PRODUCTION OF BIOGAS FROM INVASIVE WEED (*Striga hermonthica*) THROUGH ANAEROBIC CO- DIGESTION WITH COW MANURE

M.SC. THESIS

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APPROVAL SHEET

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DEDICATION

This thesis manuscript is dedicated to my dear Families

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BIOGRAPHICAL SKETCH

The author was born on June 10, 1989 from his mother W/Ro Mulunesh Agge and his father Ato Fikre Chakebo in Misha Woreda, Haddiya Zone and South Nation Nationalities People Regional State in Ethiopia. He attended his elementary and junior school education in Gemedo Primary School and secondary and preparatory school in Morsito preparatory School from 1996-2007.Then, in 2008 he joined Samera University and graduated on June 2010 with B. Ed degree in Biology. Then, after serving as a Preparatory school teacher for five years in Haddiya Zone, Soro Woreda in Gimbichu Preparatory School, he joined School of Graduate Studies on 2016, as a government sponsored student to pursue his M.Sc. study in Biotechnology at the school of Biological Sciences and Biotechnology, at Haramaya University.

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LIST OF ACRONOMYS

AD	Anaerobic Digestion
APHA	American Public Health Association
BOD	Biological Oxygen Demand
CM	Cow Manure
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
FAO	Food and Agricultural Organization
HRT	Hydraulic Retention Time
MDS	Mass of Dry Solids
M.A.S.L.	Meter Above Sea Level
MC	Moisture Content
NBP	National Biogas Program
SSAD	Solid State Anaerobic Digestion
TS	Total Solids
VS	Volatile Solids

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Production of Biogas from Invasive Weed (*Striga hermonthica)* through Anaerobic CO- Digestion with Cow Manure

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ABSTRACT

The unsustainability of conventional energy resources and their associated environmental pollutions made renewable energy the prime need of present time. Therefore, the research was conducted to investigate the production of biogas from Striga hermonthica co-digested with cow manure through anaerobic digestion with five treatment mix ratios were evaluated under mesophilic conditions (38ºC) using batch digesters in the Botanical laboratory of Haramaya University for 30 days of fermentation. In all treatments, physico-chemical parameters such as total solid, volatile solid, organic carbon, nitrogen, pH value and percent of moisture content were measured before and after anaerobic digestion. The results were indicated that, highest pH value (7.27) was observed in 10% total solid with 100% cow manure, whereas the lowest pH values (6.37) was observed in 100% Striga hermonthica with the same percentage of total solid content. Comparison of pH values between before and after anaerobic digestion showed that pH values were significantly increased after anaerobic digestion for all treatments. Similarly, maximum value of % total solid and volatile solid was recorded in 100% cow manure with 10% total solid content. In addition to this, carbon to nitrogen ratios were analyzed, the results were revealed that highest carbon to nitrogen ratio was observed in 10% total solid with 100% Striga hermonthica and lowest value in 6% total solid with mix ratio of 50% cow manure +50% Striga hermonthica. In this experimental study the result showed that co-digested substrates of the three mix ratios produced higher amount of biogas than the two sole alone. Among the different proportion of the total solid, 10%, total solid with mix ratio of 25% cow manure +75% Striga hermonthica showed the highest daily mean cumulative biogas production (2150ml) than all other treatments. 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 cow manure is co-digested with Striga hermonthica in 25%:75% mix ratio.

Key Words: Biogas, co-digestion, mesophilic, *Striga hermonthica*.

1. INTRODUCTION

Energy is one of the most important factors for human development and global economic growth. Nowadays energy crisis is a key issue all over the world. There will be severe energy shortage in the coming 50 years. According to Courtney and Dorman (2003) the crude oil will run out within 40 to 70 years and natural gas will be finished within 50 years. The high energy demand in the industrialized world as well as in the domestic sector and pollution problems caused due to the wide spread use of fossil fuels make it increasingly necessary to develop the renewable energy sources.

The ever increasing human population and the decreasing availability of fuel wood, coupled with the ever-rising prices of kerosene and natural gas in Ethiopia, draw attention of the need to consider alternative sources for domestic and cottage level industrial use in the country. Such energy sources should be renewable and should be accessible to poor. As rightly noted by Stout and Best (2001), a transition to a sustainable energy system is urgently needed in the developing countries. This will be, of necessity, be characterized by a departure from the present subsistence level energy usage levels based on decreasing firewood resources, to a situation where human and farming activities would be based on sustainable and diversified energy forms.

Renewable energy sources such as solar, wind, hydropower and biogas are potential candidates to meet global energy requirements in a sustainable way (Balat, 2007).Sustainable energy supply especially accompanied by reduction of CO_2 emission is a priority agenda worldwide.

Biogas is one of the clean and renewable forms of energy that could very well substitute (especially in the rural sector) the conventional sources of energy (fossil fuels and oil) (Santosh *et al.,* 2004). Generally, biogas consisted of methane (50- 70%), carbon dioxide (30-40%) and traces of gasses hydrogen (5-10%), nitrogen (1-2%), water vapor (0.3%), ammonia, and hydrogen sulphide (Rahmat, *et al.,* 2014). It is smokeless, hygienic and more convenient to use than other solid fuels. Biogas is a well-established fuel that can replace firewood as an energy source for cooking, lighting, heating and for other purposes in developing countries. Biogas burns very well when the CH_4 content is more than 50 %, and therefore biogas can be used as a substitute for kerosene, charcoal and firewood for cooking and lighting. This saves time and money and above all it conserves the natural resources from cutting trees to get firewood (Ayoub Mohamed *et al.,* 2002).

Biogas is produced by the microorganisms during the anaerobic digestion of biodegradable materials. Anaerobic digestion is a biochemical process in which particular kinds of bacteria digest biomass in an oxygen-free environment resulting in production of CH_4, CO_2, H_2 and traces of other gases along with decomposed mass. Traditionally, anaerobic digestions were performed using a single substrate. However, since the amount of biogas yield is largely dependent on the inoculants, microbial composition, type, quality, and amount of raw material digested, using a single substrate results in a limited biogas yield. All this has resulted in restricted popularization of biogas technology. Thus, there is a need to improve the overall efficiency of anaerobic digestion process in the biogas plants (Wei, 2000).

Insufficient biogas production necessitates wide range of studies, with the ultimate goal of assessing the effect of co-digestion of two or more substrates for biogas production. Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates (Wei, 2000). Moreover, it has been realized that an aerobic digestion as such became more stable when a variety of substrates were applied at the same time and resulted in an increased biogas yield. 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). Moreover, biogas production also directly influenced by total solid, volatile solids, loading rate, particle size, digester temperature, pH and carbon to nitrogen ratio of slurry (Adelekan and Bamgboye, 2009).

In Ethiopia, for instance, biomass in the form of wood, charcoal, crop residues, animal dung and agro- industrial wastes, accounts for more than 93 percent of the national energy supply (World Bank, 1984) and 98 percent of the rural household energy use

(Sheriff, 1987). It is, therefore, obvious that biomass represents an important part of the raw materials necessary for the satisfaction of energy needs in many developing countries including Ethiopia. This role of biomass continues to grow in Africa, where the effects of the present energy crisis are particularly acute.

Striga hermonthica commonly witchweeds, which belongs to the family Orobanchaceae, is a parasitic weed growing on the roots of cereal and legume crops in dry, semi-arid and harsh environments of tropical and subtropical Africa. *Striga hermonthica* parasitizes mainly tropical cereal crops, such as maize, sorghum, pearl millet, upland rice and cowpea (Press *et al*., 2001).

Previously there has been research on biogas production from some invasive weeds such as Parthenium, water hyacinth, Lantana and from different organic materials such as *Moringa stenopetala* seed cake powder, poultry litter, Khat (*Catha edulis*) waste, banana peels, poultry manure and Orange Peel and others was performed in Ethiopia.

However, no research has been done to evaluate the potential of biogas production from *Striga hermonthica* (witchweed) which causes considerable crop loss in many parts of Africa including Ethiopia. Therefore, this research is designed to investigate the production of biogas from *Striga hermonthica* in combination with cow manure with the following general and specific objectives.

General objective

- ✓ To determine the amount of biogas that can be produced from *Striga hermonthica co-*digested with cow manure through anaerobic digestion.

Specific objectives:

- ➢ To characterize *Striga hermonthica* and cow manure in terms of total solids (TS), volatile solids (VS), moisture content, organic carbon, nitrogen content, carbon/nitrogen ratio and pH before and after anaerobic digestion;
- ➢ To compare biogas yield from batch fermentation of individual and mixed substrates of *Striga hermonthica* and cow manure;
- ➢ To determine the average and daily cumulative biogas production from individual and mixtures of *Striga hermonthica* and cow manure combined in different proportions.

2. LITERATURE REVIEW

2.1. Biogas

Biogas, a clean, flammable and eco-friendly renewable form of energy, that produced by microbes when organic materials are fermented in a certain range of temperatures, moisture and pH under air-tight conditions (Adelekan and Bamgboye, 2009). The chemical properties of biogas are that it is very stable and does not dissolve in water, physical property lighter than air. Pure CH_4 is a colorless, tasteless, and odorless gas, but as the biogas consists of a small amount of H_2S, it smells very slightly of rotten eggs. When the CH_4 and air mixture burns, a blue flame is emitted, and it produces a large amount of heat energy (Florian, 2013). It is an energy source with a low carbon footprint (Michaela, 2010). It is about 20% lighter than air and has an ignition temperature in the range of 650°C to 750° C (Claude, 2009).

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

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

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

The primary source, which delivers the necessary microorganisms for biomass biodegradation and as well one of the largest single sources of biomass from the food/feed industry, is manure from animal production, mainly from cows and pig farms (Nielsen *et al*., 2007).

Biogas, the gas generated from organic digestion under anaerobic conditions by mixed population of microorganisms, was an alternative energy source, which has been commenced to be utilized both in rural and industrial areas at least since 1958 (Anunputtikul and Rodtong, 2004). In the complex process of anaerobic digestion, hydrolysis/acidification and methanogenesis are considered as rate-limiting steps (Juanga *et al.,* 2005; Nguyen *et al.,* 2007).

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

2.2. Anaerobic Digestion

Anaerobic digestion is a naturally occurring process where biodegradable matter is converted into biogas and a semi-solid material, by micro-organisms in the absence of oxygen (Suzie, 2013). Anaerobic digestion is a commercially proven technology and is widely used for recycling and treating animal manure, human excreta, fruit-vegetable wastes, slaughterhouse wastes and other organic wastes, which allows the production of a universal energy carrier, CH_4. 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. It is a multi-step biological process where the organic carbon is mainly converted to carbon dioxide and methane (Angelidaki *et al.,* 2003). Also the nutrient-rich solids left after digestion can be used as fertilizer (Ciborowski, 2004). There are four key stages of anaerobic digestion such as hydrolysis, acidogenesis, acetogenesis and methanogenesis (Ciborowski, 2004).

2.2.1. Hydolysis

Hydrolysis is the first step in anaerobic digestion processes. During the hydrolysis step, complex organic matters, such as carbohydrates, proteins and lipids are hydrolyzed into soluble organic molecules such as sugars, amino acids and fatty acids by extracellular enzyme, i.e. cellulose/ amylase/, protease and lipase respectively (Parawira *et al*., 2005). Hydrolytic bacteria, which hydrolyze the substrate with these extracellular enzymes, are facultative anaerobes. Hydrolysis can be the rate-limiting step if the substrate contains large molecules (particulates) with a low surface-to-volume ratio (Vavilin *et al*., 1996). While if the substrate is readily degradable, the rate-limiting step will be acetogenesis and methanogenesis (Björnsson *et al.*, 2001).

2.2.2. Acidogenesis

In the acidogenesis step, the soluble organic molecules from hydrolysis are utilized by fermentative bacteria or anaerobic oxidizers (Garcia-Heras, 2003). These microorganisms are both obligate and facultative anaerobes. In a stable anaerobic digester, the main degradation path way results in acetate, carbon dioxide and hydrogen. The intermediates, such as volatile fatty acids and alcohols, play a minor role. This degradation path way gives higher energy yield for the microorganisms and the products can be utilized directly by methanogenic microorganisms (Schink, 1997). However, when the concentration of hydrogen is high, the fermentative bacteria will shift the path way to produce more reduced metabolites (Angelidaki *et al.,* 2002). The products from acidogenesis step consist of approximately 51% acetate, 19% H_2/CO_2, and 30% reduced products, such as higher VFA, alcohols or lactate (Angelidaki *et al.*, 2002). Acidogenesis step is usually considered the fastest step in anaerobic digestion of complex organic matter (Vavilin *et al.*, 1996).

2.2.3. Acetogenesis

Intermediates formed during acidogenesis, consist of fatty acids longer than two carbon atoms, alcohols longer than one carbon atom and branched-chain and aromatic fatty acids. These products cannot be directly used in methanogenesis and have to be further oxidized to acetate and H_2 in acetogenesis step by obligated proton reducing bacteria in a dystrophic relationship with hydrogen utilizes. Low H_2 partial pressure is essential for acetogenic reactions to be thermodynamically favorable (Schink, 1997). The products from acetogenesis are then the substrates for the last step of anaerobic digestion, which is called methanogenesis.

2.2.4. Methanogenesis

In methanogenesis step, acetate and H_2/CO_2 are converted to CH_4 and CO_2 by methanogenic bacteria. The methanogenic bacteria are able to grow directly on H_2/CO_2, acetate and other one-carbon compound, such as for mate and methanol (Schink, 1997). In the normal anaerobic digesters, acetate is the precursor for up to 70% of total methane formation while the remaining 30% originates from H_2/CO_2 (Klass, 1984). Moreover, the inter-conversion between hydrogen and acetate, catalyzed by homoacetogenic bacteria, also plays an important role in the methane formation pathway. Homoacetogens can either oxidize or synthesize acetate depending on the hydrogen concentration in the system (Kotsyurbenko, 2005). Hydrogenotrophic methanogenesis functions better at high hydrogen partial pressure, while aceticlastic methanogenesis is independent on hydrogen partial pressure. At higher temperatures, the acetate oxidation pathway becomes more favorable (Schink, 1997).

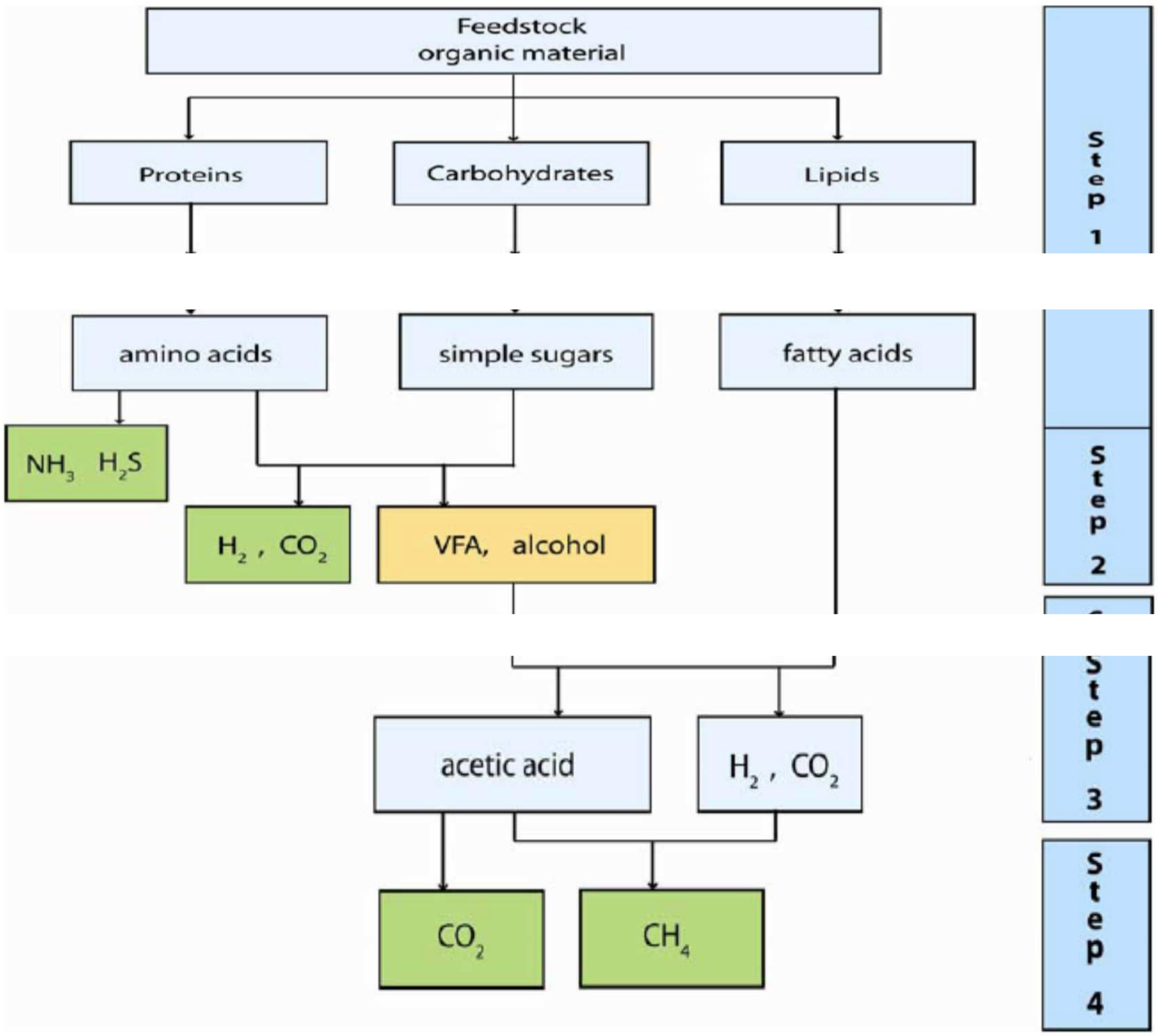


Figure 1**:** Anaerobic digestion of organic material. Step 1: Hydrolysis, Step 2: Acidogenesis, Step 3: Acetogenesis, and Step 4: Methanogenesis. (Lomborg, 2009).

2.3. Techniques Used for Enhancing Biogas Production

2.3.1. Use of Additives

Different attempts have been made in the past to increase gas production by stimulating the microbial activity using various biological and chemical additives under different operating conditions. Biological additives include different plants, weeds, crop residues, microbial cultures, which are available naturally in the surroundings. As such, generally these are of less significance in terms of their use in the habitat however, infused as additives in biogas plant could improve its performance significantly. Chemical additives are NaOH, ammonia, ammonium sulphate, H_2SO_4, HCl, H_2O_2, ClO_2 and NO_2 (Devlin *et al* 2011). The suitability of an additive is expected to be strongly dependent on the type of substrate (Yadvika *et al.,* 2004**).**

Leaves of different plant species such as *Gulmoha*r, *Leucacena leucocephala*, *Acacia auriculiformis*, *Dalbergia* and *Eucalyptus* and legumes have been found to stimulate biogas production between 18% and 40% (Yadvika e*t al.,* 2004). Increase in biogas production due to certain additives appears to be due to adsorption of the substrate on the surface of the additives. This can lead to high-localized substrate concentration and a more favorable environment for growth of microbes (Yadvika e*t al.,* 2004).The additives also help to maintain favorable conditions for rapid gas production in the reactor, such as pH, inhibition; promotion of acetogenesis and methanogenesis for the best yield.

Strains of some bacteria and fungi have also been found to enhance gas production by stimulating the activity of particular enzymes. Cellulolytic strains of bacteria like actinomycetes and mixed consortia have been found to improve biogas production in the range of 8.4–44% from cow manure (Yadvika e*t al.,* 2004).

Several inorganic additives that improve gas production have also been reported (Yadvika e*t al.,* 2004) claimed that higher concentration of bacteria could be retained in the digester by the addition of metal cations since cations increase the density of the bacteria, which are capable of aggregating by themselves. Addition of iron salts at various concentrations [FeSO4 (50 mM), FeCl3 (70 lM)] have been found to enhance gas production rate.

2.3.2. Variation in Operational Parameters

Pre-treatment of the substrate improve anaerobic digestion of lignocelluloses in order to break the polymer chains to more easily accessible soluble compounds. An ideal pretreatment will increase surface area and reduce lignin content and crystalline of cellulose (Yadvika *et al.,* 2004). Pretreatments can be carried out either physically, chemically or biologically, or as combinations of these. The most important physical pre-treatment of crop biomass is particle size reduction, leading to increase in available surface area and release of intracellular components (Yadvika *et al.,* 2004).

The production of biogas is also affected by particle size of the substrate. Too big particle size is problematic for microbes to digest and it can also result in blockage in the bio-digester. However, 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). According to the reports of Tamirat Asnake (2008) out of the five particle sizes (0.088, 0.40, 1.0, 6.0 and 30.0 mm), maximum quantity of biogas was produced from raw materials of 0.088 and 0.40 mm particle size. Large particles could be used for succulent materials such as leaves.

2.3.3. Recycling of Digested Slurry/Slurry Filtrate

The recirculation of digested slurry back into the reactor has been shown to improve the gas production marginally, since the microbes washed away are re-introduced back into the reactor, thereby providing an additional microbial population. The recycling of the digested slurry along with filtrate has also been tried out to conserve water and to enhance biogas production (Santosh *et al*., 2004).

2.4. Factors that affect the rate of Anaerobic Digestion

2.4.1. 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, at 25^0C–40^0C, or in the thermophilic range, 50^0C–65^0C. A mesophilic digester must be maintained between 30^0C and 35^0C for optimal functioning.

2.4. 2. pH

During anaerobic digestion, microorganisms require a neutral or mildly alkaline environment for efficient gas production (Augenstein *et al*., 1976). Most methanogens function only in a pH range between 6.7 and 7.4 (Buekens, 2005). A falling pH can point toward acid accumulation and digester instability. Gas production is the only parameter that shows digester instability faster than pH (Ostrem, 2004). A pH range between 6.8- 8 is suitable for most methanogenic bacteria to function normally Yadvika *et al*. (2004). The rate of methanogenesis may decrease if the pH is lower than 6.3 or higher than 7.8.

2.4.3. Loading rate

Loading rate is the amount of raw materials fed per unit volume of digester capacity per day. If the plant is overfed, acids will accumulate and methane production will be inhibited. Similarly, if the plant is underfed, the gas production will also be low (BSP, 1992).

2.4.4. Retention time

In anaerobic digestion technology, two types of reactors are used: the batch process and the continuous process. In the batch process, the substrate is put in the reactor at the beginning of the degradation period and sealed for the duration of digestion. All of the reaction stages occur more or less consecutively and therefore the production of biogas follows a bell curve. Retention time ranges from 30–60 days and only about 1/3 of the tank volume is used for active digestion (Ostrem *et al.,* 2004).

The retention time is determined by the average time it takes for organic material to digest completely, as measured by the chemical and biological oxygen demand (COD and BOD) of exiting effluent. Speeding up the process will make the process more efficient. Microorganisms that consume organic material control the rate of digestion that determines the time for which the substrate must remain in the digestion chamber and therefore the size and cost of the digester (Ostrem *et al.,* 2004). Reducing retention time reduces the size of the digester resulting in cost savings. Therefore; there is incentive to design systems that can achieve complete digestion in shorter times. A shorter retention time will lead to a higher production rate per reactor volume unit but a lower overall degradation. These two effects have to be balanced in the design of the full-scale reactor. Several practices are generally accepted as aiding in reducing retention time. Two of these are continuous mixing and using low solids (Ostrem, 2004).

2.4.5. C/N ratio

It is necessary to maintain proper composition of the feedstock for efficient plant operation so that the C/N ratio in feed remains within a desired range. It is generally found that during anaerobic digestion microorganisms utilize carbon 25–30 times faster than nitrogen. Thus, to meet this requirement, microbes need a 20–30:1 ratio of C to N with the largest percentage of the carbon being readily degradable (Santosh *et al.,* 2004). A high C/N ratio is an indication of rapid consumption of nitrogen by methanogens and results in lower gas production. On the other hand, a lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria. Optimum C/N ratios of the digester materials can be achieved by mixing materials of high and low C/N ratios (Verma, 2002).

2.4.6. Volatile solids

The weight of organic solids burned off when heated to about 550^0C 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.

2.4.7. Moisture Content

High moisture contents usually facilitate the anaerobic digestion; however, it is difficult to maintain the same availability of water throughout the digestion cycle. Initially water added at a high rate is dropped to a certain lower level as the process of anaerobic digestion proceeds. High water contents are likely to affect the process performance by dissolving readily degradable organic matter. It has been reported that the highest methane production rates occur at 60–80% of humidity. Methanogenesis processes during anaerobic digestion at different moisture levels i.e., 70% and 80%. However, bioreactors under the 70% moisture regime produced a stronger leachate and consequently a higher methane production rate (Khalid *et al*., 2011). According to Sadaka and Dan Engler (2003), water content is one of the very important parameters affecting anaerobic digestion of solid wastes. There are two reasons viz.; (a) water make possible the movement and growth of archaea facilitating the dissolution and transport of nutrient and (b) water reduces the limitation of mass transfer of non-homogenous or particulate substrate.

2.5. Types of Biogas Digesters

Different types of digesters 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 two-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). The most common anaerobic reactor type for these more diluted materials at the present time is the CSTR. In this process, the material is typically pumped into the digester continuously or semi-continuously, while digestion residues are simultaneously removed. Solid materials can be fed into a CSTR directly at the top without pumping.

2.6. *Striga hermonthica*

Striga hermonthica, commonly known as witchweed, is a genus of parasitic plants distributed across Africa, southern Europe, Australia and tropical portions of Asia including China, India and Indonesia. Natural low-density populations can be found in tropical grassland habitats across much of Africa (Mohamed *et al.*, 2001).

A witchweed, *Striga hermonthica* (Del.) Benth is a flowering root parasitic plant and it is considered as a hemi-parasitic plant. *S. hermonthica* which belongs to the family Orobanchaceae (ex. Scrophulariaceae) is deemed to be one of the most ubiquitous parasitic weed of food crops, eg, rice (*Oryza sativa* L.), millet (*Pennisetum glaucum* (L.) Leeke), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) roots (Press *et al.*, 2001).

Striga hermonthica mainly distributed in tropical arid and semi-arid zones with 400 to 1000 mm of annual rainfall (Mohamed *et al.*, 2001). The most affected countries are Mali, Upper Volta, Niger, Nigeria, Cameroon, Chad, Sudan, Ethiopia and India.

The socioeconomic impact of *Striga* infestation on cereal crops in western Africa is assessed by Obilana (1983). The success of *Striga* as a parasitic weed is due to several of its characteristics, related somehow with the farming systems in semi-arid areas where its hosts are grown, *Striga* seeds survive in arid soils for 15 years.

2.7. Feedstock's for Biogas Production

Though there can be variation in biogas production potential among feedstock, all organic materials can serve as substrate for biogas production in sole or in combination. Plant materials and animal manure have recently been used for production of biogas by co-digestion under anaerobic condition. The co-digestion of plant material and animal manure increases the rate of biogas production as compared to the sole digestion of feedstock. Because mixing substrates was found to balance between carbon and nitrogen ratio (Elmashad *et al*., 2004). There are many different types of substrates available for biogas production (Figure 2).

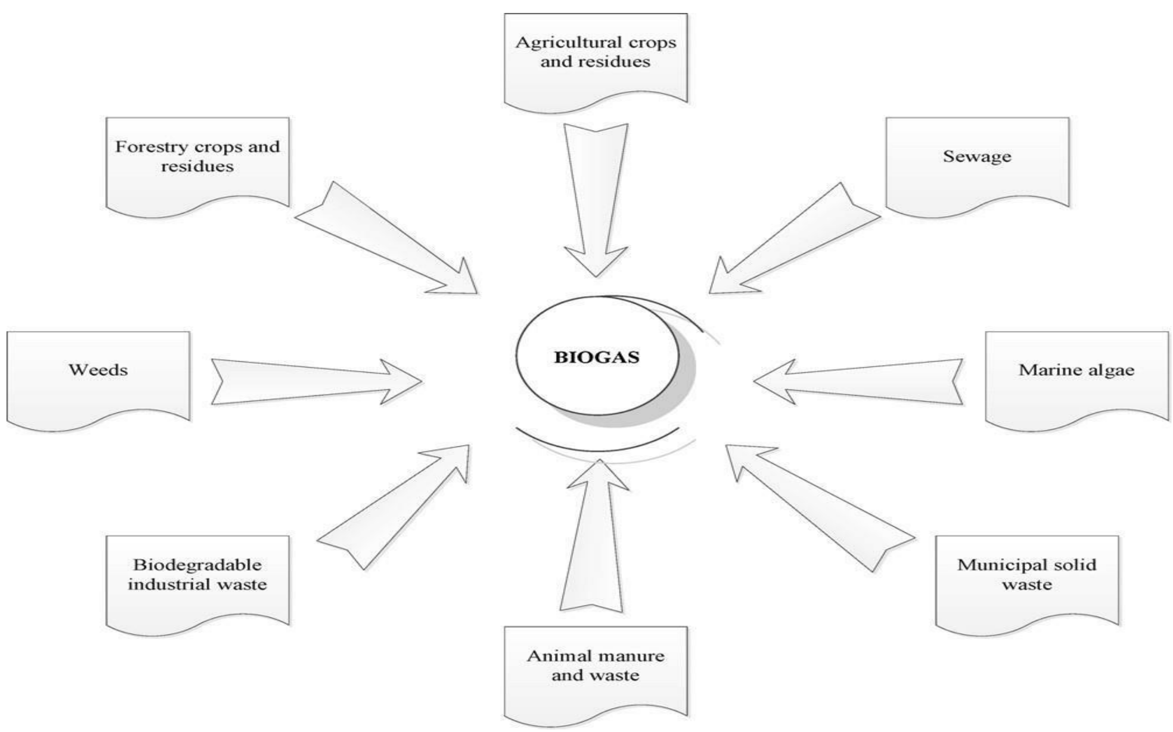


Figure 2: Feedstock's that can be used for anaerobic digestion (Abbasi *et al*., 2012).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted at Botany Laboratory of the Biological Science and Biotechnology, at Haramaya University. The University is found at 525 km away from Addis Ababa to the east. It is located at $9^{o}26'$N latitude, $42^{o}03'$E longitude and an altitude of 1980m.a.s.l. (FAO, 1990).

3.2. Design of the Experiment

The experiments were arranged in a completely randomized factorial design with three replications. All experiments were carried out at mesophilic temperature (38^{0}C) using five sets of digesters corresponding to the five-substrate mix-ratios in four types of adjusted percent of total solids.

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

The experimental setup for this study was a batch digester constructed from a 0.5L capacity plastic bottle and joined with plastic tubing in series to other plastic bottles. All plastic bottles were closed with plastic stoppers. In this arrangement, the first plastic bottle served as digester, the second contained acidified brine solution that would be prepared by dissolving NaCl in distilled water with few drops of sulphuric acid until a supersaturated solution was formed to prevent the dissolution of biogas in the water. All the three containers were interconnected with plastic tubes having a diameter of 1cm and the third received the displaced acidified brine solution. Each bottle was sealed properly at the points of insertion of tubing in order to control the entry of oxygen and loss of biogas using superglue and plaster (Taeme *et al*, 2014).

3.4. Sample Collection and Preparation of Substrate for Anaerobic Digestion

Striga hermonthica and cow manure were used as feedstock for AD process of biogas production. *Striga hermonthica* was collected from Hararghe region and cow manure was collected from the Haramaya University cattle farm. Both *Striga hermonthica* and CM were dried and crushed using all-purpose high speed smashing machine to break into smaller particles to ensure consistency of mix. Taking equal amounts of each dried substrate (20g), total solid was determined before AD. Thereafter, the two substrates were combined in different proportions so as to have five substrate treatments. These were 100% CM (A), 75%:25% mix of CM and *Striga hermonthica (B)*, 50%:50% mix of CM and *Striga hermonthica (C)*, 25%:75% mix of CM and *Striga hermonthica* (D) and 100% *Striga hermonthica (E)*.

Based on their original %TS, the substrates were adjusted to have TS of 4, 6, 8 and 10% by diluting in appropriate amount of distilled water including 100ml of fresh rumen fluid used to facilitate the start of AD (Sunarso *et al*., 2012). This helps to understand the effect of varying %TS in biogas production. Fresh rumen fluid was collected from the nearby slaughterhouse of Haramaya University and filtered through a cloth of 0.5 mm sieve diameter to separate the solid content from slurry and transported by plastic bottle and properly handled.

Anaerobic digestion of the substrates were then be done in 0.5L digester under mesophilic temperature (38^{o}C) using five sets of digesters corresponding to the five-substrate mix-ratios each with different %TS. The pH of the slurry was maintained within the pH range for optimal biogas production, by adding sodium hydroxide and hydrochloric acid to the organic substrate.i.e. About neutral (Yadvika *et al*., 2004). After the initial pH was measured and the combinations were done in five treatment proportions (table 2). To each of the treatment, appropriate amount of distilled water and inoculums' were added to achieve the recommended (4, 6, 8 and 10%) total solid content of digester (Tchobanoglous *et al.*, 1993). Each treatment had three replications in four types of adjusted total solids in five treatments.

Table 2:-Mixing ratio of the substrates

Treatments	CM	*Striga hermonthica*
A	100%	0%
B	75%	25%
C	50%	50%
D	25%	75%
E	0%	100%

3.5. Analysis of Physico-chemical Properties of the Feedstock

3.5.1. Total Solids (TS)

A clean evaporating dish (crucible) was dried at 105^{o}C for 1hr, cooled in desiccators and weighed immediately before use. Ten grams of *Striga hermonthica* and CM 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 105^{o}C. 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:

$$\%TS = \frac{mDS}{mFS} X\ 100$$

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

3.5.2. Volatile Solids (VS)

The total solid obtained was ignited at 550°C in a muffle furnace for 3hrs 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).

$$\%VS \quad = \frac{\mathrm{MDS-m(ash)}}{MDS} X\ 100$$

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

3.5.3. C: N 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 was 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 $Cr_2O_7^{-2}$ remaining, which shifts to a turbid grey before the endpoint and then changes sharply to a wine red at the end point). Use of a magnetic stirrer with an incandescent light made the endpoint easier to see in the turbid system (fluorescent lighting gives a different endpoint color).

Calculation

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

Where:

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

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

N= Normality of $FeSO_4$ (0.5N)

0.39= mill equivalent weight of C in g

mcf= moisture correction factor

Wo= 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:

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

Where,

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

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

3.5.4. Determination 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 diluted 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.

3.5.5. Moisture Content Determination

The most commonly used method to determine moisture content was the oven-drying method. In this study moisture content was determined as the percentage of wet (initial) weight of the material lost through heating. To achieve this, 10g of sample was dried in an oven at 105^0C for 24 hours and weighed. Moisture content was then calculated using the formula (Elias, 2010):

$$\%M\ C = \frac{W-D}{W} X100$$

Where, M = moisture content, W= initial weight of sample (g) and D= weight of sample after drying at 105^0C for 24 hrs

3.6. Evaluation of the Amount of Biogas

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

3.7. Liquid Content

The substrate of each bio-digester was mixed with appropriate amount of distilled water and inoculums to achieve the recommended percent of total solid. The total amount of liquid (Distilled water and rumen fluid) that was added to the bio-digester was determined by the following formula.

$$\mathbf{Y} = \frac{\mathbf{mTS - A\%\ X}}{\mathbf{A\%}}$$

Where

mTS= mass of total solid

X =mass of added fresh sample in g

A% = adjusted percents of total solids

Y= mass of fluid (water and inoculums) to be added to get 10, 8, 6 and 4% TS suspension in the digester

Then, by fixing the amount of inoculums (100 ml) that was added finally to facilitate digestion, the amount of distilled water that has to be added was then determined using the formula.

$$Z= Y-100$$

Where:

Z = amount of distilled water

Y = total amount of liquid (distilled water and rumen fluid)

Table 3: The proportion of different substrates added in the five digesters in three replicates for four types of adjusted % of TS.

Treatments in different adjusted %TS	TS of CM(g)	TS of *SH* (g)	VS of CM (%)	VS of *SH* (%)	V. Water added (mL)	V.of Inoculums added(ml)	Fresh CM added (g)	Fresh *SH* added (g)
Ts of 10% A	18	0	84.4	0	60	100	20	0
B	13.5	4	63.3	20.6	55	100	15	5
C	9	8	42.2	41.1	50	100	10	10
D	4.5	12	21.1	61.7	45	100	5	15
E	0	16	0	82.2	40	100	0	20
Ts of 8% A	18	0	84.4	0	105	100	20	0
B	13.5	4	63.3	20.6	99	100	15	5
C	9	8	42.2	41.1	93	100	10	10
D	4.5	12	21.1	61.7	86	100	5	15
E	0	16	0	82.2	80	100	0	20
Ts of 6% A	18	0	84.4	0	180	100	20	0
B	13.5	4	63.3	20.6	172	100	15	5
C	9	8	42.2	41.1	163	100	10	10
D	4.5	12	21.1	61.7	155	100	5	15
E	0	16	0	82.2	147	100	0	20
Ts of 4% A	18	0	84.4	0	330	100	20	0
B	13.5	4	63.3	20.6	318	100	15	5
C	9	8	42.2	41.1	305	100	10	10
D	4.5	12	21.1	61.7	293	100	5	15
E	0	16	0	82.2	280	100	0	20

3.8. Data Analysis

After the completion of the whole laboratory process the data was analysis of variance (one-way ANOVA) using SPSS version 16. Fishers Least Significant difference (LSD) was used to investigate statistical significance between the different bio-digester, whereas paired samples T-test was used to investigate statistical significance within a bio-digester. Difference between means was considered statistically significant at $P<0.05$.

4. RESULTS AND DISCUSSION

4.1. Characterization of Physico-Chemical Parameters of the Substrates

4.1.1. Comparison of pH and Percent of MC Before and after Anaerobic Digestion.

The initial pH value was observed for all the digester with different proportion of total solid content (4, 6, 8 and 10%). The results were indicated that, the highest pH value (7.27±.004) was observed in 10% total solid with 100%CM, whereas the lowest pH values (6.37±.005) was observed in 100% *Striga hermonthica* with 10% total solid content (Table 4). For anaerobic digestion of methanogenic bacteria the optimal pH values were Between 6.8 to 7.4. Accordingly, pH value of 100% CM was optimum for biogas production in all treatments (Table 4), whereas, 100% *Striga hermonthica* was less optimal (Thy *et al*., 2003; Yadvika *et al*., 2004). Mixing of the substrates resulted in the rise of pH compared to that of *Striga hermonthica* alone, but decreased pH from that of CM alone.

The pH before AD was found to increase with increasing of CM proportion in the mix, suggesting that CM helps to maintain the pH to meet the optimum required. Therefore, mixing of the substrates was a good way of adjusting the pH value to the optimum (Hills and Roberts, 1981). This was due to the presence of relatively high ammonia content in cow manure. Moreover, the initial pH values of mixed substrate in each digester ranged from 6.81±.001 to 7.13±.002 which were comparable with the optimum range of pH for biogas production. Comparison of pH values between before and after AD showed that pH values were significantly increased for all treatments after AD ($P<0.05$). Maximum pH value (8.57±.003) was observed in 10% total solid within 100% *Striga hermonthica*. Whereas the minimum pH value (7.67±.000) was observed in 10% total solid within 25%CM+75% *Striga hermonthica*. The reason for the increment of the pH values after AD was attributed to production of alkali compounds, such as ammonium ions during the degradation of organic compounds in the digester (Gerardi, 2003).

There was a significant (P<0.05) difference within treatments in both the initial and final %MC. In almost all treatments, %MC significantly increased after AD. Moreover, the results indicate that *Striga hermonthica* contains high moisture content than CM then mixing of substrates was balance the moisture content of the digester (Table 4). Obviously, the difference was due to the addition of inoculums and distilled water required to prepare % of adjusted total solid slurry (10, 8, 6 and 4%).

Table 4**:** Comparison of pH and % MC Before and After Anaerobic Digestion (AD) of the various substrate (values are mean± SE, n= 3) for three replication in four types of adjusted total solid, 10, 8, 6 and 4% below the table.

Treatments		Parameters			
Adjusted % of TS		pH Before AD	pH After AD	% MC Before AD	% MC After AD
For 10%	A	7.27±.004^{Aa}	7.94±.000^{Cb}	10.01±.004^{Ea}	30.01±.000Eb
	B	6.86±.001^{Ba}	8.07±.003^{Bb}	12.50±.002Da	32.01±.003^{Db}
	C	6.83±.003^{Ba}	8.12±.004^{Bb}	15.01±.001^{Ca}	34.02±.004^{Cb}
	D	6.81±.001^{Ba}	7.67±.000^{Cb}	17.50±.001^{Ba}	36.01±.005^{Bb}
	E	6.37±.005^{Ca}	8.57±.003^{Ab}	20.02±.001^{Aa}	38.02±.002^{Ab}
For 8%	**A**	7.18±.003^{Aa}	8.40±.005^{Ab}	17.50±.000^{Ea}	52.51±.001^{Eb}
	B	7.01±.001^{Aa}	8.53±.004^{Ab}	23.12±.005Da	58.50±.100^{Db}
	C	7.03±.000^{Aa}	8.25±.000^{Ab}	28.58±.001^{Ca}	64.31±.000^{Cb}
	D	6.87±.000^{Ba}	8.42±.002^{Ab}	34.50±.000^{Ba}	70.12±.002^{Bb}
	E	6.61±.001^{Ba}	8.29±.005^{Ab}	40.01±.005^{Aa}	76.01±.000^{Ab}
For 6%	A	7.22±.000^{Aa}	8.14±.001^{Bb}	30.01±.001^{Ea}	80.01±.004^{Eb}
	B	7.01±.003^{Aa}	8.54±.004^{Ab}	40.86±.001Da	83.59±.003^{Db}
	C	7.00±.001^{Aa}	8.04±.001^{Bb}	51.80±.004^{Ca}	87.50±.004^{Cb}
	D	7.10±.000^{Aa}	7.81±.002^{Cb}	62.56±.000^{Ba}	91.29±.003^{Bb}
	E	6.67±.004^{Ba}	8.23±.000^{Bb}	73.35±.000^{Aa}	95.01±.000^{Ab}
For 4%	A	7.18±.001^{Aa}	7.72±.000^{Bb}	36.02±.001^{Ea}	96.01±.002^{Cb}
	B	7.01±.000^{Aa}	8.45±.005^{Ab}	46.31±.003Da	96.75±.004^{Cb}
	C	7.06±.004^{Aa}	8.31±.001^{Ab}	57.70±.001^{Ca}	97.53±.000^{Bb}
	D	7.13±.002^{Aa}	7.99±.004^{Bb}	67.60±.000^{Ba}	98.63±.003^{Ab}
	E	6.48±.000^{Ba}	7.99±.003^{Bb}	76.81±.001^{Aa}	99.00±.002^{Ab}

Means followed by different small letters in row were significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column were significant at P<0.05%. A=100%CM, B=75%CM+25% *Striga hermonthica*, C=50%CM+50% *Striga hermonthica*, D =25%CM+75% *Striga hermonthica* and E=100% *Striga hermonthica*.

4. 1.2. Comparison of TS and VS Before and After AD

The total solids and volatile solids of the substrates were analyzed for all treatments before and after AD (Table 5). The maximum %TS before AD were measured in 100%CM within 10% total solid. Whereas the minimum %TS was measured from the digester with 100% *Striga hermonthica* within 4% total solid. Moreover, similar trend was observed for volatile solid also. However, after anaerobic digestion the maximum decrement of total solids and volatile solids were observed in 10% total solid concentration under the treatment D that contains 25%CM+75% *Striga hermonthica*. The extent of biogas production increased when feed concentration was increased from 4%TS to 10%TS. This indicated that substrate concentration has been limiting factor in anaerobic digestion for biogas production.

This study achieved highest biogas production within a feed concentration of 10%TS. In line with this Srilatha *et al*. (1995) obtained bioconversion efficiency at highest total solid percentage (8%) for biogas production. Moreover, total solids and volatile solids destruction was a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012).

Table 5**:** Comparison of % TS and % VS Before and After Anaerobic Digestion (AD) of the various substrate (values were mean± SE, n= 3) for three replication in four types of adjusted percent of total solid below the table respectively.

Treatments	Parameters			
Adjusted % of TS	%TS Before AD	%TS After AD	% VS Before AD	% VS After AD
For 10% A	90.01±.000^{Aa}	70.01±.000^{Ab}	84.45±.003^{Aa}	82.86±.000^{Ab}
B	87.50±.001^{Ba}	68.01±.000^{Bb}	83.88±.001^{Ba}	81.51±.000^{Bb}
C	85.01±.003^{Ca}	66.01±.004^{Cb}	83.33±.004^{Ca}	80.20±.004^{Cb}
D	82.51±.002Da	61.02±.001^{Db}	82.37±.001Da	75.01±.000^{Eb}
E	80.01±.000^{Ea}	62.01±.003^{Eb}	82.22±.001Da	77.41±.001^{Db}
For 8% A	64.80±.001^{Aa}	50.40±.004^{Ab}	80.31±.002^{Aa}	78.53±.004^{Ab}
B	64.61±.000^{Aa}	50.21±.000^{Ab}	76.71±.001^{Ba}	74.40±.000^{Bb}
C	64.41±.001^{Ba}	50.01±.003^{Ab}	73.12±.001^{Ca}	72.01±.004^{Cb}
D	64.01±.002^{Ba}	49.51±.004^{Bb}	63.01±.004^{Ea}	59.01±.003^{Eb}
E	64.03±.000^{Ba}	49.61±.001^{Bb}	66.01±.001Da	62.20±.000^{Db}
For 6% A	54.01±.004^{Aa}	42.01±.000^{Ab}	51.01±.003^{Aa}	50.20±.000^{Ab}
B	52.51±.001^{Ba}	40.81±.003^{Bb}	50.81±.003^{Ba}	49.51±.000^{Bb}
C	51.01±.001^{Ca}	39.51±.004^{Cb}	50.51±.002^{Ba}	49.30±.004^{Bb}
D	49.51±.003Da	38.41±.004^{Db}	50.02±.005^{Ca}	48.31±.001^{Db}
E	48.01±.001^{Ea}	37.20±.000^{Eb}	50.10±.004^{Ca}	48.80±.001^{Cb}
For 4% A	36.01±.000^{Aa}	28.00±.003^{Ab}	34.40±.001^{Ba}	32.01±.001^{Ab}
B	35.01±.005^{Ba}	27.10±.004^{Bb}	34.01±.004^{Ba}	31.90±.001^{Bb}
C	34.01±.000^{Ca}	25.70±.002^{Cb}	34.50±.001^{Aa}	32.31±.000^{Cb}
D	33.01±.001Da	24.05±.000^{Eb}	34.32±.000^{Ca}	32.21±.000^{Cb}
E	32.01±.000^{Ea}	24.00±.000^{Db}	33.40±.000^{Ca}	32.04±.000^{Cb}

Means followed by different small letters in row were significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column were significantly at P< 0.05% level of significance between treatments. A=100%CM, B=75%CM+25%*Striga hermonthica*, C=50%CM+50%*Striga hermonthica*, D =25%CM+75% *Striga hermonthica* and E=100% *Striga hermonthica*.

4.1.3. Comparison of Organic Carbon (C) and Nitrogen (N) Before and After AD

The experimental results were revealed that, percent of organic carbon was significantly reduced after anaerobic digestion in all treatments (table 6). This indicates that organic carbon was consumed by bacteria to support their metabolic activities or converted in to biogas (Gerardi, 2003; Devlin *et al*, 2011). The highest carbon reduction was record from 10% total solid with in a mix ratio of 25% CM+75% *Striga hermonthica* (50.6 ± 0.001 to 30.2 ±.0.001). Whereas, lowest carbon reduction was observed in 6% total solid within 50% CM and 50% *Striga hermonthica* (46.1±.000 to 35.81±.003).

From all treatments nitrogen contents were analyzed for all the treatments, it indicated that after anaerobic digestion most of the treatment showed highest nitrogen concentration as compared with before anaerobic digestion. Moreover, C/N ratios were analyzed, the results were revealed that highest C/N ratio was observed in 10% total solid within 100% *Striga hermonthica* and lowest value within 8% total solid with mix ratio of 50%CM+50% *Striga hermonthica*. Carbon to nitrogen ratio was one of the major factor that affecting anaerobic digestion process, which in turn affects methane yield and production rates. Therefore, balanced carbon to nitrogen in a feed material was important.

It was often suggested that an optimum C: N ratio in between 20:1 to 30:1 (Marchaim, 1992). Accordingly, all the digester C/N rations in line within the range. If the C:N ratio was very high, the nitrogen was consumed rapidly by methanogens to meet their protein requirements and was no longer act on the left over carbon content of the material. As a result, gas production was low. On the other hand, if the C: N ratio was very low nitrogen was liberated and accumulated in the form of ammonia (NH_3). The increased concentration of NH_3 was increase the pH value of the slurry in the digester and ultimately leads to the inhibition of the growth of bacteria (Braun, 1982).

Table 6**:** Comparison of Percent of Organic Carbon (%OC) and Nitrogen (N_2) before and After Anaerobic Digestion (AD) for the various substrate (values were mean± SE, n= 3) for three replication in four types of adjusted total solid in one table. This helps to understand the effect of varying %TS in biogas production.

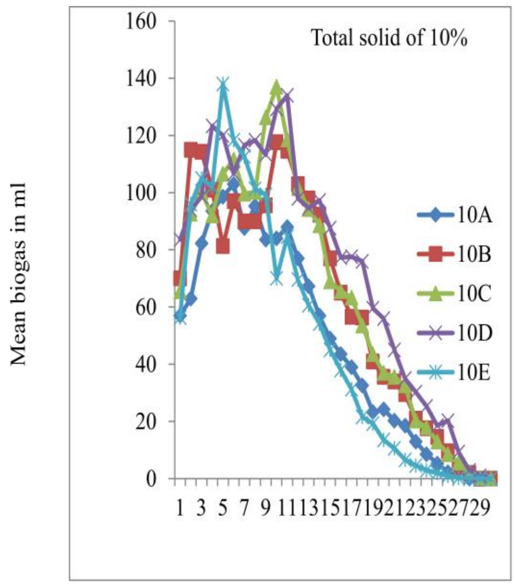
Treatments	Parameters				
Adjusted % of Ts	%C Before AD	%C After AD	N_2Before AD	N_2 After AD	C:N Before AD
For 10% A	49.98±.001^{Ca}	37.44±.004^{Ab}	2.00±.001^{Aa}	2.06±.000^{Ab}	24.99±.00:1^{Ca}
B	49.62±.004^{Ca}	35.73±.001^{Bb}	2.02±.000^{Aa}	2.21±.002^{Ab}	24.59±.00:1^{Ca}
C	46.40±.000Da	34.61±.002^{Cb}	2.01±.001^{Aa}	2.00±.000^{Ab}	23.62±.00:1Da
D	50.6±.002^{Ba}	30.20±.001^{Db}	2.00±.000^{Aa}	2.02±.003^{Ab}	25.34±.00:1^{Ba}
E	52.34±.001^{Aa}	35.92±.001^{Bb}	2.00±.001^{Aa}	2.21±.000^{Ab}	26.17±.00:1^{Aa}
For 8% A	49.90±.002^{Aa}	35.54±.000^{Ab}	2.02±.002^{Aa}	2.09±.000^{Bb}	24.96±.00:1^{Ba}
B	49.1±.001Da	35.76±.001^{Ab}	2.01±.000^{Aa}	2.41±.002^{Bb}	24.79±.00:1^{Ba}
C	45.99±.003^{Ea}	34.65±.001^{Bb}	2.00±.002^{Aa}	2.30±.000^{Bb}	23.00±.00:1^{Ca}
D	50.63±.001^{Ba}	30.90±.003^{Cb}	2.01±.000^{Aa}	2.12±.000^{Bb}	25.34±.00:1^{Ba}
E	51.54±.001^{Aa}	35.38±.001^{Bb}	2.00±.000^{Aa}	2.51±.000^{Ab}	25.77±.00:1^{Aa}
For 6% A	50.00±.003^{Ba}	35.64±.002^{Ab}	2.01±.001^{Aa}	2.16±.000^{Bb}	24.96±.00:1^{Aa}
B	49.6±.001^{Ba}	35.83±.000^{Ab}	2.00±.000^{Aa}	2.31±.002^{Bb}	24.79±.00:1^{Aa}
C	46.1±.000Da	35.81±.003^{Ab}	2.00±.001^{Aa}	2.40±.000^{Bb}	23.02±.00:1^{Ca}
D	49.30±.001^{Ca}	30.95±.001^{Bb}	2.00±.000^{Aa}	2.21±.000^{Bb}	24.46±.00:1^{Ba}
E	50.98±.001^{Aa}	35.52±.004^{Ab}	2.00±.003^{Aa}	2.51±.001^{Ab}	25.49±.02:1^{Aa}
For 4% A	48.25±.001^{Ca}	35.59±.001^{Bb}	2.01±.002^{Aa}	2.26±.001^{Bb}	24.27±.00:1^{Ba}
B	49.6±.001^{Aa}	35.33±.000^{Cb}	2.00±.000^{Aa}	2.31±.002^{Bb}	24.79±.01:1^{Aa}
C	47.03±.000Da	34.81±.001^{Db}	2.01±.002^{Aa}	2.25±.000^{Bb}	23.52±.00:1^{Ca}
D	48.90±.001^{Ba}	30.69±.000^{Eb}	2.00±.001^{Aa}	2.12±.004^{Bb}	24.44±.00:1^{Ba}
E	49.91±.000^{Aa}	36.52±.001^{Ab}	2.00±.005^{Aa}	2.61±.001^{Ab}	24.97±.00:1^{Aa}

Means followed by different small letters in the row were significant at $P < 0.05$ probability levels for paired samples T-test within treatment while means followed by different capital letter in column were significant at 5% level of significance between treatments for one way ANOVA. A=100%CM, B=75%CM+25% *Striga hermonthica*, C=50%CM+50% *Striga hermonthica*, D =25%CM+75% *Striga hermonthica* and E=100% *Striga hermonthica*.

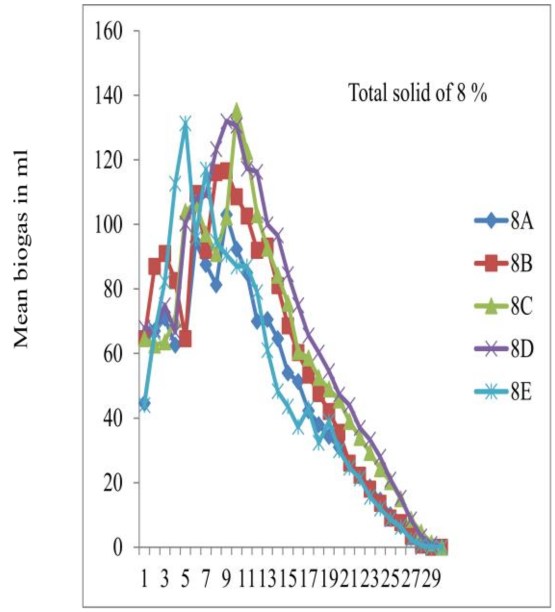
4.2. Determination of Average Daily and Cumulative Biogas Production from Solo and Co-Digestion of the Selected Substrates

Determination of average daily and cumulative biogas production from individual and co-digestion of different mixed ration were analyzed and the results were indicated in the Figure 3 and 4 respectively. The result showed that co-digested substrates of the three mix ratios (i.e. D, C and B) produced higher amount of biogas than the two individually fermented substrates (E and A), Figure 3, Appendix table 7 respectively. Similar results were reported by Tamrat Aragaw *et al*. (2013) from the co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. This was attributed to the positive synergetic effect of the co-digestion of *Striga hermonthica* and cow manure in providing more balanced nutrients, increased buffering capacity and decreased effect of toxic compounds.

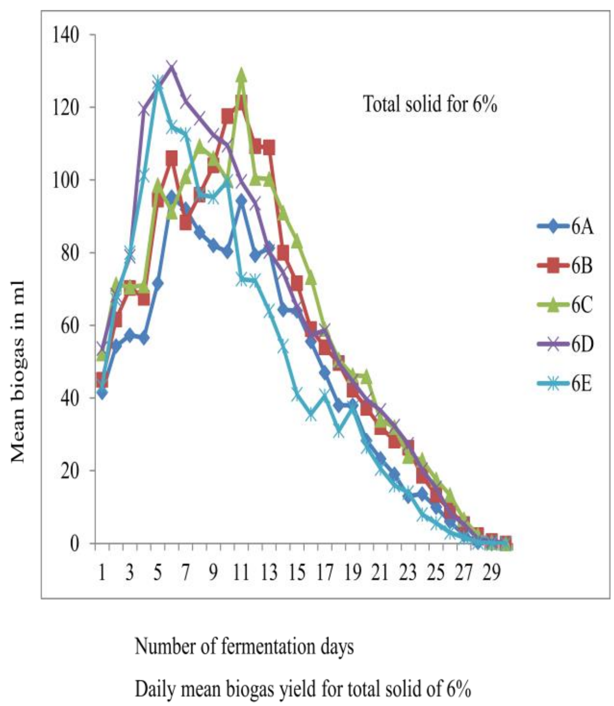
According to Jianzheng *et al*. (2011) digestion of more than one kind of substrate was establish positive synergism in the digester. Among the different proportion of the total solid, 10% total solid with mix ratio of 25%CM+75% *Striga hermonthica* showed highest production of biogas in all treatments (Figure 3, Appendix Table 7). The highest biogas production potential was due to the presence of balanced nutrient to microorganism, stable pH and good buffering capacity which was attained from the inoculation with rumen fluid (Forster *et al.*, 2008). After 11^{th} day of fermentation, biogas production was decreased and eventually reached 0 ml on 30^{th} day of fermentation in all treatments. The decline in biogas production was due to the increased bacterial population and the depletion of readily decomposable substrate (Ahn *et al*., 2009). It was also due to the increased pH value after AD which was buildup of ammonium ion (Hansen *et al.*, 1998).



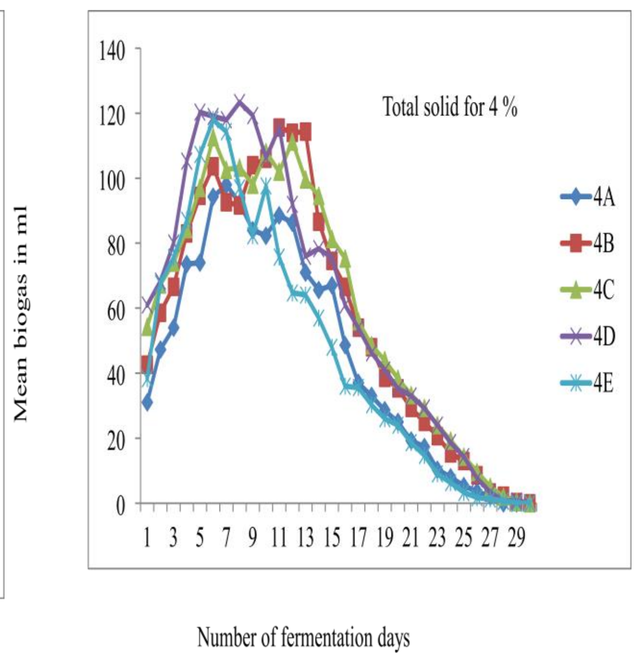
Number of fermentation days
daily mean biogas from total solid of 10%



Number of fermentation days
daily mean biogas yield for total solid of 8%



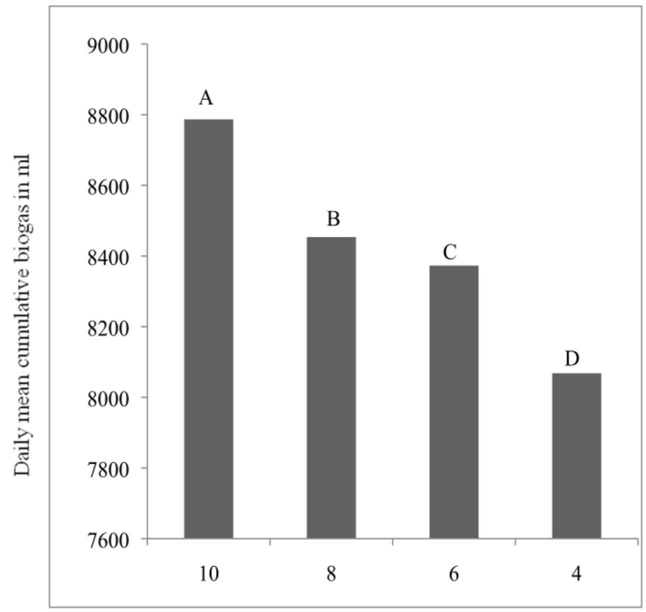
Number of fermentation days
Daily mean biogas yield for total solid of 6%



Number of fermentation days
Daily mean biogas yield from total solid of 4%

Figure 3: Daily mean biogas production for different content (for 10, 8, 6, and 4 %) of total solid concentration. A=100%CM, B=75%CM+25% *Striga hermonthica*, C=50%CM+50% *Striga hermonthica*, D =25%CM+75% *Striga hermonthica* and E=100% *Striga hermonthica*.

The daily mean average cumulative biogas productions of all the treatments were summarized in Figure 4. The results showed that 10% total solid concentration produced the highest mean cumulative biogas in all the treatment groups (Figure 4, Appendix Table 7).



Adjusted total solid concentration

Figure 4 : Cumulative biogas yield from different concentration of total solid

Moreover, biogas yield was decreasing with decreasing the proportion of total solid concentration (8786.8, 8453.7, 8373, and 8068 ml) for TS of 10, 8, 6, and 4% (Figure 4, Appendix Table 7) respectively. In line with this, Igoni *et al*. (2008) noted that the volume of biogas produced was related to percentage total solid concentration. For batch fermented systems, this relationship shows that a marginal increase in the percentage total solids results increase in the volume of biogas produced. The result also correlates with the findings of Lijuan, Yaunyuan, Chem and Ronghon, 2009, who studied biogas production at 6, 9, and 12% total solid and reported highest gas yield at 12% total solid. This trend was attributed to the fact that with increased TS, there was a concomitant increase in substrate (organic matter) and microbial flora, a consortium of which were required for biodegradation to occur (Richard, 1984).

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Currently bio-energy (energy production from biomass) have been acknowledged as one of the key options in reducing the global dependency on fossil fuel resources and to be used as one alternative energy sources. Biogas was a clean and eco- friendly renewable form of energy. The experiment was conducted under mesophilic conditions (38ºC) using five batch digesters with triplicate for different percent of total solid content (10, 8, 6 and 4%). In all treatments, %TS, %VS, % organic carbon, percentage of moisture content and pH were analyzed before and after digestion while C/N ratio before AD. The daily biogas production was measured by water displacement method for 30 days. Before digestion pH was found to increase significantly with increasing of cow manure proportion. The comparison of pH values before and after AD showed that pH values were significantly increased after AD for all the treatment.

There was a significant ($P<0.05$) difference within treatments in both initial and final %MC. In almost all treatments, %MC significantly increased after AD. Moreover, the results indicate that *Striga hermonthica* contains high moisture content than CM.The maximum %TS before AD was measured in 100%CM in 10% total solid. Whereas the minimum %TS was measured from the digester with 100% *Striga hermonthica* in 4% total solid. However, similar trend was observed for volatile solid. The result revealed that there were differences in percentage organic carbon in all mix ratios between before and after AD (paired samples-T-test, $P<0.05$). Comparison of initial and final % organic carbon 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 20:1-30:1 which was a suitable condition for methanogenic bacteria to reproduce and produce optimum biogas. The results showed that mix ratio with 25%CM+75% *Striga hermonthica* produced the highest biogas in all the treatment groups. Among, the different proportions of total solid 10% total solid produced highest cumulative biogas.

5.2. Conclusion

This research paper looks at the possibility of producing biogas from co-digestion of *Striga hermonthica* with cow manure by optimizing the parameters that affect biogas production.

The experimental results were suggested that the *Striga hermonthica* co-digested with cow manure improves the biogas production potential when compared with pure cow manure and *Striga hermonthica* alone. It was also showed that the highest biogas production was obtained in the mix ratio of 25%CM+75% *Striga hermonthica*. Thus, compared to the mono-digestion of pure cow manure and pure *Striga hermonthica*, anaerobic co-digestion of 75% *Striga hermonthica* and 25% cow manure mix ratios enhances both the rate and amount of biogas yield.

5.3. Recommendations

- Since this investigation was done at mesophilic temperature (38^{o}C), it was recommended carried out at thermophilic conditions (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 *Striga hermonthica* and cow manure, further similar studies should recommended on co- digestion of *Striga hermonthica* with other substrates.
- The percentage of methane in the biogas produced needs to be qualified for better efficiency of biogas production from *Striga hermonthica* co- digested with cow manure.

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7. APPENDIXS

- The values of physico-chemical parameters in three replications for four types of percent total solid.

Table 1: Comparison of pH and % MC Before and After AD.

Treatments	Parameters			
Adjusted % of Ts	Initial pH	Final pH	Initial% MC	Final% MC
For 10%A	7.27±.004	7.94±.000	10.01±.004	30.01±.000
B	6.86±.01	8.07±.003	12.50±.002	32.01±.003
C	6.83±.003	8.12±.004	15.01±.001	34.02±.004
D	6.81±.001	7.67±.000	17.50±.001	36.01±.005
E	6.37±.005	8.57±.003	20.02±.001	38.02±.002
For 8% A	7.18±.003	8.40±.005	17.50±.000	52.51±.001
B	7.01±.001	8.53±.004	23.12±.005	58.50±.100
C	7.03±.000	8.25±.000	28.58±.001	64.31±.000
D	6.87±.000	8.42±.002	34.50±.000	70.12±.002
E	6.61±.001	8.29±.005	40.01±.005	76.01±.000
For 6% A	7.22±.000	8.14±.001	30.01±.001	80.01±.004
B	7.01±.003	8.54±.004	40.86±.001	83.59±.003
C	7.00±.001	8.04±.001	51.80±.004	87.50±.004
D	7.10±.000	7.81±.002	62.56±.000	91.29±.003
E	6.67±.004	8.23±.000	73.35±.000	95.01±.000
For 4% A	7.18±.001	7.72±.000	36.02±.001	96.01±.002
B	7.01±.000	8.45±.005	46.31±.003	96.75±.004
C	7.06±.004	8.31±.001	57.70±.001	97.53±.000
D	7.13±.002	7.99±.004	67.6±.000	98.63±.003
E	6.48±.000	7.99±.003	76.81±.001	99.00±.002

Table 2: Percent (%) of TS and VS Before and After AD.

Treatments	Parameters			
Adjusted % of Ts	Initial %Ts	Final %Ts	Initial% Vs	Final% Vs
For 10% A	90.01±.000	70.01±.000	84.45±.003	82.86±.000
B	87.50±.001	68.01±.000	83.88±.001	81.51±.000
C	85.01±.003	66.01±.004	83.33±.004	80.20±.004
D	82.51±.002	61.02±.001	82.37±.001	75.01±.000
E	80.01±.000	62.01±.003	82.22±.001	77.41±.001
For 8% A	64.80±.001	50.40±.004	80.31±.002	78.53±.04
B	64.61±.000	50.21±.000	76.71±.001	74.40±.000
C	64.41±.001	50.01±.003	73.12±.001	72.01±.004
D	64.01±.002	49.51±.004	63.01±.004	59.01±.03
E	64.03±.000	49.61±.001	66.01±.001	62.20±.000
For 6% A	54.01±.004	42.01±.000	51.01±.003	50.20±.000
B	52.51±.001	40.81±.003	50.81±.003	49.51±.000
C	51.01±.001	39.51±.004	50.51±.002	49.30±.004
D	49.51±.003	38.41±.004	50.02±.005	48.31±.001
E	48.01±.001	37.20±.000	50.10±.004	48.80±.001
For 4% A	36.01±.000	28.00±.003	34.40±.001	32.01±.001
B	35.01±.005	27.10±.004	34.01±.004	31.90±.001
C	34.01±.000	25.70±.002	34.50±.001	32.31±.000
D	33.01±.001	24.05±.000	34.32±.000	32.21±.000
E	32.01±.000	24.0±.000	33.40±.000	32.04±.000

Table 3: Daily mean biogas yields from sole alone and co-digestion with CM and *SH* ± SE (ml) (n=3), for Ts of 10%

Days	10A	10B	10C	10D	10E
1	57.00±1.583	70.00±.000	65.67±1.933	83.67±1.371	56.33±2.215
2	63.00±3.799	115.00±3.229	92.67±1.429	94.00±.928	96.00±.000
3	82.33±1.826	114.33±1.234	100.33±1.509	98.67±.505	105.00±2.568
4	93.67±1.690	101.00±2.606	92.00±1.292	123.33±3.325	102.00±1.422
5	98.67±2.327	81.33±1.163	106.67±1.817	120.00±.000	138.00±.436
6	103.00±1.39	97.00±1.269	111.67±1.559	106.67±3.317	118.33±1.214
7	87.67±1.695	90.00±.718	99.67±3.665	116.67±1.547	112.67±.021
8	95.33±2.572	90.00±2.000	100.33±1.771	118.33±2.538	101.33±2.662
9	83.67±4.189	95.67±4.933	126.33±3.868	113.33±1.547	99.33±2.033
10	84.00±2.166	117.67±1.807	137.00±4.731	129.33±2.008	70.00±.185
11	88.00±2.165	114.67±1.015	118.67±2.505	134.00±1.698	84.67±.508
12	77.00±1.269	103.00±.083	101.67±2.887	98.00±5.292	69.67±1.528
13	67.33±1.429	98.00±1.000	94.33±3.786	94.67±4.509	60.67±1.155
14	57.00±.000	92.33±3.215	88.67±2.309	97.33±1.155	54.33±3.512
15	49.00±.568	77.00±.083	69.00±.083	87.67±.807	45.00±.000
16	43.67±.508	65.00±.245	65.67±4.933	77.33±.686	38.00±2.000
17	39.00±2.539	56.67±3.055	63.33±4.163	77.67±4.041	31.33±1.528
18	32.67±3.429	56.33±3.650	53.67±3.512	76.00±2.288	21.67±3.055
19	23.33±4.509	41.00±1.732	43.67±.508	59.67±1.528	19.33±3.512
20	24.33±.132	35.67±1.572	37.00±.557	56.00±.083	13.67±2.082
21	20.33±3.512	34.00±3.606	35.67±5.033	45.00±2.000	10.67±3.786
22	18.67±2.309	29.67±4.041	32.67±5.508	35.00±4.583	6.67±3.786
23	13.00±2.646	21.00±3.464	20.33±2.082	30.33±3.055	4.67±2.517
24	8.67±2.517	17.67±4.163	18.00±4.359	25.33±4.041	3.00±1.000
25	5.33±2.517	14.67±1.528	13.00±2.000	18.67±1.528	2.00±1.000
26	2.33±1.528	9.67±3.055	8.67±1.528	20.33±6.429	1.00±.000
27	3.67±2.887	4.00±2.646	5.67±4.619	9.33±3.786	.33±.577
28	.00±.00	2.00±1.000	2.33±2.309	3.00±2.00	.00±.00
29	.00±.00	.00±.000	.33±.577	1.00±1.732	.00±.00
30	.00±.00	.00±.000	.00±.00	.00±.00	.00±.00
total	1421.7	1844.4	1905	2150	1465.7
average	47.4	61.5	63.0	71.7	48.9

Table 4: Daily mean biogas yields from sole alone and co-digestion with CM and *SH*± SE (ml) (n=3), for Ts of 8%.

Days	8A	8B	8C	8D	8E
1	44.67±.539	64.67±1.508	64.67±1.055	68.00±.000	44.00±1.166
2	67.00±.557	87.00±.000	62.67±2.429	67.33±2.512	66.67±3.055
3	71.00±2.033	91.00±2.000	63.67±1.041	75.00±.000	82.33±.658
4	62.67±4.088	82.67±.055	70.67±1.082	66.67±.155	112.67±3.004
5	64.67±2.520	64.67±3.055	104.00±.166	100.33±.528	131.33±.429
6	94.67±.423	109.67±1.017	106.67±.859	108.00±1.817	95.67±3.650
7	87.67±3.587	92.00±1.925	97.00±1.000	109.00±.937	117.00±.000
8	81.33±4.194	116.00±.937	91.00±1.349	123.33±.275	94.67±3.051
9	103.00±.196	116.67±2.817	102.33±2.746	132.00±.165	90.67±1.372
10	92.33±.044	108.67±1.583	135.33±1.010	130.67±2.033	87.00±3.000
11	85.00±1.000	102.67±4.189	122.67±1.623	117.33±4.619	87.00±.245
12	70.00±1.000	92.00±2.298	103.00±2.478	116.33±.822	79.00±.292
13	70.67±1.824	93.33±1.719	92.67±2.939	100.00±1.000	61.00±.568
14	64.67±1.098	81.00±1.748	84.33±1.717	96.67±4.163	48.33±2.021
15	54.00±1.269	68.67±2.362	75.67±3.796	84.67±4.726	43.67±.429
16	51.33±1.786	60.33±1.060	60.67±2.082	75.00±3.606	37.33±3.786
17	42.33±.033	53.33±.506	58.67±1.528	65.67±3.055	43.00±.083
18	38.00±.000	47.67±.110	52.67±.577	60.33±1.528	32.33±3.215
19	34.33±.132	42.00±3.606	49.00±1.000	54.67±2.082	39.00±.557
20	31.00±4.000	35.67±3.215	45.67±3.055	47.33±3.786	30.00±1.732
21	26.00±5.000	26.00±5.000	39.00±2.000	44.00±2.550	24.67±3.215
22	22.33±2.082	22.33±2.082	34.00±3.000	37.00±.083	21.33±1.528
23	18.67±1.528	18.67±1.528	29.33±3.786	33.33±2.082	15.67±2.517
24	14.67±1.528	14.67±1.528	24.33±3.055	28.00±2.646	12.00±.083
25	10.00±1.000	10.00±1.000	20.33±2.082	21.00±1.000	8.67±3.215
26	6.67±1.528	7.67±1.155	15.00±2.646	15.33±1.528	6.33±2.309
27	4.00±1.000	3.33±.577	8.67±3.215	8.67±1.528	2.33±.577
28	1.00±1.00	.67±.577	4.67±2.517	3.33±1.528	.67±.577
29	.00±.00	.00±.00	1.33±1.528	1.00±1.000	.00±.00
30	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00
total	1413.7	1716.4	1819.7	198.99	1514
average	47.1	57.3	60.7	66.3	50.5

Table 5: Daily mean biogas yields from sole alone and co-digestion with CM and *SH*± SE (ml) (n=3), for Ts of 6%.

Days	6A	6B	6C	6D	6E
1	41.67±1.528	45.00±.000	52.33±.145	53.67±1.535	43.33±.609
2	54.33±1.897	61.67±.033	71.33±2.309	68.33±.429	67.00±3.606
3	57.33±.508	70.33±1.015	70.67±2.309	79.00±2.646	80.00±2.646
4	56.67±.658	67.67±.572	71.00±.245	119.67±2.670	101.33±4.846
5	71.67±1.060	94.67±.238	98.67±.767	125.33±2.106	127.00±.196
6	95.33±2.423	106.00±4.583	91.33±3.505	131.00±2.338	114.67±1.066
7	92.00±2.530	88.33±4.468	101.00±1.136	121.67±3.512	112.67±4.154
8	85.67±.083	96.00±.395	109.33±.473	117.00±1.358	96.00±1.269
9	82.00±1.581	104.00±1.193	106.00±1.823	112.33±1.408	95.33±.429
10	80.33±.652	117.67±1.807	100.00±3.211	109.67±1.017	99.67±3.22
11	94.33±2.610	121.33±1.655	129.00±2.378	99.67±2.082	72.67±.686
12	79.33±2.610	109.33±1.719	100.67±2.325	93.67±1.948	72.33±3.095
13	81.33±2.006	109.00±.550	100.33±3.271	80.33±1.258	64.00±.196
14	64.33±1.214	80.00±1.358	91.00±2.980	74.67±2.362	54.33±4.509
15	64.00±2.239	71.67±.653	83.33±2.599	65.00±2.230	41.00±1.000
16	55.67±.275	59.00±1.536	73.33±1.504	57.33±1.035	35.67±1.155
17	47.00±4.000	54.00±4.583	59.00±2.000	58.67±1.676	40.67±3.512
18	38.00±.000	49.67±4.619	50.67±1.155	49.67±1.504	31.00±1.732
19	38.00±.245	42.33±.859	46.33±4.726	44.67±1.930	37.33±.351
20	28.33±.429	37.33±2.095	46.00±3.606	39.67±1.214	26.67±4.726
21	23.33±4.933	32.00±2.550	34.00±2.646	36.67±1.846	20.67±.577
22	19.00±1.000	28.33±.807	32.00±1.732	32.33±.693	16.00±3.606
23	13.00±3.464	26.33±.970	24.00±3.464	27.33±.423	14.00±4.000
24	13.67±1.155	18.67±.508	23.00±3.464	20.33±.503	8.00±2.646
25	10.00±1.000	13.33±.774	17.67±1.528	15.33±.963	5.67±3.055
26	6.00±1.732	9.00±2.000	13.33±3.512	8.33±3.786	3.00±2.000
27	2.33±1.528	5.33±4.933	6.67±.723	5.33±3.786	1.67±2.000
28	.33±.577	2.33±2.309	2.33±2.309	1.33±.577	.33±.577
29	.00±.00	.67±1.155	.33±.577	.67±.577	.00±.00
30	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00
total	1395	1721	1806	1850	1482
average	46.5	57.4	60.3	61.7	49.4

Table 6: Daily mean biogas yields from sole alone and co-digestion with CM and *SH*± SE (ml) (n=3), for Ts of 4%.

Days	4A	4B	4C	4D	4E
1	31.00±2.000	42.67±4.509	54.33±.234	61.00±1.136	38.33±.110
2	47.33±.505	58.67±.963	67.33±3.512	68.33±4.163	67.67±.506
3	54.00±1.395	66.67±1.502	74.00±.000	80.00±2.767	75.67±3.807
4	73.67±1.214	83.00±.000	84.33±4.726	105.33±2.858	87.00±2.224
5	74.00±1.716	94.67±2.097	97.00±1.716	120.33±4.786	107.33±3.438
6	94.33±2.385	103.67±.234	112.67±1.846	119.00±2.716	118.00±.928
7	98.00±1.856	92.67±1.590	102.67±1.502	118.00±1.165	114.33±1.553
8	92.33±4.705	91.67±2.008	103.33±3.271	123.33±.572	97.00±.524
9	84.00±1.321	104.00±2.518	98.00±1.000	119.33±1.803	82.33±5.774
10	82.33±3.786	106.00±3.287	108.00±1.269	106.33±4.189	97.67±4.163
11	88.67±3.089	115.67±3.130	102.00±1.313	115.67±4.978	75.67±2.014
12	86.33±4.502	114.00±4.000	111.33±2.102	92.00±1.875	64.67±.506
13	71.00±2.138	114.33±.638	99.67±2.502	76.00±1.583	64.00±1.454
14	65.67±4.238	86.67±1.017	94.67±2.968	78.33±2.449	57.00±2.646
15	67.00±1.731	74.67±3.292	81.33±.658	75.67±1.448	48.00±1.937
16	48.67±1.504	66.67±2.423	75.33±3.095	61.00±.620	36.00±2.000
17	37.00±1.269	54.00±.292	56.00±3.000	54.00±2.288	35.67±3.786
18	33.00±2.888	48.00±.196	48.67±.859	46.33±1.786	30.33±2.309
19	28.67±5.686	38.67±6.658	44.00±1.000	41.00±1.392	26.00±4.583
20	25.00±3.000	35.33±2.028	38.33±1.528	35.33±2.963	24.00±1.550
21	19.33±3.386	29.33±.807	33.33±.309	33.00±.124	18.67±.163
22	17.33±.577	25.00±.292	29.33±2.887	29.33±.083	14.67±.163
23	10.33±1.528	20.67±5.508	24.00±3.464	24.00±.269	9.00±2.646
24	8.00±1.000	15.33±.859	19.33±1.155	18.67±.846	6.33±3.055
25	5.33±.577	13.00±.083	14.33±2.082	14.33±.815	3.33±2.082
26	3.67±.577	8.67±1.528	9.67±1.528	7.67±.110	1.67±.577
27	2.00±1.732	3.33±3.215	5.00±3.464	3.33±2.082	1.33±1.155
28	.00±.00	2.33±2.309	2.00±1.732	.67±.577	.67±.577
29	.00±.00	.33±.577	.33±.577	.67±.577	.00±.00
30	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00
total	1348	1710	1790	1828	1392
average	45	57	60	61	46.4

Table 7: Total Cumulative biogas production potential in each bio-digester for four types of adjusted percent of TS.

Treatments	Mix Ratio	Total Biogas for Ts of 10%	Total Biogas for Ts of 8%	Total Biogas for Ts of 6%	Total Biogas for Ts of 4%
A	100%CM+0%SH	1421.7 ml	1413.7 ml	1514 ml	1348 ml
B	75%CM+25%SH	1844.4 ml	1716.4 ml	1721ml	1710 ml
C	50%CM+50%SH	1905 ml	1819.7 ml	1806 ml	1790 ml
D	25%CM+75%SH	2150 ml	1989.9 ml	1850 ml	1828 ml
E	0%CM+100%SH	1465.7 ml	1514 ml	1482 ml	1392 ml
Total		8786.8 ml	8453.7 ml	8373 ml	8068 ml
Average		1757.4	1690.74	1674.6	1613.6

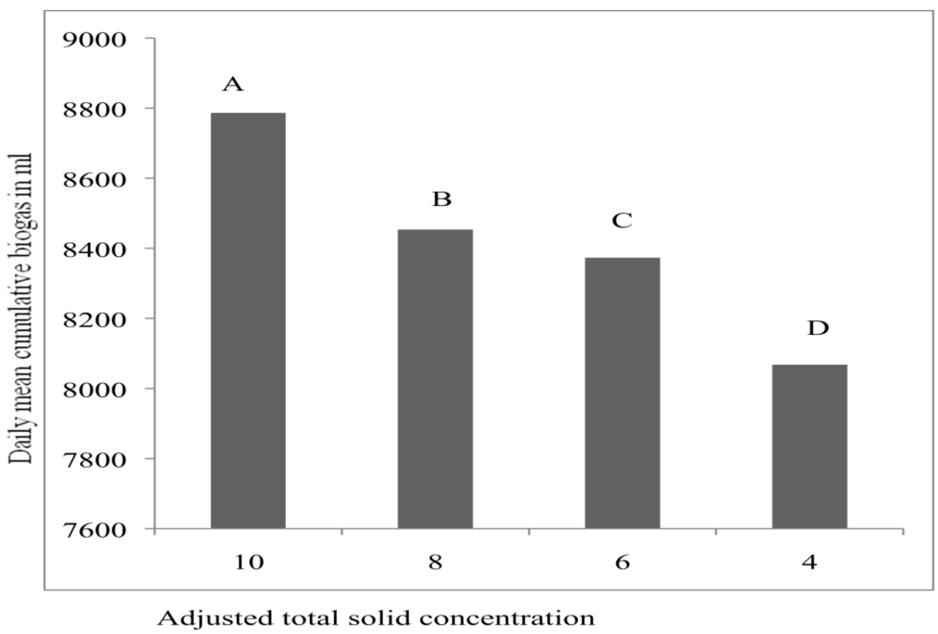

Figure 1: Daily mean cumulative biogas yield for adjusted percent of total solid concentration (for 10, 8, 6, and 4 %) respectively.

Fig 2 *Striga hermonthica (Del.) Benth:*
Source: https://www.uni hohenheim.de/ipspwww/350b/indexe.html

Figure 2: *Stiga hemonthica*

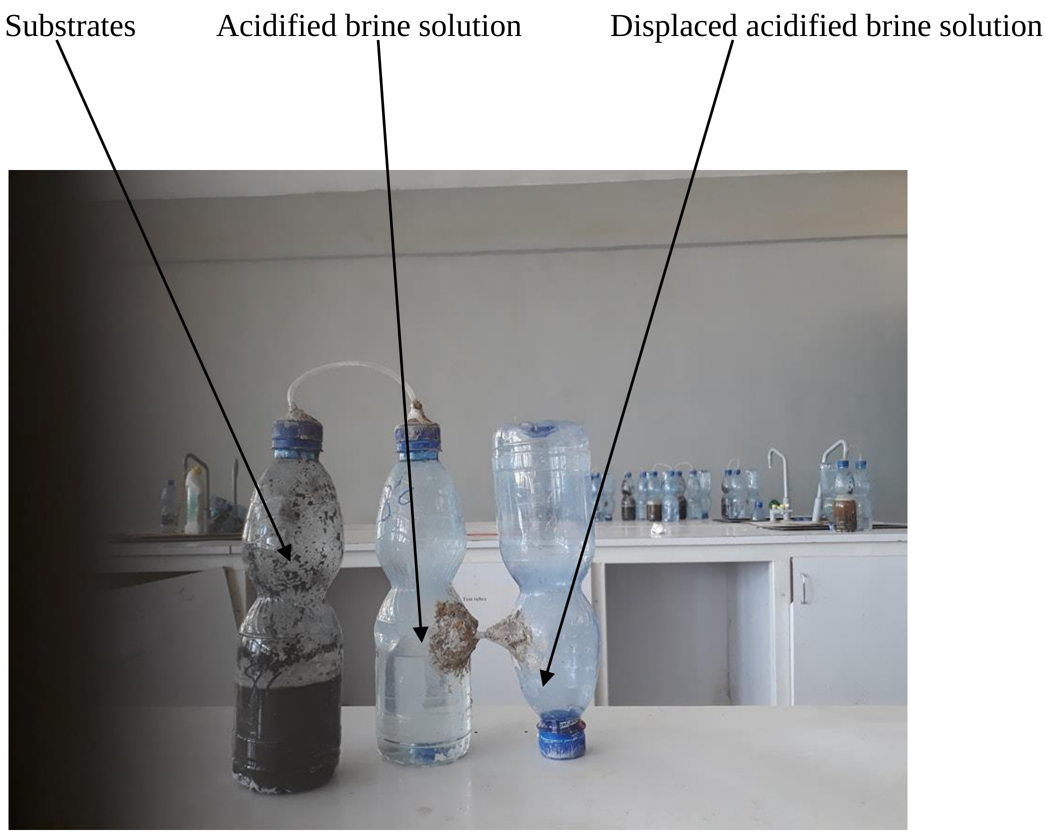

Figure 3**:** Batch form of experimental setup