulic retention time (HRT)

Hydraulic retention time (HRT) can be defined as the theoretical time of the particle or volume of liquid added to a digester and remained in it (Nijaguna, 2006). Similarly, retention time also defined as the length of time that volatile solid (VS) remain in the reactor (Dobre *et al*., 2014). Hydraulic retention time (HRT) refers to the average range that the complex compound retained in the digesters, in contact with the biomass and decomposes into metabolic products such as monosaccharide's, polysaccharides and amino acids (Dobre *et al*., 2014). 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 take 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 (Dobre *et al.* 2014).

2.6.4 Carbon to Nitrogen ratios

Necessary elements such as carbon, hydrogen, nitrogen, phosphorus and many other microelements must be present in adequate quantities for the normal growth of the micro-organisms. It has been recognized that all living organisms need nitrogen for the synthesis of protein. In the absence of sufficient nitrogen, the bacteria would not be able to utilize all the carbon present and the process would be less efficient. The carbon: nitrogen (C/N) ratio expresses the relationship between the quantity of carbon and nitrogen present in organic materials. Materials with different C/N ratios differ widely in their yield of biogas. For effective anaerobic digestion carbon to nitrogen ratio should be maintained between the ranges 20-30 (Perera, 2011). If C/ N ratios are higher than this range, biogas production will be low, because the nitrogen content of the feed material will be consumed rapidly by methanogenic bacteria for meeting their protein requirements rather than reacting on the carbon in the material. A material with high C/N ratio is typically the residues of agricultural plants. Conversely if C/N ratio is very low, that is outside the ideal range, nitrogen will be liberated and will accumulate in the form of ammonia, which raises the pH value of the slurry in the digester. To maintain the optimum C/N ratio of the influent feed, different types of material are mixed together. The microbial populations involved in anaerobic digestion require sufficient nutrients to increase in well-defined proportion. In addition to carbon and nitrogen in the digestion system, microorganisms also need micro-nutrients (trace minerals) and phosphorus for their growth (Ludwig, 1988; Jan and Felix, 2010).

2.6.5 Sulphate reduction

The effect of sulphate reduction on anaerobic system is complicated by the fact that the reduced product sulphide has inhibitory effect on almost all the microbial groups (Batstone *et al.*, 2002). The methanogenic microorganisms completing with sulphate reducing microorganisms for the common intermediate acetic acid, due to the presence of sufficiently high concentration of sulphur (Speece, 1996).

2.6.6 Volatile solids

The weight of organic solids burned off when heated to about 550ºC is defined as volatile solids. The biogas production potential of different organic materials can also be calculated on the basis of their volatile solid content. According to Santana and Pound, (1980) biogas production increase 1inearly with increasing total solids concentration.

2.6.7 Ammonium (NH4+) and Ammonia (NH3)

Ammonia and ammonium ion result from the anaerobic biological degradation of nitrogenous matter, mostly in the form of proteins and urea. Ammonia forms ammonium ions in the substrate, the extent of this depending on the pH value. Ammonia has an inhibiting effect, and with larger concentrations can even be toxic, while ammonium is innocuous. Its inhibiting effect is predominantly due to the species whose concentration depends on the pH value (Deublein, D *et al*., 2008). When hydrogen ion concentration in the solution is sufficiently high then the equilibrium is shifted to the left and ammonium ions are the main constituents of the mixture. At higher pH values this equilibrium shifts towards dissolved ammonia gas in solution. Among the four types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition. As ammonia concentrations were increased in the range of 4051–5734 mg L^{-1}, acidogenic populations in the granular sludge were hardly affected while the methanogenic population lost 56.5% of its activity. It is generally believed that ammonia concentrations below 200 mg/L are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms (Arceivala and Asolekar, 2006).

2.6.8 Agitation or stirring of the content of digesters

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).

2.6.9. Co-digestion of feed stocks

Co-digestion uses multiple feed stocks. The C/N ratio and pH of the digest can be adjusted by selecting an appropriate mixture of feed stocks. Different feed stocks have different gas yield potentials. Materials with high C/N ratios, such as waste wheat and bread, typically have a much higher gas yield than materials with a low C/N ratio, such as cattle and pig manure. Co-digestion can be used to selectively improve the biological and nutrient environment in the digester (FAO, 2011).

Fernandez *et al*. (2005) described, co-digestion as the term used to describe the combined treatment of several wastes with complementary characteristics, being one of the main advantages of the anaerobic technology. Akuzuo and Okechuku.D.O, (2010) also reported, the improvement of the buffer capacity is as a positive effect in the co-digestion process. Walker *et al*. (2009) showed, that blending the paper waste with cow dung or any other animal waste will give sustained gas flammability throughout the digestion period of the waste since plant wastes are good starters for poor producing wastes. Co-digestions of cattle slurry with poultry litter (7.5% and 15% TS) gave higher cumulative productions of methane, and the system with the lower concentration of poultry litter gave a higher specific methane yield. However, there was some evidence of ammonia inhibition. Comparing the single waste digestions with co-digestion of combined wastes, it was shown that co- digestion resulted in higher methane gas yields. In addition, co-digestion of MSW promotes synergistic effects resulting in higher mass conversion and lower weight and volume of digested residual (Macias-Corral *et al*., 2008).

2.6.10 Organic loading rate

The degree of starvation of microorganisms in biological systems is dependent on the organic loading rate (OLR). At a high OLR, a fast microbial growth (but intoxication may occur with

high quantities of organic matter) takes place whereas at a low OLR microorganism starvation takes place. However, if the applied OLR is too high, microorganism could not use up all produced organic acids and causes acidic state of the digester (Liu and Tay, 2004). OLR is mainly determined based on feeding materials and reactor temperature.

2.6.11 Particle Size

Particle size exerts less influence on gas production relative to temperature or pH of the digester contents. Large particle size of the feedstock will result in clogging of the digester thereby making difficult for the microbes to carry out the digestion function. The hydrolysis rate has been found to be directly related to the amount of substrate surface available. The surface of the particulate substrate has been reported to be a key factor for the hydrolysis process. Also, the rate of hydrolysis of particulate organic matter is determined by the adsorption of hydrolytic enzymes to the biodegradable surface sites and an increase in biodegradability results in an increase in adsorption sites for enzymes. Thus, reduced particle size could increase hydrolysis rate and shorten digestion time (Huy, 2008). Physical pretreatment such as grinding, mashing and shredding the wastes could significantly reduce the volume of digester required, without decreasing biogas production (Yadvika *et al*., 2004).

2.6.12 Nutrients

All biological processes require sufficient supply of nutrients particularly carbon and nitrogen as well as other elements are also required in trace quantities. The lack of specific elements can limit microorganism growth required for the production of biogas (Anunputtikul and Rodtong, 2004). If the manure is in dry form, the quantity of water has to be increased accordingly to arrive at the desired consistency of the substrate. When organic substance added to digester has large amount of water content, then relatively low amount of water is need to get total solid content (Jan. L, 2010).

2.6.13 Types of biogas digesters

There are different types of digesters that can be used for anaerobic digestion and they are often classified as: (1) Liquid or solid state process; (2) batch or continuous process; (3) single or double stage process. Based on the total solids (TS) content within the anaerobic digester,

the AD process can be divided into two types: liquid AD process (L-AD), with a TS content of less than 15%; and solid-state AD process (SS-AD), with a TS content of 15% or higher (Yang *et al*., 2015; Brown *et al*., 2012; Li *et al*., 2011). The L-AD type is more suitable for substrates with high moisture content, such as waste water streams (Sawatdeenarunat *et al*., 2015).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted in Botanical Laboratory of Haramaya University School of Biological Sciences and Biotechnology. Haramaya University is found at about 510 km East of Addis Ababa. The University is located at 9º24'N latitude and 42º01' E longitude with an elevation of 2044 m.a.s.l (FAO, 1990).

3.2. Experimental Design and Procedure

In this study, the total working volume was 300mL containing appropriate amounts of substrate, distilled water and inoculums. The pretreated substrates were transferred into 0.6 L plastic bottle digesters and the TS was adjusted to 8% (Tchobanoglous *et al*., 1993) by adding appropriate amount of distilled water considering the distilled water that was added for pretreatment and inoculums (100 mL) (Table 2). The pH of the slurry was maintained within the pH range for optimal biogas production (6.8 -7.2) by adding sodium hydroxide and hydrochloric acid to the organic substrate that was about neutral (Yadvika *et al*., 2004) after the initial pH was measured. Finally, the slurries containing inoculums were mixed thoroughly by shaking and AD lasted for 30 days under mesophilic (38ºC) temperature in hot air oven.

The experiment was arranged randomly in three replicates with three plastic bottles with one of the bottles containing the slurry, the second containing acidified brine solution and the third empty plastic bottle (to collect brine solution). Acidified brine solution was prepared by adding NaCl (Sodium Chloride) to distilled water with few drops of concentrated sulphuric acid until a supersaturated solution was formed (Kivuyo *et al*., 2017). This helps to prevent the dissolution of biogas in the water. The three containers were interconnected with a plastic tube having a diameter of 0.5 cm. The tube that connected the first bottle to the second was fitted just above the slurry in the first bottle to help gas collection. The lids of all digesters were sealed tightly using Amir Alpha (alpha-cyanoacrylate adhesive) and ash in order to control the entry of oxygen and loss of biogas production. The temperature of all digesters was maintained at 38°C by keeping in oven, which represented mesophilic condition

3.3. Sample Collection and Preparation of Substrates for Anaerobic Digestion

In this study, both Corn Stover (CS) and goat manure (GM) were used as feed stocks for AD process of biogas production. Approximately 4.5kg of CS was collected from farm land around Haramaya University (HU) and 3.5kg fresh GM was collected from Haramaya University goat farm by using plastic bag (kurtu). Both CS and GM were sun dried for consecutive seven days and chopped to size of 10–20mm and then crushed mechanically to 0.2–2 mm using all-purpose high speed smashing machine (Monlau *et al.*, 2013). Then after, it was sieved by a mesh that had 0.5 mm diameter to ensure consistency of the mix. Then, 800 g powder of each substrate was covered with aluminum foil and kept in a refrigerator at 4ºC until used.

Four liters of fresh rumen fluid (inoculums) used as a starter of anaerobic digestion was also collected from the nearby slaughterhouse found at Haramaya University using plastic bottles. The fluid was filtered through a cloth of 0.5 mm sieve diameter to separate solid content from slurry and pre-incubated for seven days and fully degassed at the temperature of 38ºC as selected for methane fermentation of both substrates. This was carried out to ensure degradation of easy degradable organic matter still present in the inoculums and remove dissolved methane (Lo Niee Liew, 2011). The existence of anaerobic micro- organisms in the inoculums were also examined by culturing using pours plate technique in anaerobic jar.

3.4 Physicochemical Characterization of Substrates before and after AD

The physicochemical properties were analyzed before and after AD by the standard methods. Total solid (TS) and volatile solid (VS) were determined gravimetrically (APHA (1999)), pH was determined by pH meter, Organic carbon (OC) was determined by Titrimetric method (Walkley and Black, 1934), Total nitrogen (TN) was determined by Kjeldahl digestion, TS and VS reductions were also estimated from initial and final values measured. Carbon to nitrogen ratio was then obtained from values of organic carbon and total nitrogen.

3.4.1 Total Solids (TS)

A clean evaporating dish (crucible) was dried at 105ºC for 1hr, cooled in desiccators and weighed immediately before use. Ten grams of CS and GM were weighed separately using an analytical balance and placed on a pre-dried and weighed evaporating dish. Then, the dish (crucible) was placed inside an oven maintained at 105ºC. The dish (crucible) was allowed to stay in the oven for 24hrs and then taken out, cooled in desiccators and weighed. The percentage of the TS was calculated using the formula indicated in APHA (1999) as follows:

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

Where: - %TS = percent of total solid
mDS = mass of dry sample
mFS = mass of fresh sample

3.4.2 Volatile Solids (VS)

The total solid obtained was ignited at 550ºC in a muffle furnace for 5hrs to determine the volatile and fixed solids of the sample. Then volatile solid content in the sample was determined using the formula indicated in APHA (1999).

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

Where: - %VS = percent of volatile solids
mDS = mass of dry sample
m (ash) = remaining mass after ignition =fixed solids
i.e., TS = VS + fixed solids

3.4.3 Carbon to Nitrogen Ratio

In order to determine the C/N ratio, the amount of organic carbon was first determined by Walkley-Black method while the N was determined using macro-kjedahl method. Thereafter, C/N ratio of each substrate was determined (Walkley – Black, 1934). One gram (1g) dried

organic substrate was weighed and transferred to a 500-mL Erlenmeyer flask. About 10ml of 0.167 M $K^2Cr^2O_7$ was added by means of a pipette and 20mL of concentrated H_2SO_4 was added by means of a dispenser and was swirled gently to mix thoroughly, (avoiding excessive swirling that will result in organic particles adhering to the sides of the flask out of the solution).This 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_2O7^{-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).

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

Where:

%C = percent of carbon

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 = dray sample weight in g

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 was added into a test tube and mixed carefully. Then 3.5 ml of H_2O_2 was added step by step. 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 5 to 15 minutes in the test tube rack before digestion. Then the digester was allowed to wait until its temperature reached 370°C. As the digester reached the temperature 370°C and the 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:

$$\%N = \frac{(V \times N \times 0.014 - 1 \times 100 \times mcf)}{WO}$$

Where:

%N = percent of nitrogen

V = Volume of H_2SO_4 in ml consumed during titration

N = Normality of 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: $C : N = \frac{\%C}{\%N}$

3.4.4 Measurement of pH

The pH of samples was measured using digital pH meter before and after AD. This pH meter was first calibrated at neutral pH by using distilled water before inoculation of rumen fluid was buffered the substrates. In anaerobic digestions optimal pH was between 6.8 and 7.4 (Arogo *et al*., 2009). Electrodes inserted into pH buffer solution, which was best for the single glass electrode according to 4500-H+ B standard (APHA, 1999). And the reading was taken. Measurement of pH after AD was also done using pH electrode which was inserted into samples of substrate that was digested in AD process.

3.5 Pre-treatment of Substrates for Anaerobic Digestion

First the two substrates were made into five treatments in solo and mix on TS basis and placed in 500 mL Erlenmeyer flask covered with plastic film. The five treatments were T1 (100% CS); T2 (75%: 25% mix of CS to GM); T3 (50%: 50% ratio of both substrates); T4 (25%: 75% mix of CS to GM) and T5 (100% GM). Then after, the substrates were mixed with 174 mL of distilled water and pretreated by keeping in hot water bath at 80ºC for five hours independently with intermittent shaking to ensure the homogeneity of temperatures in the flasks (Animut *et al*., 2014). After the pretreated substrates were cooled, they were then kept in a refrigerator at 4ºC until used. The controls groups were substrates without heat pretreatment.

3.6 Evaluation of the Amount of Biogas

Biogas was collected by water displacement method. 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 solution was measured using graduated cylinder to represent the amount of biogas produced daily starting from first day of incubation (Itodo *et al*., 1992).

Table2. The proportion of different substrates added in the five digesters in three replicates for both untreated and 80°C pretreated substrates.

Treatments	Fresh dried CS s ample added (g)	Fresh dried GM sample added (g)	Volume of distilled water added (mL)	Volume of inocu lums added (mL)
T_1	25.8	0	174.2	100
T_2	19.35	6.54	174.11	100
T_3	12.9	13.1	174	100
T_4	6.45	19.63	173.92	100
T_5	0	26.17	173.83	100

T_1=100%CS, T_2= 75%CS: 25%GM, T_3=50%CS: 50%GM, T_4=25%CS: 75%GM and T_5=100%GM. Note: CS= corn Stover and GM= goat manure

3.7 Data Analysis

The experimental data was subjected to analysis of variance (one-way ANOVA) using SPSS for windows version 21 to investigate statistical significance between the different treatments, whereas paired samples T-test was used to investigate statistical significance within a treatment as happened before and after AD. Differences between means were considered statistically significant at $P< 0.05$.

4. RESULTS AND DISCUSSION

4.1 Physico-chemical Characteristics of solo and Mixed CS and GM for Untreated Substrates before and after AD

4.1.1 Comparison of pH and OC values before and after AD for the Different Mix Ratios of untreated Substrates

The pH of the slurry for all treatments was measured before AD and the result showed that the pH of CS was 6.78, whereas that of pure GM was 7.8. According to Thy *et al*. (2003) and Yadvika *et al*. (2004) optimal pH for anaerobic digestion is between 6.8 and 7.2. And a slight pH deviation from the optimum negatively affects anaerobic performance (Ward *et al*., 2008). Here we note that the pH value of CS alone was at optimal level, whereas that of pure GM was not at normal. However, as the two substrates mixed with decreasing amount of the GM content, the pH level dropped to optimal level (Table 3). This is clear evidence that combination of substrates buffers pH value and compensate for the missing nutrients that help for the normal growth of microbes that aid in AD of organic substrates (Hills and Roberts, 1981).

Comparison between treatments showed that pH values were significantly varied both before and after AD. Similarly, the pH values of all substrates increased after AD (Table 3). That is, final or pH after AD became alkaline in all digesters, and alkalinity in an anaerobic digester might be derived from the degradation of organic nitrogen compounds, such as amino acids and proteins, or by the presence of ammonia in the feed stream. When amino acids and proteins are degraded, amino groups (and hence ammonia) are released and alkalinity is produced (Fang *et al*., 2010). The increment of the pH value after AD using lignocellulosic substrates co-digested with GM was also indicated in researches carried out in Haramaya University (Getu, 2016; Temesgen *et al*., 2017).

There was a significant difference between treatments in both before and after AD in %organic carbon (Table 3). The result revealed that the percentage degradation of organic carbon for treatment T_4 (25% CS+75% GM) was higher than all treatments (from 45.42±0.162 to 37.43±0.120, i.e., 17.59% reduction) (Table 3). Organic carbon can be removed in

anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or through biogas production (Gerardi, 2003). Therefore, the decrease in carbon reflects the degradation process during anaerobic digestion (Devlin *et al*., 2011). The results also revealed that there were differences in percentage organic carbon in all mix ratios before and after AD ($P<0.05$). This shows that mixing balances the percentage of organic carbon of substrates in the digester as the two substrates (CS and GM) contain different carbon content.

Table 3 Comparison of pH and %organic carbon before and after AD and among different mix ratios for Untreated Substrates (values are mean ± SE, n=3)

Treatments	Parameters			
	PH		%OC	
	before AD	after AD	before AD	after AD
T_1	6.87 ± 0.033^{Bb}	7.43 ± 0.021^{Ba}	51.16 ± 0.171^{Aa}	44.67 ± 0.226^{Ab}
T_2	7.07 ± 0.020^{ABb}	7.52 ± 0.032^{Ba}	49.60 ± 0.241^{Ba}	43.32 ± 0.037^{Ab}
T_3	7.25 ± 0.035^{Ab}	7.64 ± 0.072^{Ba}	47.29 ± 0.173^{Ca}	42.91 ± 0.021^{ABb}
T_4	7.68 ± 0.020^{Ab}	7.78 ± 0.024^{Ba}	45.42 ± 0.161^{Da}	37.29 ± 0.029^{Bb}
T_5	7.87 ± 0.013^{Ab}	8.01 ± 0.012^{Aa}	42.37 ± 0.147^{Ea}	40.73 ± 0.057^{Bb}

In the table above, capital letter superscripts compare between treatments in a column, whereas small letter superscripts compare within treatment (i.e., before and after AD). In both cases means with similar letters are not significantly different from each other, whereas those represented by different letters are statistically different at $p<0.05$. Note: T_1=100% CS, T_2=75% CS+25% GM, T_3=50% CS+50% GM, T_4=25% CS+75% GM and T_5=100%GM.

4.1.2 Comparison of %Nitrogen and %C/N Ratios before and after AD for the Different Mix Ratios of untreated Substrates

Nitrogen contents were analyzed for all treatments before and after AD. Result showed that %N and C/N ratio values significantly varied between treatments in both before and after AD. Similarly these values significantly varied within a treatment that is before and after AD of the same treatment (Table 4). Increment of total nitrogen after AD was also indicated in other researches (Smith *et al.*, 2007: Negash *et al*., 2018). Percent total nitrogen of all substrates increased after AD. This could be due to production of nitrogenous compounds through degradation of proteins. In this study, the ratio of C: N of all substrates was in an optimal

range for AD of substrates for biogas production. For effective anaerobic digestion carbon to nitrogen ratio should be maintained within the ranges of 20-30% (Perera, 2011). If the C/N ratio is very high than this, the nitrogen will be consumed rapidly by methanogens to meet their protein requirements and 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 much lower than the optimum, nitrogen will be liberated and accumulated in the form of ammonia (NH_3). The increased concentration of NH_3, thus, increases the pH of the digester to alkalinity that inhibits the growth of bacteria (Braun, 1982).

Table4. Comparison of %N and %C/N ratio before and after AD and among different mix ratios for Untreated Substrates (values are mean ± SE, n=3)

Treatment	%N before AD	%N after AD	%C/N before AD	%C/N after AD
T_1	1.84 ± 0.011^{Bb}	1.86 ± 0.008^{Ca}	27.87 ± 0.214^{Bb}	23.89 ± 0.133^{Aa}
T_2	1.86 ± 0.005^{Bb}	1.88 ± 0.008^{Ca}	26.72 ± 0.253^{Aa}	23.33 ± 0.073^{Ab}
T_3	1.92 ± 0.008^{Ab}	1.94 ± 0.011^{Ba}	24.97 ± 0.324^{Ba}	22.58 ± 0.077^{ABb}
T_4	1.95 ± 0.014^{Ab}	1.97 ± 0.012^{Ba}	23.02 ± 0.111^{Ba}	18.9 ± 0.049^{Bb}
T_5	1.98 ± 0.012^{Ab}	2.04 ± 0.017^{Aa}	20.57 ± 0.087^{Cb}	20.95 ± 0.012^{Ba}

In the table above, capital letter superscripts compare between treatments in a column, whereas small letter superscripts compare within treatment (i.e., before and after AD). In both cases means with similar letters are not significantly different from each other, whereas those represented by different letters are statistically different at $p<0.05$. Note: T_1 =100% CS, T_2 = 75% CS+25% GM, T_3 = 50% CS+50% GM, T_4 = 25% CS+75% GM and T_5 = 100%GM.

4.1.3 Comparison of %TS and %VS before and after AD for the different Mix Ratios of untreated Substrates

The TS of both sundried fresh substrates and their mixes were not statistically significant between treatments before AD (*P>0.05*). However, TS showed significant difference between treatments after AD (Table 5). Values of TS significantly decreased after AD and the amount of reductions were 9.29, 13.14, 15.99, 21.24 and 11.75% from T_1, T_2, T_3, T_4 and T_5, respectively. Highest TS reduction after AD was observed in T_4 (25%CS+75% GM) (21.24%). Highest cumulative biogas was also recorded in this substrate (1177.65 mL). Reduction in TS may have resulted due to consumption of organic matter by acidogenic and

methanogenic bacteria to produce methane. Tamrat *et al*. (2013) also reported similar trend in their experiment conducted on co-digestion of cattle manure with organic kitchen waste.

Volatile solid showed significant difference between treatments both before and after AD (Table 5). Volatile solid values of all treatments significantly reduced after AD when compared to that of before AD. As in the case for TS reduction, reduction in VS may be due to consumption of those compounds by acidogenic and methanogenic bacteria to produce methane. Similar trend was also reported by Tamrat *et al.* (2013) who did his experiment on co-digestion of cattle manure with organic kitchen waste for biogas production. Abubaker and Ismail (2012) mentioned that TS and VS destructions are good parameters for evaluating the efficiency of anaerobic digestion. Previously, GM mixed with 25% lignocellulosic material such as Parthenium was found to exhibit highest reductions in TS and VS, and these reductions were found to be a par with total biogas yield at the end of incubation (Getu, 2016).

Table5. Comparison of %TS and %VS between before and after AD and among different mix ratios for Untreated Substrates (values are mean ± SE, n=3).

Treatment	%TS before AD	%TS after AD	%VS before AD	%VS after AD
T_1	93.39 ± 0.011^{Aa}	84.66 ± 0.160^{Ab}	76.64 ± 0.174^{Aa}	72.66 ± 0.185^{Bb}
T_2	92.89 ± 0.106^{Aa}	80.68 ± 0.320^{Bb}	64.84 ± 0.156^{Ba}	62.33 ± 0.017^{Ab}
T_3	92.35 ± 0.323^{Aa}	77.58 ± 0.299^{Cb}	54.54 ± 0.172^{Ca}	50.49 ± 0.003^{Cb}
T_4	92.83 ± 0.150^{Aa}	73.11 ± 0.110^{Db}	49.65 ± 0.147^{Da}	45.53 ± 0.117^{Db}
T_5	92.03 ± 0.033^{Aa}	81.21 ± 0.210^{Bb}	33.47 ± 0.032^{Ea}	31.91 ± 0.072^{Eb}

In the table above, capital letter superscripts compare between treatments in a column, whereas small letter superscripts compare within treatment (i.e., before and after AD). In both cases means with similar letters are not significantly different from each other, whereas those represented by different letters are statistically different at $p<0.05$. Note: T_1 =100% CS, T_2 = 75% CS+25% GM, T_3 = 50% CS+50% GM, T_4 = 25% CS+75% GM and T_5 = 100%GM.

4.2 Physico-chemical Characteristics of Thermal Pre-treated Solo and CS-GM Mixes.

4.2.1 Comparison of pH and OC values before and after AD for the Different Mix Ratios of thermally (80°C) treated substrates

The pH values of thermally pre-treated substrates showed significant differences between treatments for both before and after AD (Table 6). Before AD the pH value of 100% CS was more optimal than that of 100% GM for bio-gas production as the optimal pH for biogas production ranges from 6.8 to 7.2 (Thy *et al*., 2003; Yadvika *et al*., 2004). Mixing of the two substrates brought the varying pH values of the substrate mix closer to the optimal values for anaerobic digestion. That is, mixing of the two substrates resulted in the decrease of pH compared to that of GM alone, but increased from that of CS alone. This indicates that the CS helps in buffering pH when it is used for biogas production co-digested with the GM. After AD, pH values significantly increased when compared with that of before AD (Table 6). That is, pH after AD became alkaline in all digesters, and alkalinity in an anaerobic digester might be derived from the degradation of organic nitrogen compounds, such as amino acids and proteins, or by the presence of ammonia in the feed stream. When amino acids and proteins are degraded, amino groups (and hence ammonia) are released and alkalinity is produced (Fang *et al*., 2010). Generally, except for minor variation, pH measurements were similar with that of untreated substrates shown above in table 3. Results of organic carbon also showed similar trend with that of untreated substrates.

Table 6 Comparison of pH and %organic carbon before and after AD for the different mix ratios of thermally (80°C) treated substrates (values are mean ± SE, n=3).

Treatment	pH before AD	pH after AD	%OC before AD	%OC after AD
T_1	6.42 ± 0.223^{Cb}	7.50 ± 0.095^{Ba}	51.16 ± 0.171^{Aa}	44.67 ± 0.226^{Ab}
T_2	7.05 ± 0.149^{Cb}	7.30 ± 0.109^{Ca}	49.60 ± 0.241^{Ba}	43.56 ± 0.234^{Ab}
T_3	7.37 ± 0.380^{Bb}	7.98 ± 0.445^{Ba}	47.29 ± 0.173^{Ba}	42.48 ± 0.207^{Bb}
T_4	7.66 ± 0.090^{Bb}	7.80 ± 0.0840^{Ba}	46.09 ± 0.828^{Ca}	32.29 ± 0.029^{Db}
T_5	8.01 ± 0.075^{Ab}	8.44 ± 0.168^{Aa}	42.37 ± 0.147^{Da}	40.50 ± 0.010^{Cb}

In the table above, capital letter superscripts compare between treatments in a column, whereas small letter superscripts compare within treatment (i.e., before and after AD). In both cases means with similar letters are not significantly different from each other, whereas those represented by different letters are statistically different at $p<0.05$. Note: T_1 =100% CS, T_2 = 75% CS+25% GM, T_3 = 50% CS+50% GM, T_4 = 25% CS+75% GM and T_5 = 100%GM.

4.2.2 Comparison of %Nitrogen and C/N Ratios before and after AD for the Different Mix Ratios of thermally (80°C) treated substrates

Except some minor differences, values of total nitrogen and C:N ratios of thermally pretreated substrates were more or less similar to untreated substrates and showed the same trend after AD. That is, total nitrogen showed significant differences between treatments both before and after AD (Table 7). Within treatment comparisons also showed significant differences between before and after AD in all substrate types. Total nitrogen increased after AD. Both before and after AD, C: N ratios of all substrates were in an optimal range suitable for anaerobic digestion.

Table7 Comparison of Nitrogen and C/N Ratios between before and after AD for different mix ratios of thermally (80°C) treated substrates (values are mean ± SE, n=3)

Treatment	%N before AD	%N after AD	%C/N before AD	%C/N after AD
T_1	1.84 ± 0.011^{Ba}	1.86 ± 0.012^{Cb}	27.80 ± 0.257^{Aa}	24.01 ± 0.341^{Ab}
T_2	1.86 ± 0.014^{Ba}	1.87 ± 0.012^{BCb}	26.66 ± 0.327^{Aa}	23.29 ± 0.113^{Bb}
T_3	1.91 ± 0.017^{Aa}	1.94 ± 0.011^{ABb}	24.75 ± 0.426^{Ba}	21.89 ± 0.115^{BCb}
T_4	1.94 ± 0.014^{Aa}	1.96 ± 0.011^{Bb}	23.75 ± 0.307^{Ba}	16.47 ± 0.047^{Cb}
T_5	1.98 ± 0.005^{Aa}	2.10 ± 0.008^{Ab}	21.67 ± 0.060^{Ca}	19.28 ± 0.048^{Cb}

In the table above, capital letter superscripts compare between treatments in a column, whereas small letter superscripts compare within treatment (i.e., before and after AD). In both cases means with similar letters are not significantly different from each other, whereas those represented by different letters are statistically different at $p<0.05$. Note: T_1 =100% CS, T_2 = 75% CS+25% GM, T_3 = 50% CS+50% GM, T_4 = 25% CS+75% GM and T_5 = 100%GM.

4.2.3 Comparison of TS and VS before and after AD for the Different Mix Ratios of Thermally (80^{o}C) Treated Substrates.

Except for some minor differences, values of TS and VS of thermally pretreated substrates were more or less similar to untreated substrates and showed the same trend after AD. In thermally treated substrates, TS of both sundried fresh substrates and their mixes showed significant differences between treatments both before and after AD (Table 8). Values of TS significantly decreased after AD and the amount of reductions were 12.27, 13.26, 16.94, 22.01 and 11.77% from T_1, T_2, T_3, T_4 and T_5, respectively. Highest TS reduction after AD was observed in T_4 (25%CS+75% GM) (22.01%) and highest cumulative biogas was also recorded in this substrate (1426.72 mL). Similarly, VS values showed significant difference between treatments both before and after AD (Table8). Volatile solid values of all treatments also significantly reduced after AD as compared to that of before AD although the amount of reduction varied between treatments with the highest reduction seen in T_4 (Table8).

Table 8/ Comparison of TS and VS between before and after AD and among different mix ratios for 80°C thermally pre-treatment test (values are mean ± SE, n=3)

Treatment	%TS before AD	%TS after AD	%VS before AD	%VS after AD
T_1	93.03 ± 0.071^{Ab}	81.61 ± 0.165^{Ab}	77.79 ± 0.090^{Aa}	73.67 ± 0.173^{Ab}
T_2	92.89 ± 0.106^{Bb}	80.57 ± 0.070^{Ab}	64.84 ± 0.156^{Ba}	61.31 ± 0.238^{Bb}
T_3	92.03 ± 0.000^{Bb}	76.44 ± 0.063^{Bb}	54.54 ± 0.172^{Ca}	51.20 ± 0.200^{Cb}
T_4	91.87 ± 0.103^{Cb}	71.65 ± 0.189^{Cb}	45.98 ± 0.196^{Da}	40.67 ± 0.035^{Db}
T_5	91.90 ± 0.215^{Cb}	81.08 ± 0.043^{Ab}	35.65 ± 0.192^{Ea}	30.82 ± 0.103^{Eb}

In the table above, capital letter superscripts compare between treatments in a column, whereas small letter superscripts compare within treatment (i.e., before and after AD). In both cases means with similar letters are not significantly different from each other, whereas those represented by different letters are statistically different at $p<0.05$. Note: T_1 =100% CS, T_2 = 75% CS+25% GM, T_3 = 50% CS+50% GM, T_4 = 25% CS+75% GM and T_5 = 100%GM.

4.3 Daily Mean and Cumulative Biogas Production of Untreated and Thermally Treated Substrates

The amount of biogas yield from individual and mixed substrates was measured daily starting from the first day of incubation up to 30 days of incubation period. Results showed that, in untreated substrates, biogas yield was minimum in the first three days of incubation and peaked at around 7^{th} day of incubation with gradual reduction and close to none on 30^{th} day (Fig. 2). But, gas production stopped early day 25-28 for some treatment.

For thermally pre-treated substrates, however, biogas yield was even higher on the first day of incubation and peaked earlier (on the 3^{rd} day) than untreated substrates (Fig. 3). In both untreated and treated substrates, the highest daily mean biogas yield was recorded in T_4 (25%CS &75%GM) substrate type. However, the value (118.00 mL) obtained from untreated substrate was lower than that of thermally treated substrate (152.33mL). This suggests that substrate T_4 was optimal mix ratio to produce more biogas. It can be concluded that this mix ratio presents balanced nutrients to microorganism, (Forster *et al*., 2008).

The biogas yield measured was in line with the extent of TS, VS and organic carbon reduction that shows consumption of organic materials so as to convert into biogas. The daily average

biogas yield was summed up to get the cumulative biogas yield over the entire incubation period. Results show that cumulative biogas yield of both untreated and treated substrates maximum in T_4 followed by T_3 substrate type. Compared to the untreated substrates, thermally treated substrates yielded more biogas in all mix ratios (Figure. 4).

After peak point, biogas production decreased in both untreated and treated substrates, and eventually reached 0 mL on 28^{th} and 23^{rd}day in untreated and treated substrates, respectively. The decline in biogas production could be due to the increased bacterial population and depletion of readily decomposable substrate (Ahn *et al*., 2009). The fact that maximum gas production was observed earlier in thermally pre-treated substrates than untreated ones suggests that heat treatment disintegrates the complex macromolecules into simpler ones so that microorganisms can easily decompose them (Rafique *et al*., 2010), and hence reduction in hydraulic retention time. Thus, pre-treatment does not only yield greater amount of biogas, but it also reduces hydraulic retention time needed for AD (Ferrer *et al*., 2008). Ferrer *et al*. (2008) reported that there is better biological activity of some hyper thermophilic bacteria population under heat treatment.

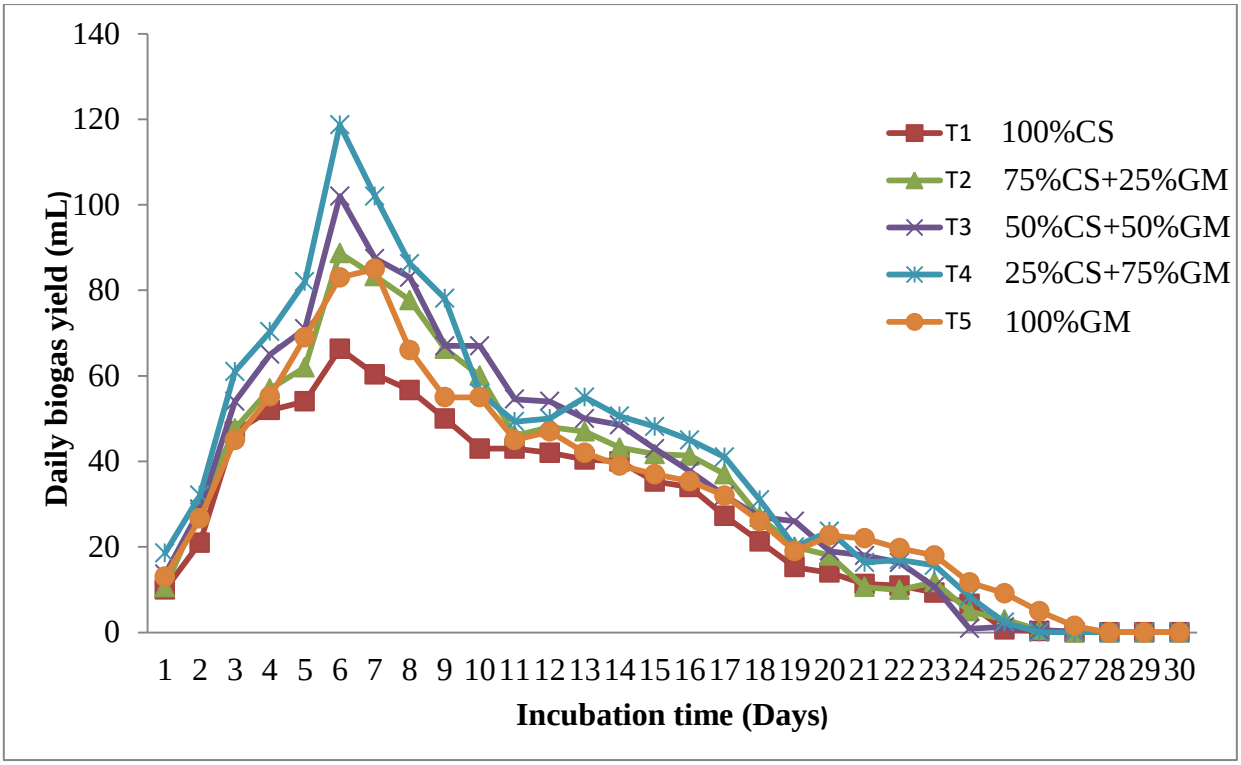

Figure 2: Daily mean biogas production from untreated substrates with different content.T_1=100%CS, T_2=75%CS+25%GM, T_3=50%CS+50%GM, T_4=25%CS+75%GM and T_5=100% GM**.**

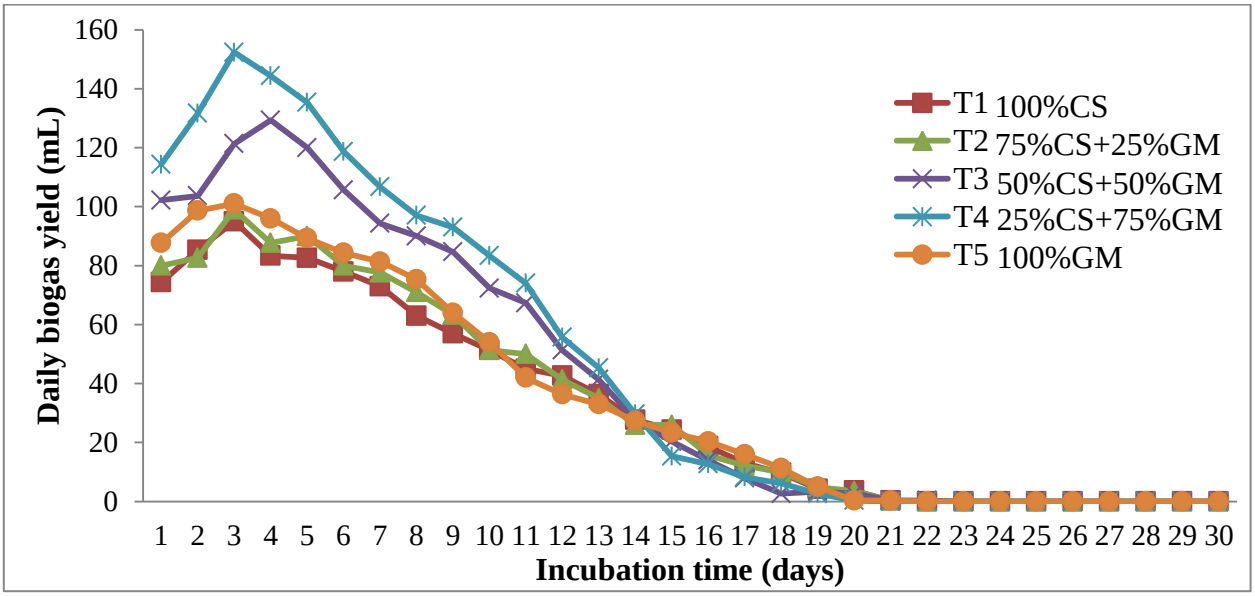

Figure 3: Daily mean biogas production for Pretreated Substrates at Temperature of 80ºc with different content. T_1=100%CS, T_2=75%CS+25%GM, T_3=50%CS+50%GM, T_4=25%CS+75% GM and T_5=100% GM.

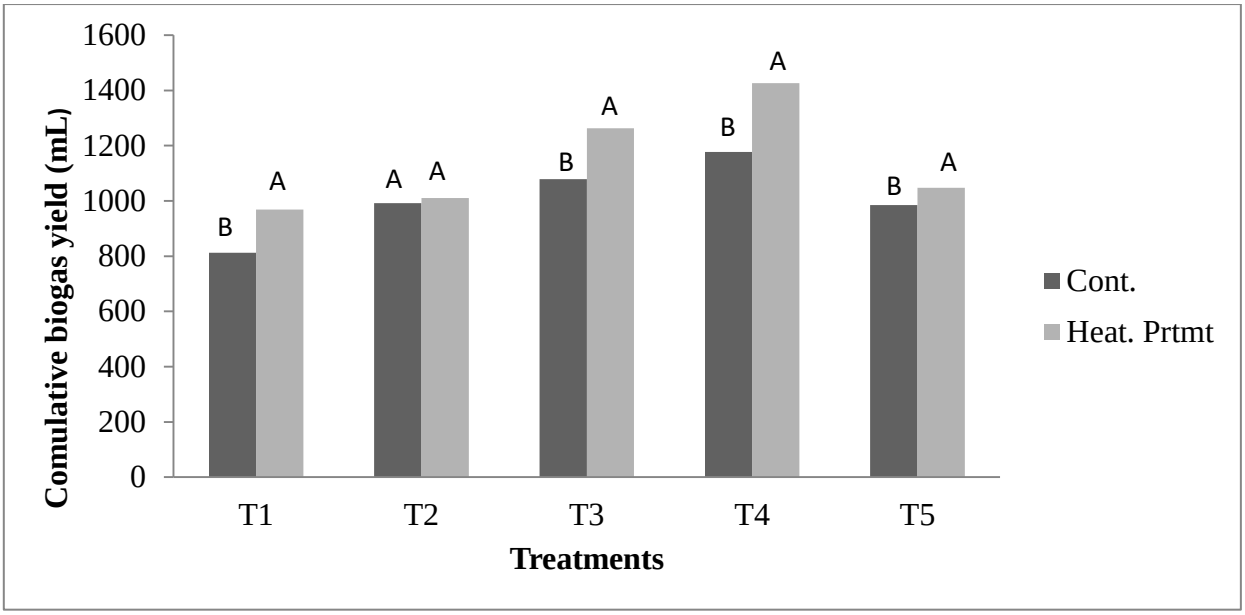

Figure 4 Cumulative biogas yields of 80°C thermally pre-treated and untreated substrates. Different capital letters for paired samples T-test within treatments shows significant difference ($P<0.05$) in cumulative biogas yield. (T_1=100%CS, T_2=75%CS: 25%GM, T_3=50% CS: 50%GM, T_4=25% CS: 75%GM and T_5=100GM

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Biogas is an alternative and renewable energy source produced through anaerobic digestion of organic matter whereby the organic matter is converted into a combustible biogas rich in methane and a liquid effluent by anaerobic microbes. With the aim of comparing between biogas yield from untreated and thermally treated substrates, a series of experiments were conducted under mesophilic conditions (38ºC) using five batch digesters (T_1=100%CS, T_2=75%CS: 25%GM, T_3=50%CS: 50%GM, T_4=25%CS: 75%GM and T_5=100%GM) with triplicate in 8% of total solid content. The daily biogas production was measured by water displacement method for 30 days. In all treatments, pH, total solid, volatile solid, organic carbon, total nitrogen, and C: N ratios were analyzed before and after AD. Before anaerobic digestion pH was found to increase significantly with increasing of goat manure proportion. The comparison of pH values before and after AD showed that pH values were significantly increased after AD for all the treatment.

Comparison of %organic carbon before AD and after AD showed that %organic carbon was significantly decreased after AD in all substrate types. In this study, C: N ratio of all treatments was found in between 20-30 which was a suitable condition for methanogenic bacteria to reproduce and produce optimum biogas. Higher reductions of TS and VS were also observed after AD of thermally pretreated substrates under temperature of 80ºC than control group. The results showed that thermal pre-treatment resulted in higher biogas yield within shorter hydraulic retention (incubation) time.

5.2 Conclusions

Anaerobic digestion of *Corn Stover* co-digested with goat manure under mesophilic conditions (38ºC) was done using batch digester in five treatments with three replications for 30 days of hydraulic retention time. In all treatments physico-chemical parameters such as total solid, volatile, organic carbon, nitrogen, C: N ratio and pH were measured both before and after anaerobic digestion. This completely randomized experimental design was carried out to achieve appropriate mix ratio for highest biogas production accordingly, the experimental data

shows co-digestion of 25% corn Stover +75% *goat manure* mix ratio gives the highest average daily and cumulative biogas yields in both untreated and thermally pretreated substrates. The experimental results suggested that the corn Stover co-digested with goat manure improves the biogas production potential when compared with corn Stover and goat manure alone. Moreover, heat pre-treatment of these substrates improves biogas yield and incubation period.

5.3 Recommendations

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

- Different combination of the substrates and thermal pre-treatments other than these used in this study should be tested to identify the most relevant one which improves the production of biogas.
- Different mix ratio other than these used in this study should be tested to identify the most relevant one which improves the production of biogas.
- Other more appropriate pre-treatment techniques should be tried out in the future to further improve biogas yield.

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7. APPENDIXS

7.1 Appendix I Tables

Table1 Daily Mean Biogas Production (mL) for Untreated Substrates (substrates ±SE (n=3).

Day	T1	T2	T3	T4	T5
1	10.06±1.659	10.60±1.249	13.67±3.282	18.60±3.124	13.00±2.081
2	21.00±1.154	29.00±1.527	28.80±3.189	32.10±1.242	26.67±1.201
3	47.00±0.577	47.67±1.452	54.03±4.409	61.00±6.928	45.00±0.000
4	52.00±1.154	57.00±0.288	65.00±0.577	70.33±1.201	55.20±1.404
5	52.66±1.732	62.00±1.322	71.00±0.577	82.00±3.464	69.00±2.309
6	66.33±0.333	88.70±3.055	102.00±4.041	118.00±10.33	83.00±4.041
7	60.33±1.763	83.30±3.511	87.43±0.218	102.00±3.055	85.00±1.732
8	56.67±1.201	77.70±4.358	83.00±2.081	86.23±0.233	66.00±1.527
9	50.00±2.645	66.33±1.364	67.00±2.516	78.00±0.100	55.00±1.527
10	43.00±1.527	60.00±1.154	67.00±0.000	56.00±3.464	55.00±1.527
11	43.00±2.081	46.00±0.577	54.53±0.290	49.30±0.173	45.00±1.732
12	42.00±1.527	48.00±1.527	54.00±0.000	50.00±3.464	47.00±4.618
13	42.53±0.218	47.00±1.527	50.00±0.577	55.00±0.577	42.00±0.577
14	40.00±0.000	43.20±0.100	48.53±0.290	50.56±0.384	39.00±0.577
15	35.27±0.133	41.03±0.000	43.00±0.000	48.13±0.296	37.00±0.577
16	34.00±0.577	41.33±0.881	37.67±1.667	45.00±0.000	35.33±0.333
17	27.23±0.120	37.00±0.577	32.00±1.527	41.00±1.527	32.00±0.577
18	21.30±0.608	27.00±1.154	27.00±0.577	31.00±0.577	26.00±1.154
19	15.33±4.141	20.00±5.131	26.00±3.055	20.00±1.732	19.00±4.725
20	14.00±0.866	18.00±3.605	19.00±3.214	23.67±5.206	22.67±3.345
21	11.32±2.781	10.63±1.122	18.00±1.527	16.33±2.027	22.00±1.527
22	10.97±1.576	9.97±0.966	16.33±1.763	17.00±2.081	19.67±2.403
23	9.32±0.889	11.77±.571	10.67±0.667	15.67±2.962	18.00±4.163
24	6.66±0.723	4.93±0.649	1.02±0.264	8.33±1.333	11.67±1.740
25	.67±0.171	3.01±0.892	1.30±0.577	2.40±1.951	9.17±2.166
26	.35±0.964	.61±0.075	.56±0.291	.00±0.000	4.93±0.635
27	.00±0.000	.00±0.000	.19±0.109	.00±0.000	1.49±0.513
28	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
29	.00±0.000	.00±0.000	.00±0.00	.00±0.000	.00±0.000
30	.00±0.000	.00±0.000	.00±000	.00±0.000	.00±0.000
total (mL)	812.73	991.78	1078.73	1177.65	984.8

Note: T1=100%CS, T2=75%CS: 25%GM, T3=50%CS: 50%GM, T4=25%CS: 75%GM and 100%GM.

Appendix Table2 Daily mean Biogas Production (mL) for Substrates pretreated at Temperature of 80ºc (substrates ±SE (n=3).

Day	T1	T2	T3	T4	T5
1	74.33±5.54	80.00±8.504	102.17±9.807	114.33±4.186	87.68±2.367
2	85.33±2.403	82.67±0.666	103.67±6.227	131.66±7.535	98.67±8.089
3	95.00±1.154	99.00±5.033	121.33±2.440	152.33±6.009	101.03±5.555
4	83.33±4.484	87.67±8.743	129.33±5.811	144.33±7.535	96.00±6.245
5	82.67±4.807	90.00±7.571	120.00±3.605	135.33±7.218	89.33±4.630
6	78.00±1.527	80.00±3.214	105.67±6.064	118.67±4.702	84.33±3.480
7	73.00±1.154	77.67±5.696	94.33±0.881	106.67±3.929	81.33±2.027
8	63.00±1.154	71.00±9.018	90.00±0.577	97.00±0.577	75.33±0.881
9	57.00±1.154	63.33±5.364	84.67±1.201	93.00±0.577	64.00±1.732
10	51.33±3.844	51.33±3.844	72.33±5.666	83.33±1.763	54.00±3.511
11	45.00±2.645	50.00±2.645	67.33±5.456	74.00±0.577	42.00±2.309
12	42.67±2.905	41.33±4.055	51.33±5.696	55.67±2.962	36.33±2.027
13	36.33±1.855	35.00±2.645	41.33±2.333	45.33±2.905	33.00±1.732
14	27.67±3.480	26.00±2.081	27.67±6.641	29.67±5.607	27.33±4.630
15	24.33±2.848	26.00±2.645	20.33±3.179	15.33±2.728	23.33±3.172
16	18.67±0.881	15.67±2.962	14.00±0.577	12.67±1.452	20.33±1.201
17	13.00±1.527	12.10±2.417	7.93±0.800	8.23±0.409	16.00±1.527
18	9.63±2.299	9.90±2.107	2.67±0.993	6.17±1.993	11.33±1.763
19	4.30±0.781	4.85±0.377	3.33±0.405	2.67±0.166	5.00±0.577
20	3.67±0.600	3.50±0.763	2.63±0.202	.33±0.033	.37±0.092
21	.30±0.057	.30±0.057	.37±0.084	.00±0.000	.33±0.030
22	.00±0.000	.00±0.000	.33±0.033	.00±0.000	.00±0.000
23	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
24	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
25	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
26	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
27	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
28	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
29	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
30	.00±0.000	.00±0.000	.00±0.000	.00±0.000	.00±0.000
total (mL)	968.56	1010.02	1262.75	1426.72	1047.05
average	32.26	33.67	42.09	47.56	34.90

Note: T1=100%CS, T2=75%CS: 25%GM, T3=50%CS: 50%GM, T4=25%CS: 75%GM and 100%GM

Appendix Table 3 the proportion of different substrates added in the five digesters in three replicates for both untreated and 80°C pretreated substrates.

Treatments	Fresh dried CS sample added (g)	Fresh dried GM sample added (g)	Volume of distilled water added (mL)	Volume of inocu lums added (mL)
T1	25.8	0	174.2	100
T2	19.35	6.54	174.11	100
T3	12.9	13.1	174	100
T4	6.45	19.63	173.92	100
T5	0	26.17	173.83	100

Appendix Table 4 Comparison of physico-chemical parameters between before and after AD and among different mix ratios for Untreated Substrates (values are mean ± SE, n=3)

Treatments	Parameters			
	pH before AD	pH after AD	%OC before AD	%OC after AD
T1	6.87 ± 0.033^{Bb}	7.43 ± 0.021^{Ba}	51.16 ± 0.171^{Aa}	44.67 ± 0.226^{Ab}
T2	7.07 ± 0.020^{ABb}	7.52 ± 0.032^{Ba}	49.60 ± 0.241^{Ba}	43.32 ± 0.037^{Ab}
T3	7.25 ± 0.035^{Ab}	7.64 ± 0.072^{Ba}	47.29 ± 0.173^{Ca}	42.91 ± 0.021^{ABb}
T4	7.68 ± 0.020^{Ab}	7.78 ± 0.024^{Ba}	45.42 ± 0.161^{Da}	37.29 ± 0.029^{Bb}
T5	7.87 ± 0.013^{Ab}	8.01 ± 0.012^{Aa}	42.37 ± 0.147^{Ea}	40.73 ± 0.057^{Bb}
Treatment	%N before AD	%N after AD	%C/N before AD	%C/N after AD
T1	1.84 ± 0.011^{Bb}	1.86 ± 0.008^{Ca}	27.87 ± 0.214^{Bb}	23.89 ± 0.133^{Aa}
T2	1.86 ± 0.005^{Bb}	1.88 ± 0.008^{Ca}	26.72 ± 0.253^{Aa}	23.33 ± 0.073^{Ab}
T3	1.92 ± 0.008^{Ab}	1.94 ± 0.011^{Ba}	24.97 ± 0.324^{Ba}	22.58 ± 0.077^{ABb}
T4	1.95 ± 0.014^{Ab}	1.97 ± 0.012^{Ba}	23.02 ± 0.111^{Ba}	18.9 ± 0.049^{Bb}
T5	1.98 ± 0.012^{Ab}	2.04 ± 0.017^{Aa}	20.57 ± 0.087^{Cb}	20.95 ± 0.012^{Ba}
Treatment	%TS before AD	%TS after AD	%VS before AD	%VS after AD
T1	93.39 ± 0.011^{Aa}	84.66 ± 0.160^{Ab}	76.64 ± 0.174^{Aa}	62.66 ± 0.185^{Bb}
T2	92.89 ± 0.106^{Aa}	80.68 ± 0.320^{Bb}	64.64 ± 0.060^{Ba}	64.33 ± 0.017^{Ab}
T3	92.35 ± 0.323^{Aa}	77.58 ± 0.299^{Cb}	54.54 ± 0.172^{Ca}	50.49 ± 0.003^{Cb}
T4	92.83 ± 0.150^{Aa}	73.11 ± 0.110^{Db}	49.65 ± 0.147^{Da}	33.53 ± 0.117^{Db}
T5	92.03 ± 0.033^{Aa}	81.21 ± 0.210^{Bb}	33.47 ± 0.032^{Ea}	31.91 ± 0.072^{Eb}

Appendix Table 5 Comparison of physico-chemical parameters between before and after AD and among different mix ratios for 80°C thermal pre-treatment (values are mean ± SE, n=3).

Treatment	Parameters			
	pH before AD	PH after AD	%OC before AD	%OC after AD
T1	6.42 ± 0.223^{Cb}	7.50 ± 0.095^{Ba}	51.16 ± 0.171^{Aa}	44.67 ± 0.226^{Ab}
T2	7.05 ± 0.149^{Bb}	7.30 ± 0.109^{Ba}	49.60 ± 0.241^{Ba}	43.56 ± 0.234^{Ab}
T3	7.37 ± 0.380^{Bb}	7.98 ± 0.445^{Ba}	47.29 ± 0.173^{Ba}	42.48 ± 0.207^{Bb}
T4	7.66 ± 0.090^{Bb}	7.80 ± 0.0840^{Ba}	46.09 ± 0.828^{Ca}	32.29 ± 0.029^{Db}
T5	8.01 ± 0.075^{Ab}	8.44 ± 0.168^{Aa}	42.37 ± 0.147^{Da}	40.50 ± 0.010^{Bb}
Treatment	%N before AD	%N after AD	%C/N before AD	%C/N after AD
T1	1.84 ± 0.011^{Ba}	1.86 ± 0.012^{Cb}	27.80 ± 0.257^{Aa}	24.01 ± 0.341^{Ab}
T2	1.86 ± 0.014^{Ba}	1.87 ± 0.012^{BCb}	26.66 ± 0.327^{Aa}	23.29 ± 0.113^{Bb}
T3	1.91 ± 0.017^{Aa}	1.94 ± 0.011^{ABb}	24.75 ± 0.426^{Ba}	21.89 ± 0.115^{BCb}
T4	1.94 ± 0.014^{Aa}	1.96 ± 0.011^{Bb}	23.75 ± 0.307^{Ba}	16.47 ± 0.047^{Cb}
T5	1.98 ± 0.005^{Aa}	2.10 ± 0.008^{Ab}	21.67 ± 0.060^{Ca}	19.28 ± 0.048^{Cb}
Treatment	%TS before AD	%TS after AD	%VS before AD	%VS after AD
T1	93.03 ± 0.071^{Ab}	81.61 ± 0.165^{Ab}	77.79 ± 0.090^{Aa}	73.67 ± 0.173^{Ab}
T2	92.89 ± 0.106^{Bb}	80.57 ± 0.070^{Ab}	64.84 ± 0.156^{Ba}	61.31 ± 0.238^{Bb}
T3	92.03 ± 0.000^{Bb}	76.44 ± 0.063^{Bb}	54.54 ± 0.172^{Ca}	51.20 ± 0.200^{Cb}
T4	91.87 ± 0.103^{Cb}	71.65 ± 0.189^{Cb}	45.98 ± 0.196^{Da}	40.67 ± 0.035^{Db}
T5	91.90 ± 0.215^{Cb}	81.08 ± 0.043^{Ab}	35.65 ± 0.192^{Ea}	30.82 ± 0.103^{Eb}

7.2 Appendix II Figures.

Dried corn Stover

Powder of corn Stover

Figure 1 Sample of corn Stover for anaerobic digestion collected from Haramaya University farm land.

Dried goat manure

Powder of goat manure

Filtered rumen fluid

Figure 2 Sample of goat manure and filtered rumen fluid used for anaerobic digestion collected from Haramaya University goat farm and slaughter house.

Figure 3 Arrangement of the pH measurement before anaerobic digestion of the substrates.

Figure 4 Analysis of carbon to nitrogen ratio.

Figure 5 Laboratory Works.