PRODUCTION OF BIOGAS FROM THE MIXTURE OF SAWDUST AND COW DUNG UNDER ANAEROBIC CONDITION

MSc. Thesis

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As thesis advisors, we hereby certify that we have read and evaluated this thesis entitled"**Production of biogas from the mixture of sawdust and cow dung under anaerobic condition**"prepared under our guidance by Aysheshm Abune Ayana. We recommend that the thesis be accepted as it fulfills the requirements.

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DEDICATION

This thesis is dedicated To my beloved parents, and my father, Abune Ayana

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own work and all resources used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for M.Sc. degree in Biotechnoloy at Haramaya University and it is deposited at the University Library to be made available to any other institutions anywhere.

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BIOGRAPHICAL SKETCH

The author, Aysheshm Abune Ayana, was born from his father Abune Ayana and from his mother Bitewush Mulu Haile and he is the third soon in their home in Ankesha Woreda, Dikuna Dereb Kebele, Awi Zone of Amhara Regional State in 1987. He attended his elementary school at Dikuna Elementary School. He attended his secondary and preparatory school education at Ankesha Secondary and Preparatory School and Injibara Preparatory School. After completing his Secondary and preparatory school, in 2010, he joined Wollo University and graduated with Bachelor of Science degree in Applied Biology in June 2012. After graduation, he was employed by Ethiopian Somali Regional State Education Bureau as a Biology teacher. He joined Haramaya University to pursue his study leading Master's degree in Biotechnology in 2015.

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ACRONYMS/ABBREVIATIONS

AD	Anaerobic Digestion
APHA	American Public Health Association
BD	Basic Density
BOD	Biological Oxygen Demand
CD	Cow Dung
CNR	Carbon to Nitrogen Content
COD	Chemical Oxygen Demand
CSA	Central Statistical Agency
CSTR	Continuously Stirred Tank Reactor
FCC	Fixed Carbon Content
FS	Fixed Solids
HHV	Higher Heat Value
HRT	Hydraulic Retention Time
MC	Moisture Content
NBP	National Biogas Program
OLR	Organic Loading Rate
TS	Total Solids
VMC	Volatile Matter Content
VS	Volatile Solids

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ABSTRACT

Biogas is eco-friendly alternative renewable energy source produced through anaerobic digestion of organic compounds. In this study, biogas production was evaluated from sawdust (SD)and cow dung (CD) co-digestion in five mix ratios under mesophilic conditions (35ºC) using batch digesters in the micro biology Laboratory of Haramaya University to evaluate biogas production potential of sawdust through anaerobic digestion alone or in combination with cow dung .In 100%SD , 100% CD, 50% SDand 50% CD, 70%SD and 30%CD, and 30%SDand 70%CD treatments, total solids%, volatile solids%, organic carbon%, moisture content% and pH were measured before and after digestion while carbon to nitrogen ratio was measured before anaerobic digestion only. The daily biogas production was measured by water displacement method. All measured physico-chemical parameters of each substrate showed significant variations. The maximum (82.02±0.24) and minimum (79.76±2.98), MC% before AD were measured in 50% SD and 50% CD) and 70%SD and 30%CD respectively.While after AD the maximum (88.9±1.59) MC% was measured in 100%CD and the minimum (83.87±1.38) MC% was measured in 100%SD. Likewise, the maximum (50.39±1.05) and minimum (46.01±0.52) C% before AD were measured in 50% SD, and 50% CD and 100%SD respectively. After AD degradation in organic carbon was highest (25.36 C%) for 70%SD and 30%CD. Generally %C after AD totaly reduced before AD. The maximum (7.51±0.37) and minimum (6.76±0.16) pH before AD were measured in 100%CD and 50%SD and 50%CD respectively. After anaerobic digestion, TS% and VS% of T1,T2,T3,T4,and T5treatments were significantly lower (32.21, 30.11, 28.36,25.8, 28.33 and 69.73, 67.1, 62.12, 42.24 and 68.29) than those of the treatments before AD. In this experiment, the carbon to nitrogen ratio of treatments T1, T2, T3, T4 and T5 before AD were24.60:1 , 31.05:1, 28.96:1 , 28.72:1 and 31.61:1 respectively. Biogas production was detected in both substrate and substrate mix ratios from the second day of digestion and declined to zero in about 31 days of incubation. The maximum (512.25ml) cumulative biogas was measured in 70%SD and 30%CD while the minimum (287.75 ml) was measured in100% CM. Assessment of cumulative biogas production revealed that the substrate mix ratio of 70% SD and 30% CD were superior (512.25ml) than others. Over all, the results of this study indicate that the increase in biogas yield and reduction in volatile solids and total solids can be significantly enhanced when sawdust was co-digested with cow manure in 70%SD and 30% CD mix ratio.

Keywords and phrases; Cumulative biogas production, Methane, Rumen fluid, saw dust, Substrate mix ratio.

1. INTRODUCTION

Biogas is a colorless, flammable gas produced via anaerobic digestion (fermentation) of animal, plant, human, industrial and municipal waste to produce methane (50-70%), Carbon dioxide (20-40%) and traces of other gases such as nitrogen, hydrogen, ammonia, hydrogen sulphide, water vapour (Ukuno, 2011).

Biogas is one of the most important renewable energy sources that contain high quality fuel that serves for various purposes such as cooking, lighting, heating, etc. It is produced from organic wastes by concerted action of various groups of anaerobic bacteria. The development of biogas technology provides an alternative source of energy in developing countries and solves environmental problems. In many developing countries, sustainable waste management has become major issue due to lack of adequate technology to handle wastes generated from daily living activities (Ofoefule *et al.,* 2013).

The majority of people in developing countries do not easily and steadily have access to advanced forms of energy such as electricity; therefore, they entirely depend on biomass of fuels like firewood to meet their basic energy needs for cooking and lighting. At the same time, over 60% of the total wood in developing countries is being used as wood fuel in the form of either charcoal, especially in the urban areas, or as firewood mostly in the rural areas. This has resulted in depleting forests at a faster rate than they can be replaced. Biogas is a well-established fuel that can supplement or even replace wood as an energy source for cooking and lighting in developing countries. Currently, as the fossil-based fuels become scarce and more expensive, the economics of biogas production is turning out to be more favorable. Biogas is a readily available energy resource that significantly reduces greenhouse-gas emission compared to the emission of landfill gas to the atmosphere (Alemayehu, 2015).

Access to modern energy services remains an issue for poor people in developing countries. In Ethiopia about 83% of the population does not have access to electricity and 93% uses biomass-based energy for cooking, which surpasses 99% in rural areas firewood

is the main fuel followed by crop residues and cows dung collected from common resource pools or own resources. Therefore, common resource pools became scarce and people travel long distances to collect firewood. Ethiopia has a population of about 90 million, of which more than 70 million live in rural areas (Gudina and Sanderine, 2015).

The energy use pattern in rural Ethiopia will bring negative environmental, economic and health impacts. The use of fuel wood and charcoal leads to deforestation, forest degradation, erosion, loss of biodiversity and other environmental problems (Cooke, 2008).

To solve these problems, it is appropriate to evaluate the potential of biogas production from different plant material and animal wastes. In this regard, biogas production from different organic materials like *Moringa stenopetala* seed cake powder (Eyasu, 2008), poultry litter (Ebrahim, 2006), *Khat* (*Catha edulis*) waste (Tesfaye, 2007) and others were performed in Ethiopia.

Plant materials and animal manure have recently been used together to produce biogas by anaerobic digestion (AD). Compared with the single digestion of feedstock, the co-digestion of plant materials and animal manures increases the rate of biogas production because of the greater balance between carbon and nitrogen (El-mashad *et al*., 2010).

Sawdust is produced as a small discontinuous chips or small fragments of wood during sawing of logs in the timber. The chips flow from the cutting edges of the saw blade to the floor during sawing operation, which is as a waste in the environment, but in recent years, researchesSaw dust like any biomass can be explored as a biogas source applying current waste to energy techniques (Olusola *et al*., 2013).

Ukonu (2011) has investigated the best ratio of water, saw dust and cow dung (based on the moisture content) for biogas production and monitored for 28 days (retention time) using six Bioreactors. However, bioreactors contain $NiSO_4$, $CoSO_4$, which reduce hydratic retention time.Those chemicals that reduce hydratic retention time,but bioreactor which contains chemicals that produce combustible biogas made up of mainly carbondioxide

(CO_2) which reduce methane quality. Therefore amount of biogas was reduced. This problem was be solved by producing biogas without chemicals (Ukonu, 2011).

According to Recebli (2015) the animal waste manure was used to obtain an alternative energy source biogas which has the lower heating value of 21,000 kJ/m3 and so usage of this energy source instead of the natural gas which has the lower heating value 34,000 kJ/m3. The problem of lower heating value is comes from the production of biogas by using single substrate. This problem was solved by the combination of the two substrates.

Manjula Das Ghatak *et al*., (*2014)* they were fill the substratesin both sawdust and cow dung At 45^{O}C, and 55^{O}C temperatures respectively which is thermophilic temperature, to reduce hydratic retantion time. Once again the highest temperature was reduced to 35ºC, which is mesophilic.

This study , therefore, meant to evaluate biogas production potential of sawdust alone and combination with cow dung without chemicals and at 35ºc (mesophilic) temprature with the following general and specific objectives

General objective

The general objective of this study was to evaluate biogas production potential of saw dust and cow dung under anaerobic condition

Specific objective

- Characterize sawdust and cow dung in terms of total solids, volatile solids, moisture content, organic carbon, nitrogen content, carbon/nitrogen ratio and pH before and after digestion;
- Compare biogas yield from batch fermentation of mixed substrates of sawdust and cow dung

2. LITRATURE REVIEW

2.1. Biogas

Biogas is a methane rich gas produced by anaerobic breakdown of organic wastes with the help of methanogen bacteria under oxygen free environment. It comprises about 60% methane, 40%carbon dioxide and 0.2 - 0.4% hydrogen sulfide (Molina *et al*.,2007).During the digestion process, microorganism produces intermediate products. Intermediate products usually are short lived and do not accumulate in the reactor. However, the production rate of intermediate products depends on the composition of the substrate and can lead to the accumulation of intermediate products. The change of operational conditions likes pH or temperature can also induce the accumulation of intermediate products. The accumulation of intermediate products can inhibit digestion process. For instance substrate containing high fats can give high production of the fatty acids and induce to decrease pH, which will inhibit the microorganism activity further (Deublein, 2008).

Biogas reduces the concentration of pathogens considerably, thereby breaking the cycle of purification and leading to improved public health. waste recycling is utilizes different kinds of wastes todirect theenvironment. And also, due to eutrophication of tropical water bodies, water hyacinth, invades them and creates an imbalance in the lifecycle of these water bodies, In order to naturally control their invasive nature of the water body (Ipeghan *et al.,* 2013).

2.2. Significance of Biogas Technology

Biogas technology has various benefits for human beings. socio-economic and environmental benefits. The environmental benefits at local perspective are related to improvement of indoor air quality, better management of animal manure and human excreta -thus improving sanitary conditions in the immediate vicinity of the rural homes. From the view of national perspective, it contributes to reduction in deforestation, better

watershed and soil management, slurry use to replenish soil nutrient and fulfill energy demand. From global perspective, it leads to reduction in the use of fuel wood, dung cakes and kerosene, In broader sense, the maintenance of environment through conservation of forest improves the environment by managing natural climates, soil erosion, floods, landslides, and global warming (Shakya and Charushree, 2009). The systems that are used to create bio-energy can greatly contribute to reducing greenhouse gases as they have the possibility of reducing the need to use fossil fuels. By providing a non-polluting energy source which is also renewable, the earth is being kept clean of harmful emissions. Biogas is also the ideal way to ensure that all areas have access to electricity. As a fairly cheap source of electricity, biogas is a fuel source that has the power to provide decent energy to the world (Oluwaleye *et al*.,2013).

Biogas is the mixture of gases, mainly methane (CH_4) and carbon dioxide (CO_2) resulting from the anaerobic fermentation of organic matter. It contains 50-70 % methane and can be used as a fuel for heating or electrical power generation. Production of biogas from agricultural plants offers several environmental benefits including production of renewable energy (CO_2-neutral), and the nutrient rich stabilized liquor (NH_4) which is directly available for plants. Further, anaerobic digestion may also assist in reducing and destroying pathogens to acceptable levels, reducing greenhouse gas emissions, and aid in reducing odors often associated with storing and handling liquid wastes. Biogas production, when compared to other biomass energies, has the advantage that it can be produced from specially grown energy crops as well as from organic waste products (Holm-Nielsen *et al*., 2009).

2.3. Description of the Feedstock

The common substrates that are used for biogas production include industrial wastes, agricultural wastes, manures, energy crops, etc. A biomass that contains carbohydrate, protein, fat, cellulose and hemicelluloses can be used as substrate for biogas production (Dioha *et al.,*2013). Fats provide the highest biogas yield but it requires a long retention

time due to their poor bioavailability. Carbohydrate and protein show much faster conversion rates but lower gas yields. All substrates should be free from pathogens and other organisms otherwise it is better to pasteurization at 70° C or sterilization at 130° C prior fermentation. The carbon - nitrogen ratio should be in the range between 20 and 30 (Dioha *et al.,*2013).Cow dung (fresh) is obtained from cows are slaughtered for human consumption. The dung is obtained after the evacuation of the dung from the intestine. Cowdung is two types; the intestinal dung and the excreted dung from cows (Ukonu, 2011).

Animal wastes containing comforter such as chicken litter with substantial quantities of wood chips or sawdust can be used successfully in anaerobic digestion. The woody material, which degrades very slowly because of its lignin structure. To enhancen energy-dense feedstocks with livestock manure is a common practice to maximize biogas production by optimizing nutrient levels and providing buffering capacity (Liu *et al.,*2009).

2.4. Cow Manure for Biogas Production

Worldwide energy crisis directed the attention to the alternative sources of energy instead of fossil fuels. Cow manure is an excellent substrate for biogas production in anaerobic digesters though the gas yield from a single substrate is not high. However, mixing cow manure with other kind of waste materials in co-digestion can optimize the production of biogas. Biogas has globally remained a renewable energy source derived from plants that use solar energy during the process of photosynthesis(Elijah *et al*.,2009).

Iqbal *et al*., (2008) reported that about 17-30% of the global methane production is from enteric fermentation. Globally, ruminant livestock produce approximately 80 million tonne of methane annually (EPA, 2009).

2.5. History of Biogas Technology

2.5.1. Biogas Technology in the World

China is the world leader in implementing biogas digester. Twenty five million people are provided energy from the first large scale biogas digester. Seven million digesters were constructed in 1970 by the government. Another large user of biogas digester is India. In this country around 280 000 small-scale digesters were installed in 1985 (WEC, 1994).

In China, India, and Nepal household and institutional bio- digesters have been constructed since 2001. China has disseminated over 2 million household digesters annually. In addition, the Chinese government has supported over 200 large and medium livestock farms and advanced biogas units. From 2001 to 2007, over 18 million households adopted the technology leading to the production of over 7 billion $m^3$of biogas. By the end of 2002, in India over 3 million domestic digesters and 3000 community and institutional plants were constructed and since 2005, more than 100,000 bio-digesters have been disseminated annually (Aggarwal, 2003).

Other successful biogas promoting Asian countries include Nepal, Vietnam and Thailand (Myles, 2008). The European biogas sector counts thousands of biogas installations, and countries like Germany, Austria, Denmark and Sweden are among the technical forerunners, with the largest number of modern biogas plants. On the other side of the Atlantic, USA, Canada and many Latin American countries are on the way to developing modern biogas sectors and favorable political frameworks are implemented alongside, to support this development (Myles, 2008).

2.5.2. Biogas Technology in Africa

As compared with other world countries, African countries biogas technology shows some limitations. The reasons for these failures are associated with poor design and construction of digesters, wrong operation and lack of maintenance by users, poor dissemination strategies, lack of project monitoring and follow-ups by promoters, and poor ownership responsibility by users. Despite the relative stagnation of biogas programs in Africa, the

future prospects are encouraging. Several biogas plants in recent years have been constructed for energy (cooking, lightning and fuel replacement), and for sustainable environmental system in several countries of Africa including Ghana, Kenya, Tanzania, Rwanda, Burundi, and South Africa (Parawira, 2009). Between 4000 and 5000 digesters are estimated in Tanzania (Marree*et al.,* 2007), while Kenya is said to have disseminated about 2000 digesters as at October 2007. In Ghana, about 200 digesters have been disseminated (Bensah, 2009).

2.5.3. Biogas Technology in Ethiopia

Biogas technology was introduced in Ethiopia in 1979. Even if biogas technology has multitude of advantages to rural household societies and for forming sustainable environment, the wider dissemination of the technology is limited until the National Biogas Program (NBP) is launched in 2008.To implement the technology widely, it needs encouraging the households because in lacking technical and financial support to rural households who are more or less unaware of the technology it is difficult to use it consistently (Eshete *et al.,*2006b). As NBP (2008) reported, around 1000 biogas plants were constructed in various parts of the country. Approximately 40% of these plants are not operational due to lack of effective management and follow-up, technical problems, loss of interest, evacuation of ownership and water problems. Other reasons for the limited success of the technology in Ethiopia include the adoption of a project-based stand-alone approach without follow-up structure in place, variations in design, and the absence of a standardized biogas technology (NBP, 2007).Ethiopia's livestock population according to 2009/10 CSA survey is about 150 million. One third of this is cattle, whose refuse can effectively be used for biogasgeneration (Jemal, 2016).

2.6. Anaerobic Digestion

Anaerobic digestion is a complex process requiring specific environmental conditions and different bacterial populations. The mixed bacterial populations degrade organic compounds producing as end product a valuable high energy mixture of gases (mainly CH_4

and CO_2) termed as biogas and a nutrient rich fertilizer (Sandars *et al*., 2003). This process occurs under managed conditions where free oxygen is absent at temperatures suitable for naturally occurring mesophilic or thermophilic anaerobic and facultative bacteria's that converts the input to biogas (WRAP, 2010). According to Liu *et al*., (2009) anaerobic digestion is considered as waste-to-energy technology and it is widely used in the treatment of different organic wastes. It also consists of mixed biological systems in which organic materials such as carbohydrate, lipids, and proteins are utilized by microorganism to produce methane and carbon dioxide in their normal metabolic activities.

2.7. Biological Stages in Anaerobic Digestion

There are four main consecutive biological and chemical stages in the process of anaerobic digestion (Drawnel, 2008). These four key biological and chemical stages are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Onojo *et al.,* 2013). Thus, four categories of microorganisms are involved in the transformation of complex organic materials into simple molecules such as methane (CH_4) and carbon dioxide (CO_2) (Bitton, 2005).

2.7.1. Hydrolysis

Hydrolysis is the first process of anaerobic digestion in which hydrolytic microorganism that secrets an enzyme to hydrolyze polymeric materials such as carbohydrate, protein, nucleic acids, fats, and cellulose into monomers such as sugar, amino acids, glycerol and fatty acid (Nasir and Beyza, 2007). Polymers like Cellulose and hemicellulose are long-chain polysaccharides that can be broken down by specific enzymes present in certain bacteria, but not in animals. Lignin has a compact structure and is practically biologically inert (Jemal, 2016).

$$(C_6H_{10}O_5)n + nH_2O \leftrightarrow (C_6H_{12}O_6)n \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

It is a slow process which depends on the nature of the particulate matter and size of Organic matters. For a complex substrates with a high solid content hydrolysisusually the slowest step and hence the rate limiting step in the overall anaerobic digestion process

(Lomborg, 2009 and Asgari, 2011). The rate of the process can be governed by substrate availability, bacteria population density, composition and particle size of substrate, temperature, pH and a high concentration of intermediate products (Jemal, 2016). During hydrolysis the complex compounds are broken down into soluble components and it is readily available for fermentative bacteria to convert into alcohols, acetic acid, other volatile fatty acids and off-gas containing hydrogen and carbon dioxide. The intermediate products of hydrolysis will be metabolized into primarily CH_4 (>60%), CO_2 (<40%) and other associated gases by methanogens (Jemal, 2016).

2.7.2. Acidogenesis

In the process of acidogenesis the remaining components of hydrolysis are further broken down by acidogenic bacteria and here volatile fatty acids are created along with ammonia, carbon dioxide and hydrogen sulfide. The acidogenesis stage is a complex phase involving acid-forming fermentation, hydrogen production and an acetogenic (acetic acid-forming) step (Bekele, 2011). In the process of acidogenesis the products of hydrolysis are converted into methanogenic substrates. The main products of acidogenesis are acetic acid, lactic acid and propionic acid. Carbon dioxide and hydrogen are evolved as a result of catabolism of carbohydrate with the additional potential for the production of methanol and other simple alcohol (Zaher *et al*., 2007). Typical reactions in the acid forming stages are shown below. In equation 2, glucose is converted to ethanol and equation 3 shows glucose as transformed to propionate (Bekele, 2011).

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

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad \text{.....(3)}$$

2.7.3. Acetogenesis

In the acetogenesis process simple molecules produced through the acidogenesis phase are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen. Acetogenic organisms are the vital link between hydrolysis-acidogenesis and the

methanogenesis in anaerobic digestion. Acetogenesis provides the two main substrates for the last step in the methanogenic conversion of organic material, namely hydrogen and acetate. Both the acidogenesis and acetogenesis produce the methanogenic substrates are acetate, hydrogen and carbon dioxide (Florian *et al.,* 2013). In general, for reactions producing H_2, it is necessaryfor hydrogen to have low partial pressure for the reaction to proceed.

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \ldots.(4)$$

Other important reactions in the acetogenic stage involve the conversion of glucose (5), ethanol (6), and bicarbonate (7) to acetate.

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$

$$2HCO_3^- + 4H_2 + H^+ \leftrightarrow CH_3COO^- + 4H_2O \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

2.7.4. Methanogenesis

The production of methane and carbon dioxide from intermediate products is carried out by methanogenic bacteria. Formic acid, acetic acid, methanol and hydrogen can be used as energy sources by the various methanogens (Dhadse *et al*., 2012). The formation of methane is the ultimate product of anaerobic treatment. The acetoclastic group comprises two main genera namely Methanosarcina and Methanothrix (Zaher *et al.,*2007). The methane former works slower than the acid former therefore the pH has to stay constant consistently and slightly basic, to optimize the creation of methane. One needs to constantly feed in sodium bicarbonate to keep pH slightly basic (Prebeheim *et al.,* 2010). These compounds involve in the conversion of simple compounds (acids) into methane and CO_2 by CO_2 utilizing anaerobic methanogenic bacteria(Catarina, 2011; Edison, 2014). The methanogenenic anaerobic bacteria are fastidious bacteria that occur naturally in deep sediments or in the rumen of herbivores. This population converts the soluble matter into methane about two thirds of which is derived from acetate conversion (equation 8 followed

by 9), or the fermentation of an alcohol, such as methyl alcohol (10), and one third is the result of CO_2 reduction by hydrogen (11) (Liu and Whitman, 2008).

$$2CH_3CH_2OH + CO_2 \leftrightarrow 2CH_3COOH + CH_4 \quad \text{……(8)}$$

$$CH_3COOH \leftrightarrow CH_4 + CO_2 \quad \text{……..(9)}$$

$$CH_3OH + H_2 \leftrightarrow CH_4 + H_2O \quad \text{…….(10)}$$

$$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O \quad \text{……(11)}$$

2.8. Factors that Affect the Production of Biogas

The performance of biogas production plants can be controlled by studying and monitoring the variation in parameters such as temperature, pH, nutrient, particle size, hydraulic retention time, total and volatile solids, water content, Organic loading rate, seeding, C/N ratios and mixing condition. Any drastic change can adversely affect the biogas production (Yadvika, 2004).

2.8.1. Temperature

Temperature is one of the major factors that can affect the fermentation processes for the production of biogas. Temperature inside the digester has a major effect on the biogas production process. There are different temperature ranges during which anaerobic fermentation can be carried out. psychrophilic (<30°C), mesophilic (30 to 40°C) and thermophilic (50 to 60°C). However, anaerobes are most active in the mesophilic and thermophilic temperature range. The length of fermentation period is dependent on temperature (Yadvika et al., 2004). The optimum temperature of digestion may vary depending on feedstock composition and type of digester but in most anaerobic digestion processes it should be maintained relatively constant to sustain the gas production rate (Alemu, 2016).

Anaerobic process like other biological process strongly depends on temperature. A thermophilic temperature reduces the required retention time. The microbial growth, digestion capacity and biogas production could be enhanced by thermophilic digestion, since the specific growth rate of thermophilic bacteria is higher than that of mesophilic bacteria (Alemu, 2016).

2.8.2. PH

The other factor that affects the production of biogas in the fermentation processes is pH For optimal performance of the microbes the pH within the digester should be kept in the range of 6.8 - 8.0. The pH value below or above this interval may impair the process in the reactor since micro-organisms and their enzymes are sensitive to pH deviation (Yadvika *et al.,* 2004). The optimal pH values are different in acidogenesis and methanogenesis stages. During acidogenesis acidogenic bacteria produce organic acids (acetic, lactic, and propanoic acids) that tend to lower the pH of the bioreactor. Theacidogens prefer a pH 5.5-6.5. methanogenesis requires a near-neutral pH (between 6.5 and 7.5). A decrease in pH can inhibit gas production and can lead to further accumulation of acids (Jonathan, 2014).

2.8.3. Nutrient

In the production of biogas Nutrient that involves a large number of chemical, biochemical and microbiological reactions taking place. Associated with the metabolism of numerous organisms and intermediates that trans-form complex organic matter into more easily de-gradable substrates by anaerobic microorganisms. As a biological process, AD requires macro and micro-nutrients. Those nutrients are essential for optimal growth and development of microorganisms. A proper nutrient balance, as well as nutrient bioavailability, and process stability (Alemu, 2016) .

Macronutrients

The most important macro nutrients are needed for all biological treatment processes are nitrogen and phosphorous. These nutrients are available for methanogens as ammonical-nitrogen and orthophosphate-phosphorus. For methane forming bacteria, NH_4^+ - N is the preferable nitrogen nutrient. The amount of nitrogen and phosphorus that must be available in the digester can be determined from the quantity of substrate or COD of the digester feed sludge (Drawnel, 2008).

Micronutrients

Methane-forming bacteria possess different types of enzymes that are provided by micronutrients. The need for cobalt, iron, nickel and sulfide is critical. For successful digestion, incorporation of micronutrients in enzyme system is important. These micronutrients are required by methane- forming bacteria inorder to convert acetate in to methane (Funda, 2011). Anaerobic bacteria utilize carbon and nitrogen content in the feedstock by different rate for the purpose of energy and building cell structures, respectively. The optimum value of carbon and nitrogen ratios in anaerobic digestion should be from 20:1 to 30:1 (Ezekoye, 2013). A high carbon to nitrogen ratio indicates rapid consumption of Nitrogen in the stages of methanogens and results in a lower gas production. On the other hand a low carbon to nitrogen ratio causes ammonia accumulation and pH values exceeding 8.5 which are toxic to methanogenic bacteria (Monnet, 2003).

2.8.4. Particle size

The particle size of the substrate is affecting the yield of biogas. The big particle size of the substrates are difficult for microbes to digest and it can also result in blockage in the digester, whereas small particle size gives a large surface area for substrate adsorption and thus allows the increased microbial activity followed by increase in the production of gas (Yadvika *et al.*, 2004).

2.8.5. Hydraulic retention time

Hydraulic Retention time is the time required for completion of the anaerobic digestion, and varies with technologies, temperature, and waste composition. Lower retention times are required in digesters operated in the thermophilic range. A high solid reactor operating in the thermophilic range has usually shorter retention time (Demetrides, 2008). The retention times of mesophilic and thermophilic digesters range between 25-35days (Bouallagui *et al.,* 2003).

According to Shuaibu, (2011) Kinetic studies show that 90% or more of the biologically available solids was mesophilically (35^{o}C) biodegraded within 12 days At a retention period of 20 days, methane production from dairy cattle is essentially complete.

2.8.6. Total and volatile solids

The typical animal and human wastes consist of about 15-48 percent of total solids and 77 percents of volatile solids (Fulford, 1988). Nallathambi and Lakshmanaperumalsam, (1990) studied the biogas production potentials of parthenium in batch digester. They observed that the maximum gas production was 35 liters per kg fresh plant at a total solids concentration of 5 % and the methane content of the biogas was 75 %.

2.8.7. Water content

Water is an essential element for the growth and the activity of micro-organisms.Bacteria utilize substrates in dissolved form. When the amount of water content in feedstock is below 20% by weight, hardly any biogas will be produced (Rilling, 2005). The movement of bacteria and activity of extra cellular enzymes are highly determined by the water content in the digester (Jemal, 2016). Optimum moisture content has to be maintained in

the digester and the water content should be kept in the range between 60-95 % (Demetriades, 2008).The water content for each digester was determined according to the recommendation of (Ituen *et al*., 2007). Feed stocks were mixed with distilled water to get about 8% of TS suspension. The amount of water to be added was then determined using the following formula.

$$8\% = \frac{mFTS}{A + B}$$

Where, mFTS = mass of fixed total solid, A = mass of fresh sample added, B = mass of water and inoculums to be added to get 8% TS suspension in the digester.

2.8.8. Pre-treatment

The main goal of any pre-treatment is to change or remove structural impediment to hydrolysis and subsequent degradation processing in order to enhance digestibility, to improve the rate of enzyme hydrolysis and increase yields of intended products (Mosier *et al.,* 2005). Hendriks and Zeeman, 2009) Pre-treatment methods include mechanical, chemical and biological means to bring change in the nature of plant biomass in order to achieve the desired products. Pre Treatment by thermal pressure hydrolysis (23°C, 20-30 bars) results in the splitting of organic polymers by hydrolysis into short chain, biological available compounds which increase the biogas yield while the retention time in digester can be reduced drastically (Mosier *et al.,* 2005).

2.8.9. Organic loading rate (OLR)

Loading rate is the amount of raw material fed per day per unit volume of digester capacity. It is the rate at which substrate is supplied to the digester and is usually expressed in terms of Kg volatile solids cubic meter per day. Organic loading rate is an important parameter that affects gas yields. If the plant is over fed, the acid will be accumulated and methane production will be inhibited since bacteria cannot survive in acidic situation and if the plant

is underfed the gas production will also be low because of alkaline solution, which is not favorable condition for anaerobic bacteria (Jemal, 2016). Volatile Solids contain largely carbon, oxygen, and nitrogen which burn off from an already dry sample in a laboratory furnace at 500-600°C, leaving only the ash which contains largely calcium, magnesium, phosphorus, potassium, and other mineral elements that do not oxidize. In general, materials with high volatile-matter content produce more biogas (Arogo *et al.,* 2009).

2.8.10. Seeding

The common seeding materials used for biogas production include digested sludge from a running biogas plant or material from well-rotted pig manure or cow dung slurry (Yadvika et al., 2004), Forster *et al.,* (2007) indicates that digested sludge is the best inoculum source for anaerobic thermophilic digestion of the treatment of organic fraction of municipal solid waste at dry conditions (30% TS). Sunarso *et al*., (2012) states that Rumen fluid inoculums caused biogas production rate and efficiency increase more than two times compareto manure substrate without rumen fluid inoculums. Rojas *et al*., (2010) states that the addition of manure slurry to the batch reactor as part of the starter improved the biogas production.

2.8.11. Carbon- nitrogen ratio

A suitable C/N ratio plays an important role for the proper proliferation of the bacteria for the degradation process. Depending upon the relative richness in C and N content, feed material can be classified as nitrogen or carbon-rich. It is generally found that during digestion microorganisms utilize carbon 25-30 times faster than nitrogen, i.e. carbon content in feedstock should be 25-30 times faster than nitrogen (Manyi-Loh *et al*., 2013; Krishania *et al*., 2013). To meet this requirement, constituent of feedstock are chosen in such a way to ensure a C/N ratio of 25.1to 30.1 and concentration of dry matter as 7-10%. Even in situation where C/N ratio is close to 30.1, the biomass can undergo efficient anaerobic fermentation only if waste materials are also biodegradable at the same time (Behling *et al.,* 1997; Uzowuru *et al*., 2011).

The digestion of livestock waste containing high nitrogen to carbon ratios is more likely to result in toxic conditions for bacteria arising from the concentration of free ammonia (Arogo *et al.*, 2009). The solid waste substrate with high C/N ratio is not suitable for bacterial growth due to deficiency of nitrogen. As a result, the gas production rate and solids degradability will be low. On the other hand, if the C/N ratio is very low, the degradation process leads to ammonia accumulation which is toxic to the bacteria (Hartmann and Ahring, 2006). Carbon and nitrogen tests were carried out using the CHNS machine (Musa *et al.,2014).*

2.8.12. Mixing

The close contact between micro-organisms and substrate material is important for an efficient digestion process. The mixing of the digester contents has a number of benefits one of the most obvious being that it helps to mix up material evening out any localized concentrations, thus also helping to stop the formation of dead zones. In addition it uses increase the waste's availability to the bacteria which helps remove and disperse metabolic products and also act to ensure a more uniform temperature within the digester (Gareth and Judith, 2003). Mixing also promotes heat transfer, particle size reduction as digestion progresses and release of produced gas from the digester contents (Prasad *et al*., 2008).

2.8.13. Co-digestion process

Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. It is a waste treatment method where different types of wastes are treated together. Co-digestion offers several ecological, technological and economical advantages. The process provides improved nutrient balance from a variety of substrates which helps to maintain a stable and reliable digestion performance and produce a good fertilizer quality of the digestion (Feng and Rundong, 2010). Advantage of co-digestion includes better degradability, enhanced biogas production methane yield arising from availability of additional nutrients, as well as more efficient utilization (Elijah *et al*., 2009).

2.9. Types of Anaerobic Digestion System

A wide variety of systems have been developed to anaerobically treat agricultural wastes. They can be split into different categories as follow.

- Continuous versus batch process.
- Mesophilic versus thermophilic.
- Single stage versus two stage digestion.

2.9.1. Continuous versus Batch Process

The process may be operated either continuously or in batches depending on the substrate being digested (Lettinga, 2005 and Sakar *et al.,* 2009). With continuous digestion, new material is continuously pumped into the digestion tank. Creating a very smooth inflow of raw material, and hence also a smooth production of gas. It is possible to do this for substrates in liquid form that have a dry solids content of less than 5%, such as municipal and industrial waste water. Material in the form of sludge with a dry solids content that is between 5% and 15%, such as slurry and sewage sludge can also be added to the process more or less continuously. In addition to being more practical for the operator, this is also advantageous for the microorganisms because they get a more uniform supply of a substrate. This helps the interaction between various groups of microorganisms in the breakdown chain and reduces the risk that microorganisms will become overloaded due to the addition of a large quantity of substrate at one time. In this manner, the steady addition of substrates can make a higher total load possible (Sakar *et al*., 2009).

In contrast, in batch digestion all the material is digested at once and the material remains undisturbed in the same place throughout the entire digestion process. No new material is added nor is any digestion residue removed during the process. Methane production is generally highest at the beginning and then decreases over time. When the material is digested, the entire container is emptied of its contents and a new batch of substrate is added. An example of batch digestion, when waste is treated in the same place for a long

time, is in landfills. Batch digestion is also common in connection with biogas production for individual households, which is particularly common in Asian countries. Batch digestion is advantageous from a microbiological point of view because the organisms have plenty of time to break down the organic matter. Also, the organisms do not get washed out of the system. However, sometimes it can be difficult to achieve a high and even digestion rate, particularly if the substrate has a high content of dry solids (Bjorsson and Bergland 2006; Nordberg, 2007).

2.9.2. Mesophilic versus Thermophilic

The biodegradation of hand sorted organic fraction of wastes in batch type digesters at 55^0C resulted in a maximum methane yield ranging from 0.39 to 0.43 m^3 kg-1 VS added without paper and wood and VS reduction ranged from 63 to 69%. In the thermophilic high solids anaerobic digestion, higher OLR and methane production rate can be achieved at reduced HRT. Nordberg, (2007) studied that the methane yield was around 0.2 m^3kg-1 VS added. Digestion under thermophilic condition has many advantages such as higher metabolic rates and a high destruction of pathogens and weed seeds. On the other hand, thermopilic treatment has some drawbacks such as less stability compared to mesophilic conditions. Furthermore, the energy requirements of thermophilic systems are higher than those of mesophilic systems. The effect of temperature is particularly important on the hydrolysis step. The hydrolysis rate of cellulose in thermopilic conditions is about 5-6 times higher than that observed in mesophilic condition (Biey *et al*., 2003). The anaerobic digester that operates at the mesophilic temperature range (35-38 degree centigrade) is known as mesophilic digestion. Mesophilic anaerobic digestion is most common system which has a more stable operation (WEF, 2004).

2.9.3. Single Stage versus Two Stage Digestion

The simplest model for biogas production is to use a single digestion tank for the entire process, so-called one step digestion. With one step digestion, all stages in the microbial breakdown process, i.e. hydrolysis, fermentation, anaerobic oxidation and methane

production take place at the same time and in the same place. It is common for one step digestion to take place in total mixed process. A common type of biogas reactor is the Continuously Stirred Tank Reactor (CSTR). The substrate is completely mixed by various mixers. It is often used in one stage process for treating sludge, food waste, manure, etc. sometimes some of the residues/process liquids are returned to the process. This increases the retention time of material and helps more microorganisms to remain in the process (Nordberg *et al*., 2007).

An alternative to a single-stage process is to divide the process into two parts, called two-stage digestion. In two stage digestion, the first step is to load raw material into a digestion tank where the process is focused on hydrolysis and fermentation. This primarily results in acid formation. But a certain amount of biogas is normally also produced, because it is difficult to completely divide the process. Then, the digester or the process liquid from this process is separated and added to another digestion tank that is specially adapted for methanogenesis (Mshandete *et al*., 2008). This type of process may be appropriate when a substrate contains material that is easy to break down and the hydrolysis stage is fast (Anthony *et.al.,* 2008).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Microbiology and Central Laboratory of Haramaya University main campus which is found around 510 km away from Addis Ababa. The University is geographically located at a latitude of 9°26’ N, longitude of 42°03’E and has an altitude of 1980 m.a.s.l (FAO, 1990) and the mean annual temperature is 23.4ºC (HUA,1998).

3.2. Substrates

Sawdust and cow dung were the main substrates of this study and they were used as feedstock for anaerobic digestion process of biogas production. Both Saw dust and cow dung collected were from Haramaya University campus. Both substrates were oven dried for 72 hours in 105ºC to constant weight and crushed to smaller size using mortar and pestle to facilitate homogeneity for anaerobic digestion.

3.3. Experimental Design

Anaerobic digestion was conducted inbatch mode in 0.5 L digester using the two substrates, i.e., sawdust (SD) and cow dung (CD) in five proportions to have five substrate treatments (Table 1).

Table 1. The proportion of the two different substrates added into the five digesters

Treatments	Dry sawdust(gm.)	Dry cow dung (gm.)	Distilled H_2O added (ml)	RF(ml)	Percentage of	Percentage of CD
T1	10	0	114.9	100	100.00	0
T2	0	10	114.9	100	0.00	100
T3	5	5	114.9	100	50.00	50
T4	7	3	114.9	100	70.00	30
T5	3	7	114.9	100	30.00	70

T1=100%SD, T2=100%CD, T3=50%SD50%CD, T4=70%SD30%CD,and T5=30%SD70%CD, RF=Rumen fluid

To have 8% TS in fermenting slurry, appropriate amount of distilled water and rumen fluid (100ml) were mixed (Tchobanoglous *et al*., 1993). Treatments were randomly arranged in the lab and done in three replicates. The temperature of bio-digester was maintained at 35^0c by keeping in oven, which represents mesophilic condition (Knottier, 2003). The pH of the slurry was also maintained within the pH (between 6.5 and 7.5) range for optimal biogas production, i.e. about neutral (Thy *et al*., 2003; Yadvika *et al*., 2004).

3.4. Digester Configuration and Setup for Biogas Production

The three plastic bottles of 0.5ml were arranged in order so that the first bottle contained a slurry, the middle contained acidified brine solution and the last in order was empty for collecting the brine solution that was expelled out from the second container. The acidified brine solution was prepared by dissolving NaCl in distilled water with few drops of sulphuric acid until a supersaturated solution 40g salt /100 mL of water. is formed to prevent the dissolution of biogas in the water. All the three containers were interconnected with plastic tubes having a diameter of 1 cm. The tube connecting the first bottle to the second was fitted just above the slurry in the first bottle to help gas collection. Thus, the

biogas produced by fermentation of the slurry was driven from the first bottle to the second bottle that contained a brine solution so as to displace a volume of the brine solution equivalent to the volume of biogas produced. The lids of all digesters were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas.

Figure 1. Biogas production setup

3.5. Parameters measured

3.5.1. Analyses of Physico-chemical Characteristics of Substrates

Both Substrates were analysed for various physico-chemical parameters before and after AD as follows based on the Standard Methods for the Examination of Water and Wastewater (APHA, 1999).

3.5.2. Total solids (TS)

A clean evaporating dish (crucible) was dried at 105ºC for 1hr, cooled in desiccators and weighed immediately before use. 10g of SD and CD 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.

$$\%TS = \frac{MDS}{mFS} X100$$

Where, mDS = mass of dry sample, mFS = mass of fresh sample.

3.5.3. Volatile and fixed solids

The substrates were ignited at 550 °C in a muffle furnace (BiBBY, Stuart) for 3 hours to determine the volatile and fixed solids. The following formula was used to calculate the percentage of volatile solids content of the TS (APHA 2540 E, 1999).

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

Where, % VS = percentage of volatile solids

mDS = mass of dry solids in gram

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

i.e., TS=VS + fixed solids

Then percentage VS removal was calculated using the equation below

$$\%VS\ removal = \frac{VSi - VSf}{VSi} \times 100$$

Where Vsi = initial volatile solids before AD

Vsf = final volatile solids after AD

3.5.4. Determination of pH

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

3.5.5. Organic carbon

The carbon content of the substrates were obtained from volatile solids data using an empirical equation as reported by Badger *et al.* (1979).

$$\text{Carbon} = \frac{\%VS}{1.8}$$

Where,VS= Volatile solids

3.5.6. C : N Ratio

The organic carbon was determined using the data from volatile solids and employing the formula suggested by Haug, (1993).

$$\%\text{Carbon} = \frac{\%VS}{1.8}$$

%VS = percentage of Volatile Solid

The total nitrogen in the sample was determined using the Kjeldahl method. This method has three main steps. These are digestion, distillation and titration. One gram of sample and 6 ml of concentrated H_2SO_4 were added into a tecator 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 has 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 tecator rack before digestion. Then the digester was switched on and allowed to wait until its temperature reached 370°C. As the digester gained this temperature the rack was placed in it and the digestion continued for about 4 hours until a clear solution was observed.

The tube in the rack was transferred to the fume hood for cooling. About 50 ml of distilled water was added and shaken by hand to avoid sulphateprecipitation 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 were 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 HCl to a reddish color and %Nitrogen was calculated using the following formula.

$$\%\text{Nitrogen (N2)} = \frac{Vx\ 0.1\ x14\ x\ 100}{Wo}$$

Where,

V = Volume of HCl in Liter consumed to end point of titration

wo = Sample weight on dry matter basis and

14 = the molecular weight of nitrogen

0.1= Normality of HCl

Finally, the ratio of carbon to nitrogen was calculated as.

$$\frac{\%C}{\%N} = \text{C : N}$$

3.5.7. Measurement of Biogas production

Daily biogas production was measured following the method suggested by Itodo*et al*. (1992). As biogas production commenced in the fermentation chamber, it was delivered to the second chamber which contained the acidified brine solution. Since the biogas is insoluble in the solution, a pressure build-up provided the driving force for displacement of the solution. The volume of the displaced solution was measured to represent the amount of biogas produced.

3.6. Data Analysis

Mean values between treatments were analyzed using analysis of variance (one-way ANOVA) using SPSS for windows version 20) to investigate statistical significance between the different treatments. Paired samples T-test used wereto investigate statistical significance within a treatment, i.e., values between before and after AD. Difference between means were considered statistically significant at $P<0.05$.

4. RESULTS AND DISCUSSION

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

The moisture content, organic carbon content, and pH of the two substrates and substrate mix ratios were determined before and after anaerobic digestion and the results were compared statistically. The data are shown in Table 2below.

Table 2. Comparison of % MC, %Organic carbon and pH between before and after AD

Treatments	%MC initial	%MC Final	pHinitial	pHfinal	%C initial	%C final
T1	81 ± 1.08^{Ca}	83.87 ± 1.38^{Ab}	6.93 ± 0.17^{Aa}	7.34 ± 0.15^{Ba}	46.01 ± 0.52^{Ab}	38.69 ± 1.02^{Ea}
T2	77.89 ± 2.87^{Aa}	88.9 ± 1.59^{Db}	7.51 ± 0.37^{Bb}	6.47 ± 0.15^{Aa}	46.63 ± 0.93^{Bb}	37.28 ± 0.31^{Ca}
T3	82.02 ± 0.24^{Da}	86.71 ± 0.44^{Bb}	6.76 ± 0.16^{Aa}	6.67 ± 0.15^{Aa}	50.39 ± 1.05^{Db}	34.53 ± 1.06^{Ba}
T4	79.76 ± 2.98^{Ba}	87.9 ± 0.13^{Cb}	7.27 ± 0.02^{Bb}	6.59 ± 0.14^{Aa}	48.82 ± 0.77^{Cb}	23.46 ± 0.61^{Aa}
T5	80.51 ± 1.33^{Ca}	86.44 ± 0.75^{Bb}	6.89 ± 0.1^{Ab}	6.48 ± 0.14^{Aa}	50.04 ± 1.21^{Db}	37.92 ± 0.81^{Da}

T1 = 100%SD, T2 =100% CD, T3 = 50% SD and 50% CD, T4 = 70% SD and 30% CD, and T5 = 30% SD and 70% CD.

Means followed by the same small letters in row are not significant at $P< 0.05$ for paired samples T-test within treatment while means followed by the same capital letter in column are not significantly different at $P<0.05$ between treatments for one way ANOVA. In each case, different letters show the presence of significant difference between means at $P<0.05$.

As can be seen from Table 2 there was a significance difference ($P<0.05$) with in treatments in both initial and final % MC Table 2.all most all treatments %MC significantly increased after AD.The incensement of in % MC resulted when the adjustment of total solid in the digester was made to 8% as the recommendation of Elijah *et al.,*(2009) and Ituen *et al*., (2007) Thus it is possible to conclude that %Mc of these treatment groups were adjusted to suitable MC for better biogas production and it was with in the suitable range (65-95) recommended by Demetriade, (2008) In the treatment initial MC were to low to the

previous researchers (Ukonu, 2011) . because the substrate were very dried by the sun before inter to the oven.

There were significant differences ($P<0.05$) between before and after AD in %C in all substrates. After AD, %C was found to decrease in all substrates (Table 2). The decreasein %C reflects thedegradation of compounds during anaerobic digestion to be converted into biogasor to maintain the growth of bacteria (Gerardi, 2003; Abdel-Hadi *et al*., 2008). Compared to all other substrates, the extent of carbon reduction was highest (25.36) in substrate mix ratio of 70%SD and 30%CD followed by 50%SD and 50%CD mix ratio, which were (15.86). This mix ratio was also found to yield more biogas than the rest of the treatments as shown in Fig 2.

There were also significant differences at ($P<0.05$) within treatments in both the initial and final %C. The %C generally showed a significant decrease as a result of AD in all treatments (Table 2). The decrease in C% reflects the occurrence of degradation during anaerobic digestion (Abdel-Hadi *et al*., 2008). The degradation is meant for conversion of the substrates to cellular materials for growth and reproduction of bacteria or as a result of biogas production (Gerardi, 2003). However, degradation of organic carbon was highest (25.36 C%) for T4 (i.e. 70%SD and 30%CD). This mix ratio was also found to yield more biogas than the rest of the treatments as shown in Fig. 2.

Likewise, significant variations were shown between the initial and final pH values of in T1 and T3 treatments (Table 2). As can be seen from Table 2, the final mean pH values of 100%SDand 100% CD were 7.34±0.15 and 6.47±0.15, respectively. However, when the two substrates were mixed, their pH values were found to de crease form that of solo substrates. The pH of the slurry of cow dung alone (T2), 6.47, is similar to 6.38 as reported by Fokhrul .This result suggests that the PH range of anaerobic digestion occurred between 6.5-8.0 (Adrian *et al.,*2012).

4.1.1. Analysis of VS Values of Substrate before and after AD

The volatile solids of substrates were analyzed for all treatments both before and after AD (Tables 3), and values showed significant differences ($P<0.05$) between treatments and within treatments (between before and after AD).The maximum %VS before AD was measured in 50%cow dung and 50% sawdust, whereas the minimum %VS was measured from the digester with 100% SD (Table 3).

Table 3. mean value of %VS of substrate before and after digestion

Treatments	%VS Initial	%VS Final	%VS removal
T1	83 ± 2^{Ab}	69.73 ± 0.43^{Ea}	13.27±1.8
T2	84 ± 0.04^{Bb}	67.1 ± 0.56^{Ca}	16.9±0.55
T3	90.8 ± 0.28^{Eb}	62.12 ± 1.01^{Ba}	28.68±0.98
T4	87.9 ± 0.29^{Cb}	42.24 ± 0.75^{Aa}	45.66±3.32
T5	90.1 ± 0.17^{Db}	68.29 ± 0.86^{Da}	21.81±0.52

Means followed by the same small letters in row are not significant at $P< 0.05$ probability levels for paired samples T-test within treatment while means followed by the same capital letter in column are not significantly different at $P< 0.05$ level of significance between treatments for one way ANOVA.
T1 = 100%SD, T2 =100% CD, T3 = 50%SDand 50% CD, T4 =70%SDand 30%CD, and T5 = 30%SD and 70%CD.

After anaerobic digestion, %VS of all substrate and substrate mix ratios were significantly lower than before AD (Table 3).However, more %VS reduction was observed in 70%SDand30% CD mix ratio. This volatile reduction was also found to be on a balance with the amount of biogas measured. This suggests that most of this substrates mix were converted to biogas (Gerardi, 2003; Tamrat *et al*., 2013) and this mix ratio of the two substrates is high yielding compared to solo and other mix ratios. Reduction in %VS after AD is a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012).

4.1.2. Analysis of TS Values of Substrate before and after AD

The total solids of substrates was analyzed for all treatments both before and after AD (Table4) The percentage of TS showed significant difference between before and after AD at $P<0.05$. The maximum TS% before AD was measured in 50% SD and 50% CD whereas the minimum TS% was measured from the digester with 100% cow dung. The observed maximum values of TS% in 50% SD and 50% CD may suggest the presence of more biodegradable substrates for biogas production but it produce lower biogas production than 70%SD and 30% CD.

Table 4. Mean values of %TS of substrates before and after digestion

Treatment	Initial TS%	Final TS%	TS%Removed
T1	88.01 ± 0.94^{Cb}	32.21 ± 1^{Da}	55.8
T2	83.91 ± 1.56^{Ab}	30.11 ± 0.72^{Aa}	53.8
T3	90.1 ± 0.84^{Db}	28.36 ± 0.92^{Ca}	61.74
T4	87.87 ± 1.01^{Cb}	25.8 ± 0.76^{Ca}	62.02
T5	85.78 ± 1.81^{Bb}	28.33 ± 1.23^{Ba}	57.45

Means followed by the same small letters in row are not significant at $P< 0.05$ probability levels for paired samples T-test within treatment while means followed by the same capital letter in column are not significantly different at $P< 0.05$ level of significance between treatments for one way ANOVA.
T1 = 100%SD, T2 =100% CD, T3 = 50%SDand 50% CD, T4 =70%SDand 30%CD, and T5 = 30%SD and 70%CD.

After anaerobic digestion,TS%of all substrate type were becomesignificantly lower (Table 4).But more reduction was observed in T4 (70%SDand 30%CD), T3 (50%SD and 50%CD), T5 (30%SD and 70%CD)than in T1 (100%SD and T2 (100%CD). In T4, 70%SD and 30%CD there was a relatively higher percentage of TS removal then it produce high amount of biogas. Less biogas production observed from CD 100%can be attributed to its toxicity or conversion of the substrates to cellular materials for growth and reproduction of

bacteria (Gerardi, 2003; Tamrat *et al*., 2013). Thus, total solids destruction is a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012).

4.1.3. Carbon to Nitrogen (C : N) Ratio of Substrates before AD

Table 5. The carbon to nitrogen ratio of SD and CD when used singly and in different combinations

Treatments	% Carbon	%Nitrogen	C.N ratio
T1	46.11±0.72	1.87±0.08	24.66:1
T2	46.21±1.13	1.48±0.06	31.22:1
T3	50.44±1.44	1.74±0.13	28.99:1
T4	48.83±0.89	1.70±0.3	28.72:1
T5	50.05±1.06	1.58±0.11	31.61:1

T1 = 100%SD, T2 =100% CD, T3 = 50%SD and 50% CD, T4 =70%SD and 30% CD and T5 = 30%SD and 70% CD.

In this experiment, the carbon to nitrogen ratio of treatments T1, T2, T3, T4 and T5 before AD were 24.66:1, 31.22:1, 28.99:1, 28.72:1 and 31.61:1 respectively (Table 5).

The results unfold that the yield of biogas depends on C/N ratio of the various feedstock. The optimum yield of biogas is in the range of C/N ratio of 20 – 30:1. The variation of the C/N values can affect the pH of a slurry. The increase in carbon content will give rise to more carbon dioxide formation and lower pH value, while high value of nitrogen will enhance production of ammonia gas that could increase the pH to the detriment of the micro-organisms (Dioha *et al*., 2013).

The result was in agreement with the optimum C:N ratio (20:1 to 30:1) reported by Dioha (2013). It is also comparable with the carbon to nitrogen ratio of cattle manure (22.22:1) mixed with rumen content (26.17:1) reported by Solomon (2014). And also it is comparable with the carbon to nitrogen ratio of paddy straw (26:1) and groundnut shell (27:1) mixed

with cow dung in pre-digested slurry reported by (Oghenero *et al.,* 2016). For good biogas production the adjusting of C/N ratio is desirable and this can be achieved by mixing wastes of high ratio with those of low ratio (FAO/CMS, 1996).

4.2. Analysis of Average Daily and Cumulative Biogas Production from Solo and Co-Digestion of the Selected Substrates

The results showed that the co-digested substrates of the three mix ratios (i.e. T4, T3 and T5) produced larger amount of biogas than the two individually fermented substrates (T1 and T2)
that were used as control groups. Similar results were reported by Tamrat *et al*. (2013) from the co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. This might be attributed to the positive synergetic effect of the co-digestion of *sawdust*and cow dung in providing more balanced nutrients, increased buffering capacity and decreased effect of toxic compounds. Previous studies have revealed that the digestion of more than one kind of substrate could establish positive synergism in the digester (Li *et al*., 2009; Danqi, 2010; Jianzheng *et al*., 2011).

Of the three mix ratios, digester with 70%SD + 30% CD (T3) produced biogas 512.25ml which is larger than the other two mix ratios. The rapid biogas production in the digester containing 70% SD + 30% CD might be also due to the shorter lag phase in the growth of bacteria, the availability of readily biodegradable organic matter in the substrate. The highest biogas production was also observed from this digester probably due to the availability of balanced nutrient composition for microorganism and the existence of stable pH which was attained as a result of the addition of inoculum and the particular mix ratio.

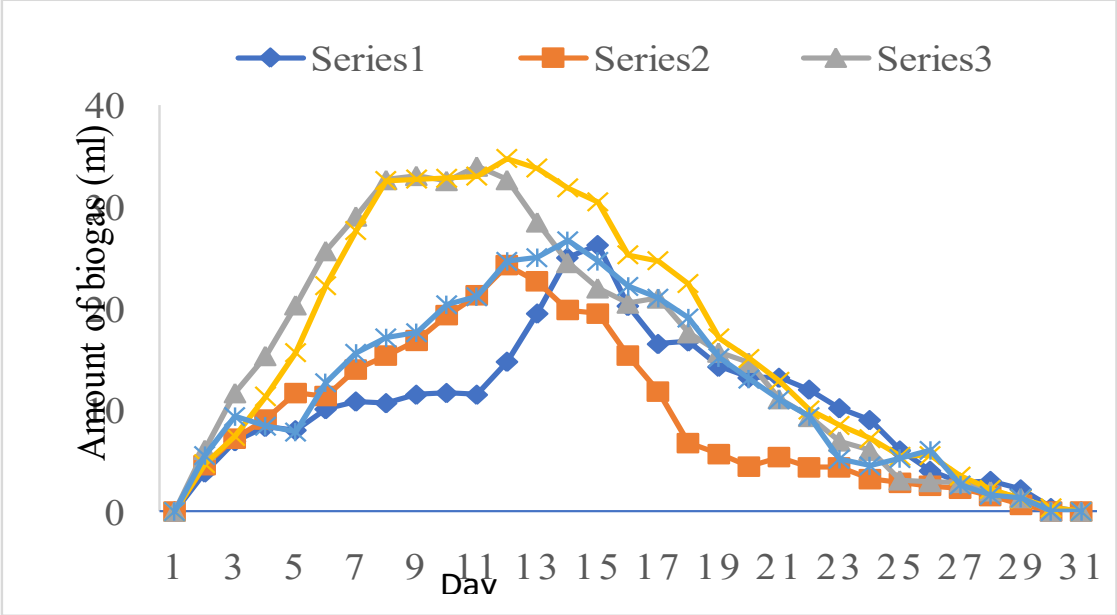

Figure 2. Daily mean biogas yield of the five treatments in 31 days with ml.

There was a significant difference between the substrates in the overall biogas yield (Figure 2, $p<0.05$). However, there is no biogas production observed in 1st day, this may be due to the fact that the microorganisms were in theirincubation period (the period of apparent inactivity in which the cells are adapting to a new environment and preparing for reproductive growth). Cells are usually synthesizing new components (Cundiff and Mankin, 2003).

After 18 days, more or less biogas production showed decrease presumably when the microorganisms and the nutrients were entereddecline (Cundiff and Mankin 2011). At the end of the 30th and 31th day biogas production ended and stopped. This may be due to the fact that the microorganisms entered the death phase, the period in which the cells are dying at an exponential rate. Some of the reasons for entering this phase could becontinued accumulation of wastes, loss of cell's ability to detoxify toxins, the depletion of the necessary nutrients from the digesters and the increase in ammonium concentration that resulted in an increased pH values (Hansen *et al*., 1998).

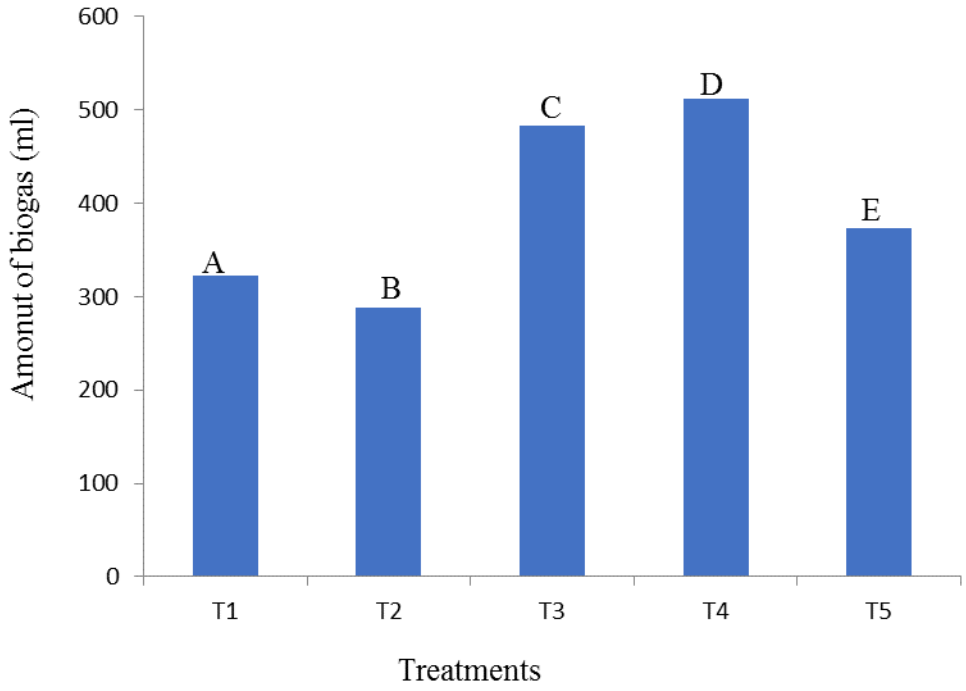

Figure 3. Mean cumulative biogas yield of the five treatments

Bar graphs with different letters indicate significant differences between means while those with same letters show no significant difference between means. T1 = 100%SD, T2 =100% CD, T3 = 50%SD and 50% CD, T4 =70%SD and 30%CD, and T5 = 30%SD and 70%CD.

The cumulative biogas productions of the five treatments are summarized in Figure 1. The results showed that T4 produced the highest mean cumulative biogas, which was 512.25 ml within 31 days of incubation period. This was followed by T3 which produced 483.08 ml gas. The 5th and 1st highest biogas production, yielding 373.75 and 322.42ml, were obtained in T5 and T1, respectively. Cow dung alone (T2) yielded less biogas, which was 287.75 ml. As it is a product of a substrate that has already undergone partial fermentation in the intestinal tract of the animal and as it contained less degradable macro and micro nutrients (Chawala, 1986; Deublein and Steinhauser,

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Production of biogas through anaerobic digestion of organic waste materials provides an alternative energy. The experiment was conducted under mesophilic conditions (35ºC) using five batch digesters of triplicates. In all treatments, TS%, VS%, Organic carbon%, percentage of moisture content, and pH were analyzed before and after digestion, but C/N ratio were analyzed before AD. The daily biogas production was measured by water displacement method for 31 days.

The pH was found to increase significantly with increasing of saw dust proportion in the mix, suggesting that saw dust helps to maintain the pH to meet the optimum required.The comparison of pH values between before and after AD showed that pH values are btween 6.5-8.0 for all the treatments. It showed the pH ranges between 6.47 for 100% CD and 7.34 for 100% SD.

The %initial highest moisture content (82.02) was observed in 50%*CD*and 50% SD the %initial lowest moisture content (79.76) in 70% SD and 30%CD.

The results also showed that there are differences in percentage organic carbon in all mix ratios between before and after AD ($P<0.05$). Comparison of initial and final %organic carbon showed that %organic carbon significantly decreased AD in all substrate types.

In this study, C :N ratio of almost all treatments was found in between 20:1-30:1 which is a suitable condition for methanogenic bacteria to reproduce and produce optimum biogas. High reduction of VS was measured in 70% saw dust and 30% of cow dung substrate compared to the rest of substrates after AD, since at initial VS was 87.9 but after AD it became 42.24 The biogas in T4 (70% cow dung+ 30% CD produce higher (512.25 ml)

amount of biogas than others at 31 days. But the minimum amount of biogas produced in T2 (100%) cow dung(287.75ml

5.2. Conclusion

The results showed that there is a strong possibility to enhance the biogas production. Use of certain organic additives seem to be show potential for enhancing biogas production. In this result sawdust have been found to enhance the gas production significantly. The general outcome of this study suggested that the *sawdust* co-digested with cow dung improved the biogas potential compared to cow dung and sawdust alone. The experimental data also showed that the highest gas production was obtained in the mix ratio of 70% *SD*and 30% CD (512.25 ml). Thus, compared to the mono-digestion of pure cow dung and pure *sawdust* anaerobic co-digestion of 70% *sawdust* and 30% cow dung mix ratios enhances both the rate and amount of biogas yield.

5.3. Recommendations

- Since this investigation was done at mesophilic temperature (35^{o}C), it is recommended that further study be carried out at room temperature (25^{o}C) and at thermophilic conditions (Above 55^{o}C).
- Parameters such as design of digester should be taken into account as sampling was unsatisfactory and that different designs should also be evaluated for improving the yield of biogas.
- Since this experiment was carried out on the co- digestion of saw dust and cow dung further similar studies should also be made on the co- digestion of *saw dust* with other substrates.
- The percentage of methane in the biogas produced needs to be qualified for better efficiency of biogas production from *sawdust* co-digested with cow dung.

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7. APPENDIX

Appendix table 1. Daily biogas yield in 31 days fermentation time

Days	Treatments				
	T1	T2	T3	T4	T5
D1	0	0	0	0	0
D2	3.83	4.58	6.08	4.67	5.5
D3	6.92	7.17	11.67	7.42	9.33
D4	8.33	9.08	15.33	11.33	8.42
D5	8	11.67	20.33	15.67	7.83
D6	10.08	11.33	25.67	22.25	12.67
D7	10.83	14	29.08	27.67	15.58
D8	10.67	15.33	32.67	32.58	17.08
D9	11.50	16.83	33.08	32.75	17.58
D10	11.67	19.33	32.58	32.83	20.33
D11	11.5	21.25	34	33	21.17
D12	14.75	24.25	32.67	34.75	24.67
D13	19.5	22.67	28.5	33.83	25
D14	25	19.83	24.5	31.83	26.67
D15	26.25	19.5	22	30.5	24.67
D16	20.25	15.33	20.5	25.25	22.17
D17	16.5	11.83	21	24.67	21
D18	16.75	6.75	17.58	22.42	19.08
D19	14.25	5.67	15.67	17.08	15.17
D20	13.17	4.42	14.67	15.08	13.08

D21	13.17	5.33	11.08	12.83	11
D22	12	4.33	9.33	10	9.33
D23	10.17	4.33	6.92	8.5	5.17
D24	9	3.17	6	7.17	4.5
D25	6	2.83	3.08	5.42	5.25
D26	4	2.5	2.92	5.5	6
D27	2.83	2.25	2.83	3.5	2.58
D28	3	1.5	2	2.17	1.58
D29	2.17	0.67	1.33	1.25	1.33
D30	0.33	0	0	0.33	0
D31	0	0	0	0	0

Appendix table 2. Total Biogas production from five treatments

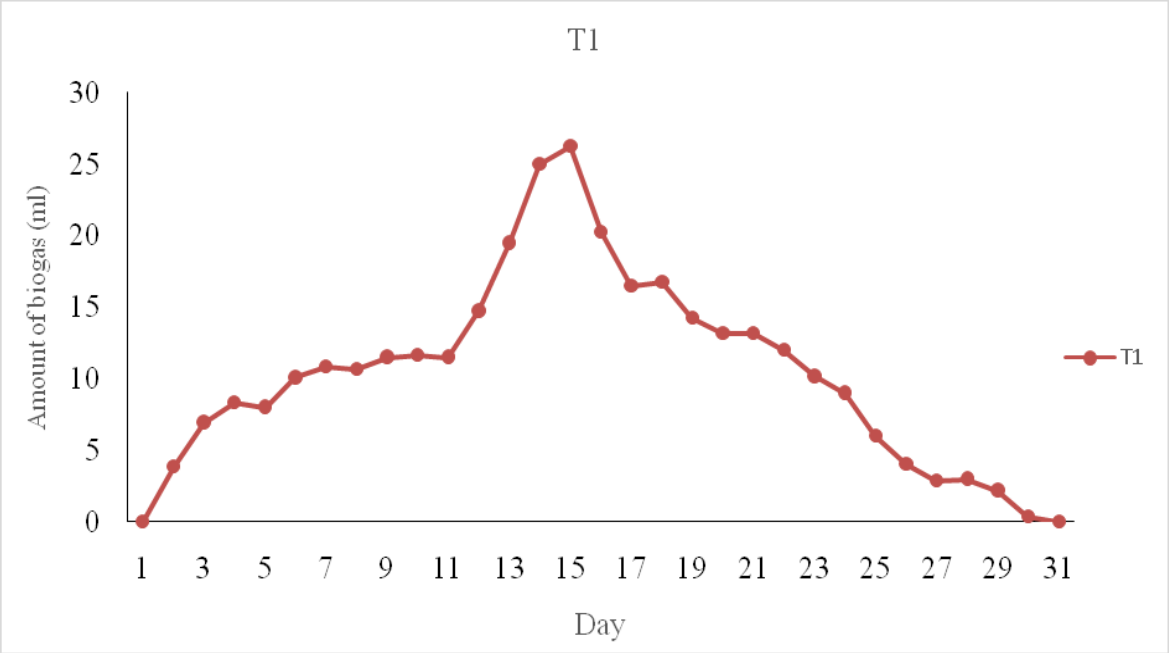
Treatments	Mix ratio	Total biogas(ml)
T1	100% *Sawdust*	322.42
T2	100% Cow dung	287.75
T3	50% SD + 50%CD	483.08
T4	70% *SD* + 30% CD	512.25
T5	30 SD + 70 % CD	373.75

Appendix table 3. Average percentage composition of TS Values of Substrate before and after AD

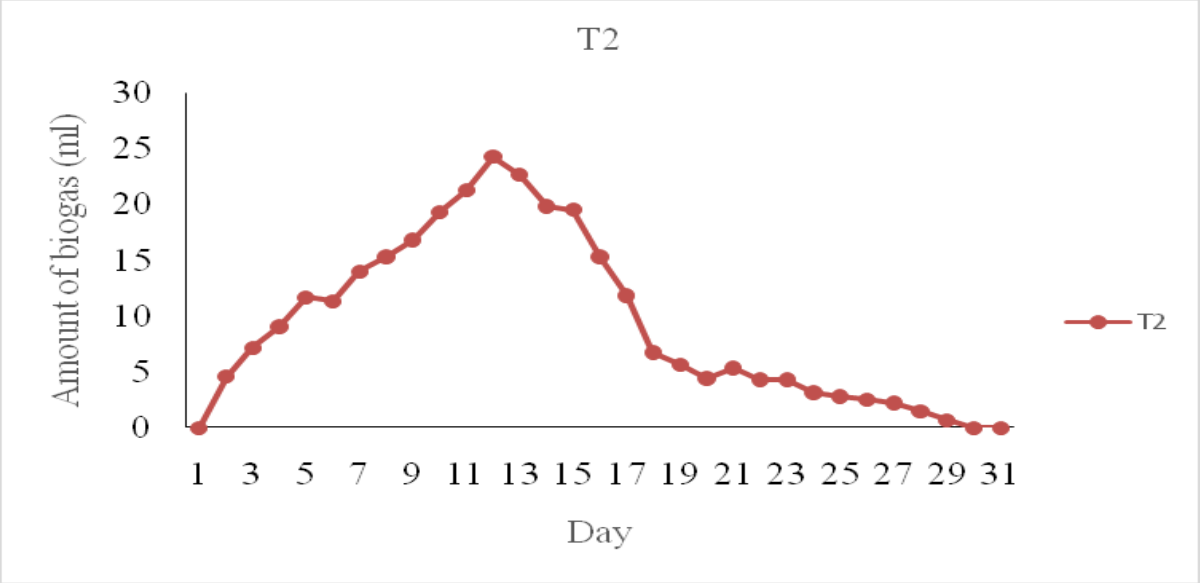
Treatments	%TS Initial	%TS final	%TS Removal
T1	$88.01.\pm0.89^{Cb}$	32.21 ± 1^{Da}	55.8
T2	83.91 ± 1.56^{Ab}	30.11 ± 0.76^{Aa}	53.8
T3	90.1 ± 0.84^{Db}	28.36 ± 0.92^{Ca}	61.74
T4	87.87 ± 1.01^{Cb}	25.8 ± 0.76^{Ca}	62.02
T5	85.78 ± 1.81^{Bb}	28.33 ± 1.23^{Ba}	57.45

Appendix table 4. Average percentage composition of VS Values of Substrate before and after AD

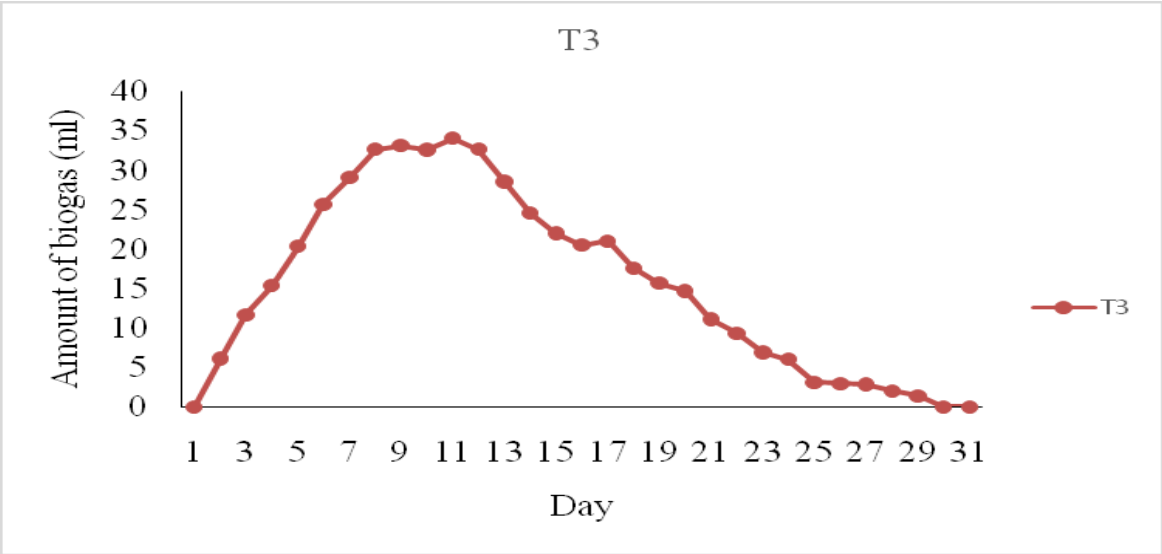
Treatments	%VS Initial	%VS final	%VS removal
T1	83 ± 2^{Ab}	69.73 ± 0.43^{Ea}	13.27
T2	84 ± 0.04^{Bb}	69.3 ± 0.56^{Ca}	16.9
T3	90.8 ± 0.28^{Eb}	62.12 ± 1.01^{Ba}	28.68
T4	87.9 ± 0.29^{Cb}	42.24 ± 0.75^{Aa}	45.66
T5	90.1 ± 0.17^{Db}	68.29 ± 0.86^{Da}	21.81



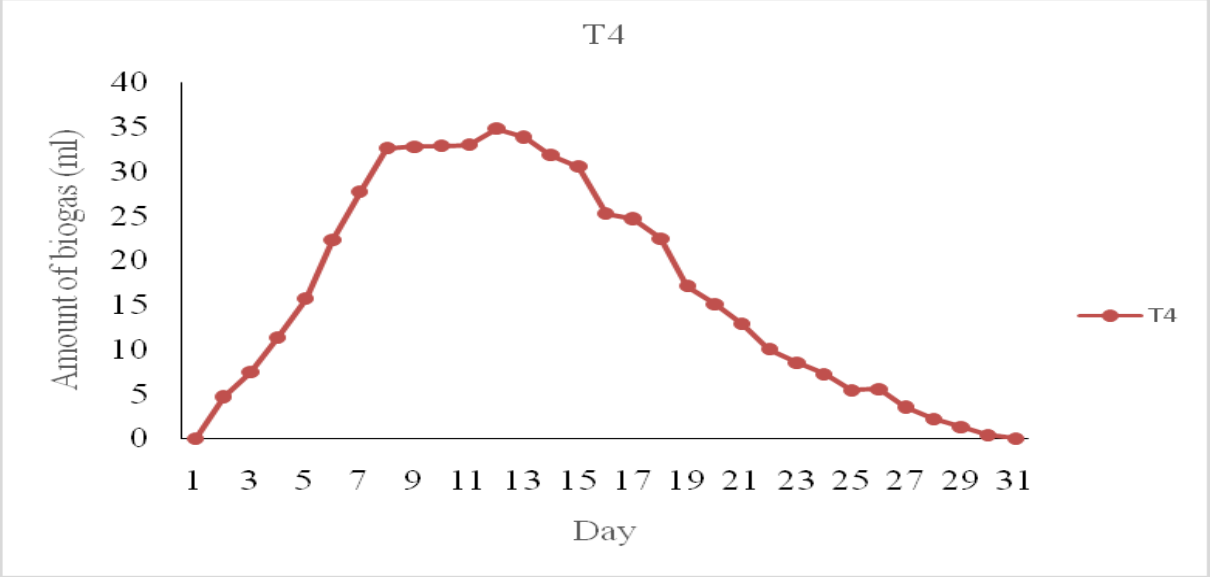
Appendix figur 1. Daily biogas yield in ml from T1



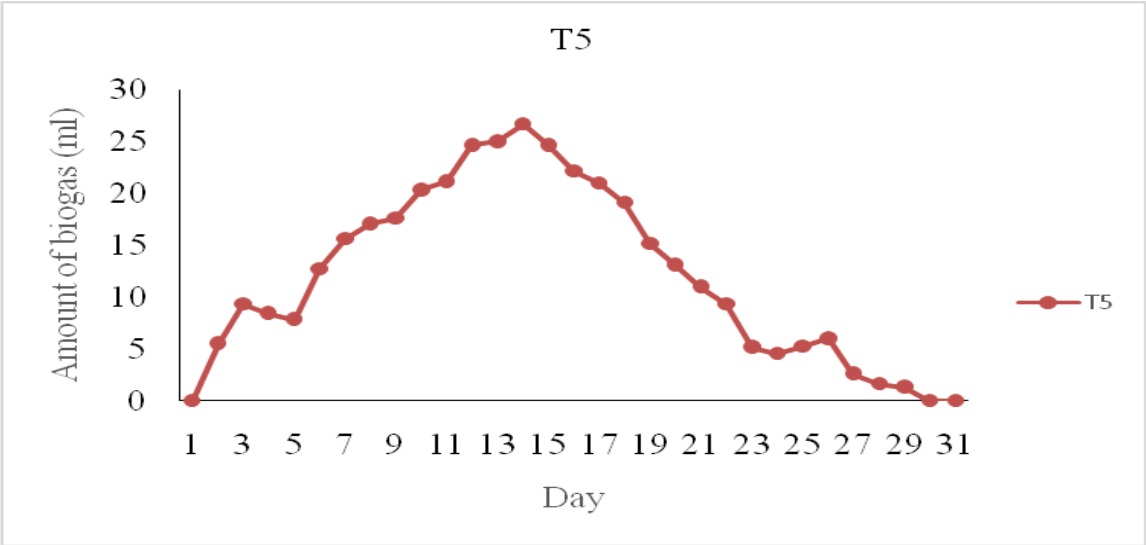
. Appendix figur 2. Daily biogas yield in ml from T2



Appendix figur 3. Daily biogas yield in ml from T3



Appendix figur 4. Daily biogas yield in ml from T4



Appendix figur 5. Daily biogas yield in ml from

Appendix figur 6. Production of biogas during pretreatment, setup and measurement.