BIOGAS PRODUCTION FROM *Justicia schimperiana* LEAVES CO-DIGESTED WITH COW DUNG

M.Sc. THESIS

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Biogas Production from *Justicia schimperiana* leaves Co-digested with Cow Dung

A Thesis Submitted to the School of Biological sciences and Biotechnology, Postgraduate Program Directorate, College of Natural and Computational Sciences
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Postgraduate Program Directorate

We here by certify that we have read and evaluated this thesis entitled, "**Biogas production from *Justicia schimperiana* Leaves Co-digested with cow dung'.** Prepared under our guidance by Mola Erbeto. We recommended that it be submitted as fulfilling the thesis requirement.

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Final approval and acceptance of the thesis is contingent up on the submission of its final copy to the postgraduate program directorate (PGPD) through the candidates department or Postgraduate program Directorate committee(PGPD).

DEDICATION

I dedicate this thesis to my beloved wife Rahele Desta for her patience and special care.

STATEMENT OF THE AUTHOR

I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment for the requirement of M.Sc. Degree at Haramaya University and is deposited at the university library to be made available to borrowers under the rules of the library. I declare that this thesis is not submitted to any other institution for award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author was born in small village called Koecho in Hadiya Zone, on June 20,1984. He attended his elementary school education at Koecho Junior Secondary School, and High School Education at Wachamo Senior Secondary School. He completed his High School Education and Joined Awasssa College of Teachers Education and graduated with diploma in Biology(from 2003-2004). Then he was employed as Biology teacher in Hadiya Zone and worked for two years and joined Haramaya University and graduated with bachelors degree (Biology Education) in 2010. At present the author is teaching in Homecho preparatory School, Hadiya Zone Gibe Woreda. He continued studying M.Sc. in the School of Postgraduate Program Directorate of Haramaya University by being self Sponsored to pursue his postgraduate study in 2015 in the School of Biological Science and Biotechnology, M.Sc. in Biotechnology.

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LIST OF ABRIVATION AND ACRONYMS

AD	Anaerobic digestion
APHA	American Public Health Association
C/N	Carbon to nitrogen ratio
EEMBPM	European Energy Manager Biogas Production Material
FAO	Food and Agricultural Organization
NBPE	National Biogas Program of Ethiopia
NRCS	Natural Resource Conversion Strategy
OC	Organic Carbon
TN	Total Nitrogen
TS	Total Solid
VFAs	Volatile Fatty acids
VS	Volatile Solid

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ABSTRACT

Production of biogas through anaerobic digestion of organic waste materials provides an alternative environmentally eco-friendly renewable energy. In this study, biogas production from co-digestion of the Justicia schimperiana *and cow dung in five mix ratios,A100%JS, B 75%JS: 25%CD, C 50%JS:50%CD, D25%JS:75%CD, E100%CD was evaluated under mesophilic conditions (38ºC) using a batch digester in microbiology laboratory of Haramaya University. In all treatments, Total solid, Volatile Solid, organic carbon, percent moisture content and pH were measured before and after digestion, while, Organic carbon to Nitrogen ratio was measured before anaerobic digestion, and found in the range of optimal nutrient requirement for anaerobic microorganisms. The daily biogas production was subsequently measured by water displacement method for 28 days. All measured Physico-chemical parameters of each substrate were significantly different between before and after anaerobic digestion. Gas production was noticed in all of the substrate variation from the first day of digestion experiment and became almost zero at the 28th day in all substrates. Assessment of cumulative biogas production revealed that substrate in a mix ratio of 75% JS and 25% CD showed the highest cumulative biogas production (924.11mL), suggesting this mix ratio of the two substrates is an optimal mix ratio to yield better amount of biogas. Overall results indicate that the increment of biogas yield, and VS and TS reduction can be significantly enhanced when* Justicia schimperiana *and Cow dung are co- digested in 3:1 ratio.i.e.75%JS:25 %CD.*

Key Words: Biogas, co-digestion, Cow dung, *Justicia schimperiana*

1 .INTRODUCTION

According to a prognosis from the United Nations (2013), the world population will likely increase by 2.4 billion over the next 38 years, passing from the current 7.2 billion to 9.6 billion in 2050. Energy is one of the most important limiting factors to global prosperity. Currently, the global mix of fuels comes from fossil (78%), renewable (18%) and nuclear (4%) energy sources. As far as fuel is concerned, the rural population in developing countries heavily depends on traditional fuels, such as fire wood, animal wastes and agricultural residues. Biomass mainly in the form of firewood is the main energy source in Ethiopia. It accounts for about 94.7% of the total energy supply (World Bank, 2001). The dependence on fossil fuels as primary energy source has led to global climate change, environmental degradation, and human health problems. Moreover, the recent rise in oil and natural gas prices may drive the current economy towards alternative energy sources such as biogas (Sunarso *et al.,* 2012).

Biogas production technology has been introduced to various applications such as production of energy from organic wastes, organic fertilizers and improvement of the hygienic condition by reducing pollution (Bhumiratana *et al.,* 1984). Biogas, apart from its benefit for cooking and lighting, the effluent that comes as slurry is rich with various plant nutrients such as potash, phosphorus, nitrogen which are essential for plant growth. Well fermented bio-slurry improves the physical, chemical and biological properties of soil resulting in enhancing the quantitative yield of food crops. The slurry is free of parasites and pathogens, it is highly recommended for use in farming. The economic value of slurry is high because it saves the money for importing inorganic fertilizers by adding micro and macro nutrient to the soil (Fisseha and Fantaw, 2010).

Moreover, use of biogas reduces the carbon dioxide emissions through reduction of the demand for fossil fuels. At the same time, by capturing uncontrolled methane emissions which is the second most important green house gases next to carbon dioxide (Laichna and wafula, 1997). Furthermore biogas is also improving the environment of indoors and outdoors. The indoor environment is enhanced by reduction of the incidents of illness from pollution of fire wood and dung outdoors by reduction in carbon dioxide and methane emission (Siltan, 1985). Biogas production in smaller agricultural units can reduce the use of forest resource for house hold energy purpose therefore slow down deforestation. This will maintain water cycle and alternating periods of drought and flood (Dagnachew *et al*..2003).

The National Biogas Program (2007) of Ethiopia, which aimed to establish 14000 biogas plants between 2008 and 2012 in different parts of the country, utilizes cattle manure as the feedstock for biogas production (EREDPC, 2008). However, the efficient use of this technology is limited by the low degradability of manure, which is only in the range of 30–43% (Møller *et al*., 2004). About 25% of the unused methane potential is bound in the biofibers (Hartmann *et al*., 2000). Increasing rate of lignocelluloses (the major component of animal manure) hydrolysis improves biogas production (Malik *et al*., 1988).

Most of the developing countries are suffering from energy crises which is due to depletion of locally available energy resources, forests, for example. Energy consumption has increased steadily over the last century as the world population grew and more countries have become industrialized. Biogas, a renewable bio fuel, is becoming increasingly important as a consequence of major concern for depleting oil reserve and green house effect of using fossil fuel. Deforestation is a very big problem in developing countries like Ethiopia where most of the people depend on firewood for fuel supply which requires cutting of forests. As more and more trees are cut down in unsustainable manner, the impact on the environment including soil erosion lead to decreasing soil fertility, land slide, etc. Furthermore, use of crop residues and dung cakes as a substitute for fuel wood reduces soil fertility and agricultural productivity (Asnake *et al*., 2006). Use of dung as firewood for source of energy is also harmful to human health since smoke arising from the burning cause air pollution. Thus, the situation calls for the use of an eco-friendly substitute for energy source (Dawit, 2011).

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

General Objective was to:

- evaluate the biogas production potential of *Justicia schimperiana* and its combination with cow dung through anaerobic digestion.

Specific Objectives were to:

- ✓ characterize *Justicia schimperiana* and cow dung in terms of total solids (TS), volatile solids (VS), fixed solids, organic carbon, and pH .
- ✓ find out the optimal *Justicia schimperiana* and cow dung mix- ratio for high biogas production.

2. LITERATURE REVIEW

2.1. Biomass Energy and the Environment

One of the main environmental problems of today's society is the continuously increasing production of organic wastes and release of toxic gases from fossil fuels. In many countries, sustainable waste management as well as release of toxic gases prevention and reduction has become major political priorities, representing an important share of the common effects to reduce pollution and greenhouse gas emissions and mitigation of global climate change (Teodorita *et al.*, 2008).

2.2 Fuel Wood crises in Developing Countries

Two billion people, about 40% of the total world population, depend on fire wood and charcoal as their primary energy source. Of these people, three-quarter (1.5 billon) do not have an adequate and affordable supply. Most of them are in less developed countries where they face a daily struggle to find enough fuel to warm their homes and cook their food. The problem is intensifying because rapidly growing populations in many developing countries create increasing demand for fire wood and charcoal from a diminishing supply (Cunningham *et al*., 2003).

Globally, 55% of the wood extracted from forests is for fuel, and fuel wood is responsible for 5% of global deforestation (UNFECC, 2010). Over 80% of Sub-Sahara Africa relies mainly on solid biomass, that is to say fire wood, charcoal, agricultural byproducts and animal waste in order to meet basic needs for cooking and lighting (IEA,2011).

All in all, the efficiency and the environmental concerns of energy use can be progressed in one of the strategies in warded b y UNDP (2004), i.e., the energy ladder. It is a framework for examining trends and impacts of household fuel use and ranks these fuels along a spectrum running from simple biomass fuels (dung, crop residues, wood) through fossil fuels (kerosene and gas) to the most modern form (electricity). The energy assessment further elaborated that the fuel-stove combinations that represent rungs in the ladder tend to become cleaner, more efficient, more storable, and more controllable in moving up the ladder.

2.3 Biogas Composition

Biogas is reported to be composed of components indicated in Table2 below. However, Jemmett (2006) stated that depending on the digestion process, the methane content of biogas is generally between 55-80%. The remaining composition is primarily CO_2 with trace quantities (0-

15000ppm) of corrosive H_2S and H_2O.

Table 1 Biogas Composition

Component	Percentage composition
Methane	50-75
Carbon dioxide	25-50
Steam	0-10
Nitrogen	0-5
Oxygen	0-2
Hydrogen	0-1
Ammonia	0-1
Hydrogen sulfide	0-1

Source : EEMBPM, 2002; Marshal,2004

2.4. Biogas technology in Ethiopia

Initially, biogas use in Ethiopia was began around in 1979. Even if biogas technology has a diversified advantages to rural households societies and for forming sustainable environment, the wider dissemination and installation of the technology is limited to few areas until the National Biogas Program of Ethiopia (NBPE) is launched in 2008, and came into operation and installation of biogas plants with two phase programmers' primarily in four regional states of Oromiya, Amhara, Tigray and South Nations Nationalities and People's Region (SNNPR) and second phase supposed to be scaled up into other regions. According to NBPE,(2014) report, during phase 1 (2009-2013) the NBPE program constructed 8,063 biogas plants against a target of 14,500 in 163 woredas (districts) (NBPE, 2014). Amhara region installed 1,892 plants, SNNPR 1,699 plants, Tigray 1,992 plants and Oromiya 2,480 plants. While, in the second phase (2014-2017) the program has targeted to install 20,000 plants. In 2014 alone, a total of 1762 plants had been constructed summing up to 9825 plants constructed since 2009(NBPE, 2014).

As NBPE.(2014) report showed, however, over 1000 plants had been established (constructed) by 2008, approximately 50% of the installations were not functional. About 77% of agricultural families have cattle and many agricultural wastes and are eligible for biogas installation, but, they did not produce and get significant benefit from biogas energy so far. This is 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 as stated by (NBPE, 2014).

As NBPE, (2014) report suggested installation and dissemination of plants can be implemented via multi-actor through the active participation of different stakeholders, such as government, private sector, civil society, cooperatives, donors and users. It can also be implemented via private partnership with government, NGOs, private sector involvement and make for strong partnerships for the successful dissemination of the technology in the country. And via market-oriented ways in which users buy the inputs (construction materials, appliances and labor) at a reasonable cost from the competitive market.

2.5. Principal Uses of Biogas

Manure production in Ethiopia is assumed to be one of the largest, as livestock population of the country is estimated at over 42 million cattle (FAO, 2009). In rural Ethiopia, where firewood is scarce, cattle dung cakes are the main sources of household fuel. Cattle in direct combustion are not only unclean and unhealthy, but also inefficient source of energy. The burning of dung also excludes its contribution to increase agricultural production (Wudnesh and Belay, 1994).

Domestic biogas digesters convert animal manure into small, but precious amounts of combustible methane gas, known as biogas. Biogas can be used in simple gas stoves for cooking and in lamps for basic lighting. This digestion process generates a potent organic fertilizer, known as bio-slurry, which can be used to increase agricultural productivity. On average; farmers with at least four heads of local breed cattle can generate sufficient biogas to meet their daily basic cooking and lighting fuel needs (SNV, 2010).

Biogas can basically be used in all applications that have been developed for natural gas. It has the advantage that it can be stored relatively easily and that it can be used where it has been generated. Alternatively it can be transported to the site where it is needed. There are four basic ways of biogas utilization; production of heat and steam, electricity generation/cogeneration, use as a vehicle fuel and (possibly) production of chemicals (Annaschnurer, 2010).

Without any treatment such as the removal of sulphur, biogas can only be used at the place of production. By increasing the energy content of biogas, such as through compression, the biogas can be transported over larger distances in pressure tanks. The enrichment and enhancement of the use of biogas can be achieved after removing the $CO_2$and other contaminants. Strachan *et al*. (2007) described the use of landfill gas in the Durban area to fuel a micro turbine thus generating power, which is added to the electrical grid. Applying this technology reduced the emission of greenhouse gases (GHG), for which carbon credits were earned.

2.6. Description of Feed Stocks

2.6.1 Botanical background of *Justicia schimperiana*

The family and genus of *Justicia*

The family *Acanthaceae* is a taxon of dicotyledonous flowering plants containing almost 250 genera and 2500 species. Most are tropical herbs, shrubs or twining vines, some are epiphytes. Only a few species are distributed in temperate region. The four main centers of distribution are Indonesia, Malaysia, Africa, Brazil and Central America (Reddy *et al*., 2013). *Justicia* is the largest genus of *Acanthaceae*, with approximately 600 species that are found in pan tropical and tropical regions (Corrêa and Alcântara, 2012; Hedberg *et al*., 2006). The species of *Justicia* can be easily recognized by their bilabial corolla, with a posterior lip that is generally two-lobed, an anterior lip that is three lobed, two stamens, a capsule with four seeds, and a basal sterile portion (Corrêa and Alcântara, 2012).

Justicia Schimperiana

Synonyms: *Adhatoda schimperiana/Gendarussa schimperiana*, local name ‗dhumuugaa' in Afan Oromo, ‗Sensel' or ‗simiza'in Amharic, ‗surpa', ‗kasha' or ‗keteso' in Sidama. It belongs to the family of *Acanthaceae*. It is a shrub with much branched stems 2-3 m high, with slightly unpleasant smell. It grows in moist manatee forest usually near stream/river, evergreen shrub on hill slopes, waste ground, and village & house hedge from altitude ranging 1300-2700 m (Hedberg *et al*., 2006).

2.6.2 Cow dung/manures

In the absence of appropriate disposal method cow dung can cause a diverse environmental and health problem such as pathogenic contamination, odor, Greenhouse gas (Harikishan *et al*., 2003).Also in regard to animal wastes, it is important to note pathogens destruction. Manure can contain numerous pathogenic organisms that are associated with human diseases including *Salmonella*, *E. coli*, *Giardia*, *Campylobacter*, and *Cryptosporidium* (Klein *et al*., 2010).

2.7. Biochemical Processes in Anaerobic Digestion

Anaerobic digestion is a complex process consisting of a mixed biological system in which organic materials such as carbohydrates, lipids, and proteins are utilized by microorganism to produce methane and carbon dioxide rich biogas in their normal metabolic activities (Kamthunzi, 2008). It occurs in a basic biological and chemical step of anaerobic digestion as a result of the activity of a variety of microorganisms (Ciborowski, 2004).

The anaerobic digestion process involves a large number of microorganisms, which convert the feedstock to the methane and carbon dioxide-rich biogas through a number of different processes. These microorganisms include hydrolytic bacteria, acetic acid-forming bacteria (acetogens) and methanogenic bacteria (archaebacteria) (Ciborowski, 2004).

According to Budiyano *et al*. (2010), anaerobic digestion that utilizes manure for biogas production is one of the most promising uses of biomass wastes because it provides a source of energy while simultaneously resolving ecological and agrochemical issues. The anaerobic fermentation of manure for biogas production does not reduce its value as a fertilizer supplement, as available nitrogen and other substances remain in the treated sludge (Budiyano *et al*., 2010).

Biogas production is a complex biochemical reaction that takes place under the action of delicately pH sensitive microbes mainly bacteria in the presence of little or no oxygen. Three major groups of bacteria (hydrolytic, acidogens/acetogens and methanogens) are responsible for breaking down the complex polymers in biomass waste to form biogas at anaerobic conditions and animal manure has been established as major source of this gas (Bori *et al*., 2007). Specific groups of microorganisms are involved in each individual step. These organisms successively decompose the products of the previous steps. Anaerobic digestion process can be described by a four-stage scheme namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Bori *et al*., 2007; Ciborowski, 2004; Demirbasa Balat, 2009).

2.7.1 Hydrolysis

It is the first process in which hydrolytic microorganisms secrete an enzyme to hydrolyzee polymeric materials into monomers such as glucose, amino acids, and fatty acids. The microorganisms producing these enzymes can be obligate or facultative anaerobes. (Nasir and Beyza,2007).

During hydrolysis the complex compound are broken down into soluble components, thus they are readily available for fermentative bacteria to convert into alcohols, acetic acid, other volatile fatty acids (VFAs), and off-gas containing H_2 and CO_2. These intermediate products are metabolized into primarily CH_4 (60%), CO_2(<40%) and other associated gases by methanogenesis (Garba, 1999).

A complex consortium of microorganisms participates in the hydrolysis and fermentation of organic material (Rojas *et al.,* 2010). This step is inhibited by lignocelluloses containing materials, which are degraded only very slowly or incompletely (Riling, 2005).

2.7.2 Acidogenesis

The biological process of acidogenesis is where there is further break down of the remaining components by acidogenic (fermentative) bacteria, here, volatile fatty acids (VFAs) are created along with ammonia, carbon dioxide and hydrogen sulfide as well as other by products. The acidogenesis stage is complex phase involving acid-forming fermentation, hydrogen production and an acetogenic (acetic acid-forming) step. Once complex organics are hydrolyzed, the hydrolysis product which are relatively small soluble compounds can diffuse inside the bacterial cell through cell membrane. Acidogenic (acid-forming) bacteria convert sugars, amino acids and fatty acids to smaller organic acids, hydrogen, and carbon dioxide (Zinder; 1998). The community of bacteria that are responsible for acid production may include facultative anaerobic bacteria, strict anaerobic bacteria, or both. Hydrogen is produced by the acidogenic bacteria including hydrogen producing acetogenic bacteria.

2.7.3 Acetogenesis

The third stages of anaerobic digestion is acetogenesis. Here simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen. Acetogenic organisms are 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 materials, namely hydrogen and acetate. Both the acidogenics and acetogenesis produce the methanogenic substrates, acetate, H_2 and CO_2. The important distinction between these two types is that the fermentative bacteria have the possibility of using various electron acceptors from the disposal of electrons. The acetogenesis is an obligate proton-reducer and can utilize only protons as electron acceptors and only when the hydrogen –concentration is low. At very low H_2 concentrations, however, methanogenesis from H_2 and CO_2 becomes unfavorable (Chynoweth and Isaacson,1987). Acetogenic bacteria such as *Syntvobacter wolini* and *Syntrphomonas wolfei* convert volatile fatty acids (e.g. Propionic acid and butyric acid) and alcohol in to acetate, hydrogen and carbon dioxide, which are used in methanogenesis (Zaher *et al*., 2007).

The acetogenesis is regarded as the thermodynamically unfavorable unless the hydrogen partial pressure is kept below 10^{-3}atm, pathway efficient removal of hydrogen-consuming organisms such as hydrogenotrophic methanogens and/or homoacetogens (Zinder,1998).

2.7.4 Methane Formation

Methane formation involving conversion of simple compounds in to methane (CH_4) and CO_2, utilizing anaerobic methanogenic bacteria. The formation of methane, which is the ultimate product of anaerobic treatment, occurs by two major routes. Formic acid, acetic acids, methanol and hydrogen can be used as energy sources by the various methanogens (Zaher *et al*., 2007).

The primary route of methane production is the fermentation of the major product of the acid forming phase, acetic acid, to methane and carbon dioxide (Zaher *et al.*, 2007). Bacteria that utilize acetic acid are *acetoclastic* bacteria (acetate splitting bacteria). The *acetoclastic* group comprises two main genera: *Methanosarcina* and *Methanothrice.* During the thermophilic digestion lignocelluloses waste, *Methanosarcinais* the dominant *acetoclastic* bacteria is encountered in the bioreactor (Zaher *et al* ., 2007).

2.8. Factors Affecting Bio-digestion

The main factors that affect biogas productions are, temperature, pH or hydrogen ion concentration and nutrient and inhibitor concentration. Any drastic change in these factors can adversely affect the biogas production (Chatterjee, 2007 and Marchaim, 1992).

2.8.1 Temperature

Methane bacteria work best at a temperature of 35-38^{0}C (Rai, 2004). The fall in gas production starts at 20^{0}C and stops at a temperature of 10^{0}C. There are two significant temperature zones in anaerobic digestion and two types of microorganisms mesophilic and thermophilic are responsible for digestion at the two temperature ranges. The optimum mesophilic temperature lies at about 38^{0}C while the thermophilic temperature is 55^{0}C. Most of the sewage digestion tanks are heated at 35^{0}C so as to reduce the time required for digestion and minimize the capacity of the tanks (Rai, 2004).

According to Dahalman and Forst (2001), the most favorable temperature for methanogenesis is the mesophilic range (20^0C to38^0C). Similarly, Velsed *et al.* (1979) stated that at a digestion temperature of 13^0C, no methane was produced, while in mesophilic range (20 to 45^0C) methane production increased with temperature and under thermophilic conditions (55^0C) decreased (Rai, 2004).

Wilkie (2008) described this factor in a more widened manner as; biological methanogenesis exist at a temperature ranging from 2^0C (in marine sediment) to over 100^0C (in geothermal areas but , most applications of this fermentation have been performed under ambient (15 to 25^0C), or thermophilic (50 to 60^0C) temperatures. In general the overall process kinetic, doubles for every 10^0C increase in operating temperature, up to some critical temperature (about 60^0C) above which a rapid drop-off in microbial activity occurs. Most anaerobic digestions are operated at mesophilic or ambient temperatures as higher operating temperature permits reduced reactor size and higher energy requirements. This author further recommended that since methanogenesis is sensitive to temperature fluctuations, effective insulation should be done during the digestion process. According to NRCS (2005), the daily fluctuations of digester temperature should be limited to less than 1^0C.

2.8.2. pH or Hydrogen ion concentration

pH of slurry changes at various stages of digestion. In the initial acid formation stage, in the fermentation process, the pH is around 6 and much of CO_2 is given off. In the later 2-3 weeks time, the pH increases as the volatile acids and N_2 compounds are digested and CH_4 is produced. To maintain constant supply of gas, it is necessary to maintain a suitable pH range in the digester. The digester is usually buffered to maintain the pH at 6.5 to 7.5. In this pH range, the microorganisms will be very active and bio digestion will be very efficient (Rai, 2004). Dahlman and Forst (2001) also discovered the favorable pH for digestion ranges in between 6.8 to 7.2. The proper pH range for anaerobic fermentation is between 6.8 to 8.0. Acidity higher or lower than this will hamper fermentation. The introduction of too much raw material can cause excess acidity and the gas producing bacteria will not be able to digest acids quickly enough. The addition of a little ammonia can raise the pH value very fast. If the pH grows to high (not enough acids), fermentation will slow until the digestive process forms enough acidic carbon dioxide to restore balance (Saxon, 1998).

2.8.3 Carbon to Nitrogen ratio of the input material

Besides carbon the quantity of nitrogen present in the input material is a crucial factor in the production of biogas. The compounds of carbon in the form of carbohydrates and nitrogen as protein and ammonium nitrates are the main foods of anaerobic bacteria. Carbon is used for energy and nitrogen for building the cell structure. The bacteria use up carbon about 30 times faster than they use up nitrogen. So, carbon and nitrogen should be present in the proper proportion i.e. C:N is 30:1 (Rai, 204). If the ratio is higher, the nitrogen will be exhausted while there is still a supply of carbon left. This cause some bacteria to die, releasing the nitrogen in their cells and eventually restoring the equilibrium. Digestion proceeds slowly as this occurs. On the other hand, if there is to much nitrogen, fermentation will stop when the carbon is exhausted and will be incomplete and the left over nitrogen will not be digested. This will lower the fertilizing value of the slurry (Saxon,1998). Dahalman and Forst (2001) forwarded the favorable carbon to nitrogen ratio ranges 20:1 to 30:1. Hills (1979) reported that the greatest methane production per unit occurred when the C:N ratio of the feed was 25:1.

According to (Rai, 2004), substituting a production of vegetable waste instead of dung will enable to get more gas for the same amount of substrate but it is necessary to maintain the C/N ratio between 30 and 35 by properly varying the quantities of other biodegradables (EEMBPM, 2002).

2.8.4 Total solid (TS) and Volatile Solid (VS) Content

Ghose *et al*. (1979) reported that the presence of digestible volatile solids in the plant wastes when added with cow dung and subjected to anaerobic digestion led to more methane production. Anand *et al.* (1991) carried out experiments on solid phase fermentation of leaf biomass to biogas. Fulford, (1988) reported that the composition of typical animals and human wastes consists of 15-48 percent of total solids and volatile solids is 77-90 percent of total solids. For cow dung, the total solids are in the range of 15-20 percent, and the volatile solids as percent of total solids is 77. Nallathambi and Lakshmanaperumalsamy (1990) studied the biogas production potential 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 %. Cusin *et al*. (1992) conducted an experiment on anaerobic digestion of cassava peel and observed that the composition of cassava peel was 25-35 % TS, VS was 90-97 % of TS.

2.8.5 Retention Time or Rate of Feeding

The period of retention of the material for biogas generation depends on the type of feed stocks and the temperature (Rai, 2004). Normal retention period is between 30 and 45 days and in some case 60 days (Rai, 2004). Depending on the waste material and operating temperature, a batch digester starts producing biogas after two or four weeks, slowly increasing in production then dropping off after three or four months (Jemmett,2006). According to G annon (2005), the amount of time the substrates spend in the digesters is one of the critical factors in methane production. Too short retention time means an efficient extraction of methane, so full revenue is not realized. Too long retention time means too much is spent on surplus capacity or not enough substrate is being added to maximize revenue.

2.8.6. Feed Stocks and Co-digestion

According to Wilkie (2008) the feed stocks for biogas generation can be composed of carbohydrate, lignocelluloses, protein, fats or mixture of these components.

Table 2.Theoretical Methane Yield of Biomass Component

Components	Max. methane yield (m^3/t vs.)
Proteins (leucine)	998
Fats (lauric acid)	585
Carbohydrates	370
Plants	470

Source : Wilkie (2008); EEMBPM (2002)

All plant and animal wastes may be used as the feed materials for a digester when feed stocks is woody or contains more of lignin, then bio-digestion becomes difficult. Cow dung and buffalo dung, human excreta, poultry dropping, pig dung, waste materials of plants, cobs, etc can all be used as feed stocks. To obtain an efficient bio-digestion, these feed stocks are combined in proportions, pre digesting and finely chopping will help in the ease of some materials (Rai, 2004).

The feed stocks for anaerobic digestion vary considerably in composition homogeneity, fluid dynamics and biodegradability. In intensive animal farming, pig and cow slurries are reported to contain dry matter contents in the range of 3 to 12%. Chicken manure contains 10 to 30% TS. Some agro-industrial wastes may contain less than 1% TS, while other contain high TS contents of more than 20%. This result in some substrate being able to be fermented only when mixed with other substrate or diluted (Braun and wellinger, 2002).

Thus a combination of two or more substrates, co-fermentation, will optimize the degradation properties of the feed stocks and hence increases the methane yield. It has been reported that the performance of digesters could be considerably improved by means of co-substrate addition and hence increase degradation efficiency and biogas production (Kaparaju *et al.;* 2001).

Shivappa *et al,* (1980) studied different plant wastes, sunflower wastes, and banana trashes. Similarity, Rai (2004) recommended that the weight of dung in a dung vegetable mixture should be maintained above50%. Anand *et al,* (1991) carried out an experiment on anaerobic digestion of leaf biomass and water hyacinth for the production of biogas. They reported that the decomposition of the leaf biomass and water hyacinth substrates used was rapid taking 45 and 30 days respectively for production of 250 and 235 L biogas per kg of TS, respectively. In addition, Rajasekaran *et al*, (1989) studied the anaerobic digestion of *Euphorbia triucally L.* they stated that the slurry consisting of 375g fresh cow dung, 375g of one cm bits of *Euphorbia triucally* and 750g of water produced 19.2 liters of biogas in nine weeks compared to 16.5 liters produced by cow dung alone. The carbon dioxide content of the biogas was 38 percent from *Euphorbia triucalli* , plus cow dung and 35% from cow dung alone.

2.8.7. Pre-treatment of Feed Stocks

Plant biomass mainly consists of cellulose, hemicelluloses and lignin which are poorly degraded in anaerobic conditions, and the rate and extent of lignocelluloses utilization is very severely limited due to the intense cross-linking of cellulose with hemicelluloses and lignin as these materials form a scum and can easily clog the system. So that increasing surface area and reducing lignin content is highly important. (Fan *et al*., 1981; *et al*., 2009).

Treatments may be physical, biological or chemical and 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 (Pelmowski and Muller, 1991).

According to Bader *et al.* (1979), mechanical chopping and grinding could provide greater volumes gas from carbonaceous residues.

2.8.8 Mixing of the Contents of the Digester

According to Rai (2004), since bacteria in digester have very limited to reach to their food, it is necessary that the slurry is properly mixed and bacteria get their food supply. He reported that slight mixing improves the fermentation; however a violent slurry agitation retards the digestion. Some method of stirring the slurry in a digest is always advantageous. If not stirred, the slurry will tend to settle out and form a hard scum on the surface, which will prevent release of the biogas. This problem is much greater with vegetable waste than with manure, which will tend to remain in suspension and have better contact with the bacteria as a result (Saxon, 1998).

2.8.9. Particle size

The production of biogas is also affected by particle size of substrate. Too big particle size is problematic for microbes to digest and it can also result in blockage in the digester, were as small particle size gives a large surface area for substrate absorption and thus allows the increased microbial activity followed by increase in the production of gas (Yadvika *et al*.,2004). The size

of the feed stock should not be too large otherwise it would result in the clogging of the digester and also it would be difficult for microbes to carry out their digestion. Smaller particles on the other hand would provide large surface area for absorption of the substrate that would result in increased microbial activity and hence increased gas production. Large particles could be used for succulent materials such as leaves. However, for other materials such as straws, large particles could decrease the gas production. The results suggested that a physical pretreatment such as grinding could significantly reduce the volume of digester required, without decreasing biogas production (David and Kouich,1984).

2.8.10.Water content

Bacteria take up the available substrates in dissolved form. Therefore, biogas production and the water content of the initial material are interdependent. Rilling (2005) reported that when the water content is below 20% by weight, hardly any biogas is produced. Optimum moisture content has to be maintained in the digester and the water content should be kept in the range of 60-95% (Demetriades, 2008).

Anaerobic digestion of organics will proceed best if the input material consists of roughly 8 % solids. Buysman (2009) pointed out that TS content is important for two practical reasons. For domestic digesters, TS content should not be too high, otherwise substrate would not slide easily through the inlet of the digester and if toxins are present, such as ammonium in high concentrations, high TS is likely to affect bacteria more than when the substrate is diluted. Alternatively, TS content should not be too low, otherwise the feedstock is very dilute, and a large digester volume (V) is required. According to Sadaka and danEngler (2003), water content is one of the very important parameters affecting AD of solid wastes. There are two main reasons viz.; (a) water makes possible the movement and growth of bacteria facilitating the dissolution and transport of nutrient and (b) water reduces the limitation of mass transfer of non-homogenous or particulate substrate.

2.8.11. Inhibitors

Organic wastes from livestock farms and plants contain a variety of poisonous substances, such as disinfectants, pesticides, and heavy metals, which will inhibit the growth, metabolism, and propagation of the anaerobes (Chen *et al*., 2008). A variety of substances have been reported to be inhibitory to the AD processes. A material may be considered inhibitory when it causes an adverse alteration in the microbial community or inhibition of the bacterial growth. Inhibition is usually revealed by a decrease in methane production and an accumulation of volatile fatty acids. Considerable variations in the inhibition/toxicity levels of some substances (e.g., ammonia, certain light metal ions such as Na, K, Mg, Ca, and Al, heavy metals at high concentrations, chlorophenols, halogenated aliphatics, N-substituted aromatics including nitrobenzene's, nitrophenols, aromatic amines, long-chain fatty acids including lauric and oleic acid, and lignin related compound such as hydroxylmethylfurfural and others) have been reported in the literature (Chen *et al*., 2008; ward *et al*., 2008 ; Benjamin *et al*., 1984;).

2.8.12. Inoculation

The use of a source high in anaerobic microbes (digester effluent for example) the start up an anaerobic system is called inoculation. According to Wilkie (2008), the quality and quantity of inoculums are critical to the performance, time required, and stability of bio- methanogenesis during commissioning (start up) or restart of anaerobic digester. In manure and some wastes the microbes needed for digestion ma y be already present in the waste in small numbers, albeit sufficient to act as inoculums, and will develop in to a full y functional bacterial population if the right conditions are provided.

2.9. Biogas Digester

Unlike other plants for using renewable energy, a multitude of systems and processes exist for biogas plants and these are classified according to the various criteria. The selection of any particular system is always decided on an individual basis and is dependent up on various factors (Bourn, 2002). For example, depending on the feeding style of the digester, it can be classified in two as batch and continuous feeding.

2.9.1.Batch Feeding

There is biogas system designed for solid vegetable waste alone. Since plant solids will not flow through pipes, this types of digester is best used as a single batch digester. The tank is opened, old slurry is removed for use as fertilizer and the new charge is added. It then released and ready for operation. Batch digesters are therefore best operated in groups, so that a least one is always producing useful quantities of gas (Jemmett, 2006).

2.9.2.Continuous digestion

The complete anaerobic digestion of cow manure takes about eight weeks at normally warm temperatures. One third of the total biogas will be produced in the first weeks another quarter In the second weeks and the remainder of the biogas production will be spread over the remaining six weeks. Gas production can be accelerated and made more consistent by continuously feeding the digester with small amounts of waste daily. This will also preserve the nitrogen level in the slurry for use as fertilizer (Jemmett, 2006).

Chynoweth and Isaacson (1987) stated that if a continuous feeding system is used, it is essential to ensure that the digester is large enough to contain all the material that will be feed through in a whole digestion cycle. One solution is to use a double digester, consuming the waste two stages, with the main part of the biogas (methane) being produced in the first stage and the second stage finishing the digestion and a slower rate, but still producing another 20% of total biogas.

2.10. Use of Domestic Biogas in the Rural House holds

2.10.1. Cooking

Biogas cooking is generally quicker than fire wood and pots need far less cleaning after use In practice, this reduces time women spend on cooking. In addition the availability of biogas reduce the working load of women and children in rural areas who traditionally depends on fire wood for cooking purpose, where the collection of fire wood takes significant time and effort (Shrestha *et al.*,2010).

The advantage of using biogas for cooking has higher net efficiency, 5 times higher stove efficiency than traditional fire wood stove. Cooking on biogas has significant health advantage over traditional cooking on open fire. The major point is the fact that cooking is smokeless and will diminishes the number of eye infections and respiratory problems in particular among women usually in charge of cooking and small children being near their mothers, Also the danger that children burn themselves while cooking is less when using biogas stove (Jan *et al.*,2010).

2.10.2. Lighting

The luminous flux (light output) is measure in lumino meter (lm). At 400 to 500lm, the maximum light flux values that can be achieved with the biogas lamps are comparable to those of normal 60 to 70 watt light bulb. Their luminous efficiency ranges from 1.2 to 2 lm/w. By comparison, the overall efficiency of the light bulb comes to 3-5lm/w, and that of fluorescence lamp ranges from 10 to 15lm/w. One lamp consumes about 1.20- 1.50 litter of biogas per hour. However biogas lamp is not very energy efficient. This means that they, beside light, also generate a lot of heat. The bright light of biogas lamp is the result of incandescence, like thorium, cerium, lanthanum, etc at a temperature of 1000- 2000^0c. If it hung directly below the roof, they can cause fire hazard (Jan *et al.*,2010).

2.10.3. Fertilizer

The organic, nutrient rich bio-slurry is also used as a fertilizer that enhance agricultural productivity and promote organic farming, it has more nutrient especially nitrogen than farm yard manure or compost (Karki, 2006 ; Eije, 2007).

2.11. Benefit of using Domestic Biogas

Biogas contributes to the three aspects of society, economic, social and environmental which are the pillars of sustainable development. These benefits can be direct or indirect and can occur at international, national, regional, household or individual levels of society.

2.11.1. Economic Benefit

By switching to biogas , households are gaining as excess to improve energy services and this can lead to livelihood diversification, bio gas reduce expense energy sources for cooking and lighting (electricity, LPG, charcoal, fire wood purchased from middle men etc), because it made from organic wastes readily available in the surrounding for free. Improved lighting can extend working day and lead again to higher income. It also provide an income generating opportunity for local businesses and improve security of energy supply locally, regionally or nationally. More financial resource can be saved on chemical fertilizer and increased crop yields through bio-slurry use. Some user even try to gain additional income by selling surplus biogas or bio-slurry (Tereza *et al.*,2011). Additional economic impacts of biogas attract financial contribution from multi-sector revenues from CO_2 emission reduction, creation of sustainable employment opportunities for farmers and stimulus for regional economic development (Huang,2010).

2.11.2. Health and Social Benefits

The health benefit of biogas are related to a substaintional reduction of smokes and indoor air pollution compared to traditional wood fire. Mostly women and children benefit from this as they spend lot of their time indoor cooking (Tereza, 2011 and Shrestha, 2010). It reduces the respiratory infections and eye ailments associate to smoky environment. Biogas plants change the traditional live stock keep in and manure treatment modality, animals are kept more in confined space and dung is fed regularly to the plant ,as a result hygiene in the house and on the farm land benefit significantly of smell and amount of flies can be reduced because manure handling is more controlled. Where culture allows the connection of toilets to the biogas digester, sanitary condition and comfort further improved. It can reduce infestation of water -born diseases because around 90% of the parasite egg are destroyed in the digester (Shrestha, 2010, BSP, 2007 and Kark, 2000).

Most importantly, biogas means significant time and work load saving for those biogas user ,especially for women and children, who use to collect their fire wood themselves in the past and benefit to income diversification, opportunity for users in saved time. All together, these time saving can be used for other activity taking care of family, studying or gaining additional income (Karki *et al.,* 2006).

2.11.3. Environmental Benefits

From national perspective the process of substituting biogas for fire wood help to reduce deforestation this in turn has important implication for water shied management and erosion. Treating manure in biogas digester also reduce risk of contamination of soil and water. In addition use of bio-slurry reduce the depletion of soil nutrient by providing organic rich nutrients resulting increasing crop field and hence reduce the pressure of expanding crop lands.

The use of slurry improver nutrient recycling in agriculture and can substitute chemical fertilizer, thus reducing the related Environmental problem (Shrestha and Heong, 2010).

2.12. Limitation and Challenges of Biogas Technology

There are some problems of the bio digester during the construction, maintenance and operational activities. One of the biggest challenge of biogas technology is still relatively high initial investment in biogas plant construction. Especially poorer households hesitate greatly to invest loan money, because without any cash directly generated by the plant they are worried that they will not be able to pay back (Shakaya, 2005;Jan, 2010).

In some countries the farm yard especially in densely populated area, are very confine and the construction of biogas digester can be difficult. Often in such case the plant will be positioned underneath the pigsty or cattle stable. Domestic biogas may not be efficiently save time if it is installed in distance place from the available source of input like water and dung. It is not recommendable to build a digester if the nearest permanent water source is more than 30 minutes away (Jan, 2010). In Ethiopia the common type of animal husbandry is to let the livestock's left to graze freely in a common ground . This make collection of animal dung and taking it into a location of bio digester very difficult since this common ground is usually far from their house. Social and cultural barriers may also hinder harnessing fully the benefits of the technology, in some culture ,resistance exist against using human extract in bio gas digester, as this is seen as making the biogas „unclean" for cooking (Jan, 2010).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in microbiology laboratory of Haramaya University main campus, which is located at latitude of 9^{o}26'N, longitude of 42^{0}03'E and altitude of 1,980m.a.s.l. The mean annual temperature is 17^{o}c , with mean minimum and maximum temperatures 3.8 and 25^{0}C (FAO, 1990).

3.2. Experiment Materials

In this study two main substrates *Justicia schimperiana* leaf (JS) and cow dung (CD) were used as feed stocks for AD process of biogas production. *Justicia schimperiana* leaves were obtained from around Haramaya Town while CD was collected from the Haramaya University animal farm. Both the JS and CD were sun dried to a constant weight and crushed to smaller size using mortar and pestle to ensure homogeneity for AD. Fresh rumen fluid (to be used as starter of AD of the feed stocks) was obtained from slaughter house in the Haramaya University. The rumen fluid was filtered through a cloth of 0.5 mm sieve diameter to separate solid content from slurry. Prior to use, the inoculums was starved for 1 week by incubating at 38ºC to remove the easily degradable VS present in inoculums (Lo Niee Liew, 2011). The substrates and rumen fluid were stored in a refrigerator until use.

3.3 Design of the Experiment

Anaerobic digestion was conducted in batch mode in 0.5 L digester using the two substrates, i.e., JS and CD in five proportions to have five substrate treatments. The five substrate treatments were 100% JS, 75%:25% mix of JS: CD, 50%:50% mix of JS: CD, 25%:75% mix of JS: CD and 100%CD. To have 8% TS in fermenting slurry, appropriate amount of distilled water and rumen fluid (100ml) were mixed (Techobanoglous *et al*.1993). Treatments were randomly arranged in the laboratory and done in three replicates. The temperature of bio-digester was maintained at 38°C by keeping in oven, which represents mesophilic condition (Knottier, 2003). After measuring the initial pH values of all the digesters, their pH values were adjusted to be between 6.5 and 7.5 (7.0) by adding buffer solution. In this experiments H_2SO_4 and NaOH, which are strong acid and strong base, respectively were used to adjusted the pH value.

3.4. Digester Configuration and Setup for Biogas Production

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

3.5 Parameters to be measured

3.5.1 Analyses of Physico-chemical Characteristics of Substrates

Both Substrates were analyzed 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).

- **Total solids**

A clean evaporating dish was dried in an oven at 105^0C for 1hour, cooled in a desiccator and weighed immediately before use. Then 10g of freshly collected samples of JS and CD were weighed using a digital balance, and placed into a pre-dried and weighed evaporating dish. Then, the dish was put inside an electric hot air-oven maintained at 105^0C using a crucible. The crucible was allowed to stay in the oven for 24 hours, and then was taken out, cooled in desiccators and weighed (APHA 2540 B, 1999). Then, the percentage of the TS was calculated as:

$$TS\% = \frac{(A-B)}{(D-B)} x100$$

Where:

A= weight of dish + dry sample (in grams)

B= weight of dish (in grams)

C= weight of wet sample (in grams)

❖ **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).

$$VS\% = \frac{mDS - m(ash)}{mDS} x100$$

Where:

VS=volatile solid

mDS=mass of dry solids in gram

m(ash)=remaining mass after ignition i.e. TS=VS+ fixed solid

Then percentage VS removal were calculated using the equation below.

$$VS\%\ \text{removal} = \frac{VSi - VSf}{VSi} x100$$

Where,

VSi= initial volatile solids before AD

VSf=final volatile solids after AD

- **Determination of pH**

The pH values were determined using digital pH meter before and after AD (HANNA HI8314). In the case of before AD, an electrode was inserted into samples of substrate that were diluted using distilled water before inoculation of rumen fluid. pH measurement after AD were done using pH electrode which was inserted into samples of substrate that is digested at the end of the experiment.

- **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).

$$OC\% = \frac{VS\%}{1.8}$$

Where,

VS= Volatile solids

1.8 = Denominator given by Badger for VS%

3.5.2 Biogas production

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

3.6. Data Analysis

Data were first checked for their normality. Data that are not normally distributed were lo-transformed and thereafter were to analysis of variance (one-way ANOVA) using SPSS. Paired samples T-test was used to investigate statistical significance within a treatment. Difference between means was considered statistically significant at $p<0.05$.

4. RESULTS AND DISCUSSION

4.1. Physico-chemical Properties of the Substrates

pH is important parameter for assessing the efficiencies of anaerobic digester. The pH of 100% CD slurry before anaerobic digestion was about 7.24, whereas that of 100% JS was 6.57 (Table 3). The pH value of 100% CD was optimum for biogas production, whereas that of 100% JS was less optimal (Yadvika *et al*.; 2004; Thy *et al*., 2003)**.** Mixing the substrates resulted in the rise of pH compared to that of JS alone, but decreased pH from that of CD alone. The pH was found to increase significantly with increasing of CD proportion in the mix, suggesting that CD helps to maintain the pH to meet the optimum required (P<0.05). Mixing substrates is a good way of adjusting the pH value to the optimum (Hills and Roberts, 1981). Significant difference in pH values of anaerobic digestion between before and after were seen by using paired samples T-test, (P<0.05).

After anaerobic digestion, the pH values of all substrates increased when compared to before digestion. This may be attributed to the production of alkali compounds (e.g., ammonium ions) during the degradation of organic compounds in the digester (Gerardi, 2003).The high pH value recorded after AD for Treatment A, B, C, D and, E in this study might be attributed to increased production of ammonia resulting from high organic matter available in *Justicia schimperiana* than cow dung (Gray *et al*., 1971). The pH value increases through ammonia accumulation during degradation of protein while accumulation of VFA (volatile fatty acid) resulting from degradation of organic matter decreases the pH value.

Table.3. Comparison of pH between before and after AD of the various substrates (values are mean ±SE, n=3

Treatment		Parameter		
		Ph		
		Initial	Final	Difference
A	100%JS	6.57±0.011^{Aa}	8.73±0.012^{Ab}	2.16
B	75%JS+25%CD	6.78±0.015^{Aa}	8.71±0.011^{Ab}	1.93
C	50%JS+50%CD	6.77±0.015^{Aa}	8.26±0.011^{Ab}	1.49
D	25%JS+75%CD	6.84±0.015^{Aa}	7.94±0.023^{Ab}	1.1
E	100%CD	7.24±0.011^{Aa}	8.63±0.014^{Ab}	1.39

Means followed by different capital letters in column are significantly different at 0.05 level of significance between treatments. Mean followed by different small letters in row are significantly different at5% level of significance within treatments, JS=Justicia schimperiana, CD=cow dung.

Table 4. Comparison of % organic carbon between before and after AD of the various substrates (values are mean ±SE, n=3

Treatments		Parameters		
		OC%		
		Initial	Final	Differences
A	100%JS	46.78±0.011^{Aa}	44.56±0.018^{Ab}	2.22
B	75%JS+25%CD	46.24±0.020^{Aa}	43.15±0.017^{Ab}	3.09
C	50%JS+50%CD	46.03±0.017^{Aa}	43.44±0.020^{Ab}	2.59
D	25%JS+75%CD	45.73±0.088^{Aa}	42.92±0.017^{Ab}	2.81
E	100%CD	46.06±0.023^{Aa}	43.45±0.017^{Ab}	2.61

Means followed by different capital letters in column are significantly different at 0.05 level of significance between treatments. Mean followed by different small letters in row are significantly different at 5% level of significance within treatments, JS=Justicia schimperiana, CD=cow dung.

There were significant ($P<0.05$) differences between the substrates in %OC both before and after AD. However %OC significantly decreased in all substrates after AD when compared with that of before AD (Table 4). The percent degradation of organic carbon was higher in 75%JS+25%CD (3.09) mixed substrate than all other substrates. Compared to the sole substrates, this mix ration facilitated carbon degradation, suggesting that mixing may enhance degradation of substrates for biogas production. Organic carbon can be removed in anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or biogas production (Gerardi, 2003; Devlin *et al*., 2011).

4.2. Values of total solid and volatile solid of substrate co-digestion

The percentage composition of the initial total solids of the 100% JS,75% 75%JS+25% CD, 50%JS+50% CD, 25%JS+75% CD and 100% CD were about22.05±0.029%, 21.82±0.057% 21.06±0.017%, 18.52±0.057% and 18.06±0.002 % respectively. The total solid content of all mix before AD was between 18.10±0.05% (i.e., 1.81 gram of TS from 10 gram sample) and 21.30±0.05 %(2.1 gram of TS from 10 gram sample).The TS content of 22.05±0.0029% to 18.06±0.002% used for this experiment is in the range o f 5 to 70 percent from different feedstock as described by Steffen *et al*. (2000). The maximum TS were measured in *Justicia schimperiana* (22.05±0.0029%), whereas the minimum TS were measured from Cow dung (18.06±0.002%) (Table5). Although JS alone has the highest TS in percent than all mix, the mixture with 75% JS and 25% CD resulted in a high reduction of the amount of TS (21.82±0.057 to 9.62±0.014). As shown in the Table 6, addition of 50% JS to 50%CD alone resulted in an increase of the amount of total solid reduction, from 21.06±0.017 to 11.64±0.024% (i.e., 2.1 gram to1.16 gram of TS from 10 gram sample) Compared to the values measured before digestion, TS significantly (P<0.05) decreased after digestion for all mix, high decrease was observed in mixed substrates with 75% JS+25% CD than the other substrates.

Table 5. Comparison of total solid and volatile solid before and after AD of the various substrates (values are mean ±SE, n=3)

	Treatments	**Parameters**			
		TS%		**VS%**	
		Initial	Final	Initial	Final
A	100%JS	22.05±0.029^{Aa}	12.44±0.020^{Ab}	84.33±0.024^{Aa}	79.64±0.025^{Ab}
B	75%JS+25%CD	21.82±0.057^{Aa}	9.62±0.014^{Ab}	84.67±0.015^{Aa}	77.53±0.06^{Ab}
C	50%JS+50%CD	21.06±0.017^{Aa}	11.64±0.024^{Ab}	84.06±0.017^{Aa}	79.56±0.012^{Ab}
D	25%JS+75%CD	18.52±0.057^{Aa}	10.06±0.021^{Ab}	84.16±0.017^{Aa}	78.26±0.024^{Ab}
E	100%CD	18.06±0.02^{Aa}	10.06±0.027^{Ab}	83.16±0.04^{Aa}	79±0.015^{Ab}

Mean followed by different capital letters in a column are significantly different at 0.05 level and small letters in row significantly different at P<0.05. JS= *Justicia schimperiana*, CD=Cow dung.

The result also revealed that significant differences were observed between substrates in VS before and after AD. High reduction of VS was measured in75%JS + 25%CD (7.14) mix substrates compared to the rest of substrates after AD (Table5). The TS and VS values before digestion was found to vary significantly (P<0.05) with increasing of JS proportion in the mix, suggesting that mixing helps to adjust the TS and VS. Removal of VS after AD suggests its conversion to biogas. Total solids and volatile solids destruction is a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012).

When determining TS and VS it is important to understand that high content of volatile fatty acids (VFAs) in the substrates can produce misleading results since they might volatilize from the substrate when they are first heated and thus give total solids and volatile solids values that are too low. This in turn can produce incorrect estimate of biogas production, which depends on volatile solids (Annaschnure, 2012).

4.3. Average Daily and cumulative Biogas Production of the Treatments

The biogas from each digester was measured for about four weeks (28 Days) until it stops to produce any more gas. Though there was variation between digesters, gas production was noticed from day one of the experiment in all digesters and peaked between the 6th - 10th days of fermentation. Eventually, gas production went decreasing became zero after the 28th day of AD (Figure. 1). This might be due to the depletion of readily decomposable substrate after the first day (Ahn *et al*., 2009) and/or an increase in ammonium concentration that resulted in an increased pH values (Hansen *et al*., 1998). . Previous studies have revealed that the digestion of more than one kind of substrates could establish positive synergism in the digester (Li *et al*., 2009, Danqi, 2010; Jianzheng *et al*., 2011).

After the 6th -10th day, the Biogas content of the treatment B (75%JS+25%CD) increased and remained in the range of 50ml to 62ml which agrees with the literature value of 50ml to 75ml (EEMBPM, 2002) and 55ml to 88ml (Jemmet, 2006).

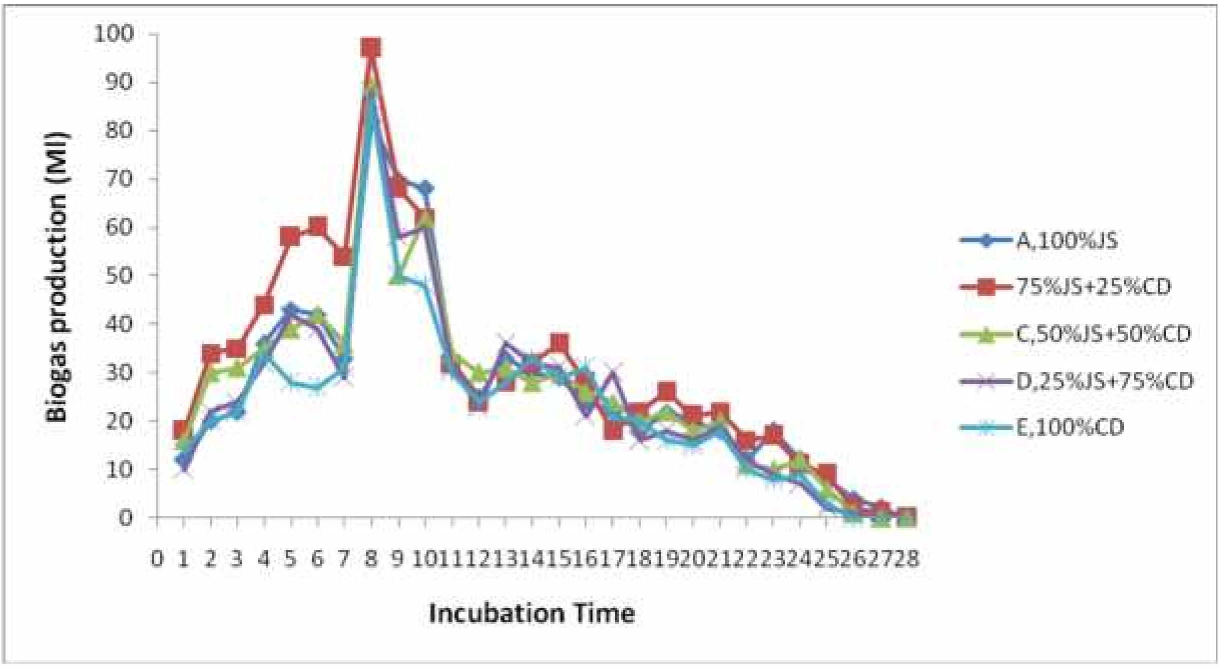

Figure 1Daily mean biogas yield of the different substrate proportion in each treatment

Cumulative biogas yield of the three mixtures of JS and CD was significantly ($p<0.05$) higher (924.11ml) than CD substrate alone (646ml) (Table7). This may be described to the low content of biodegradable material in CD (Hobson, 1981). Cow dung alone yielded less amount of biogas may be due to the partial fermentation it has undergone in the intestinal tract of the animals, and contains less degradable materials mainly composed of structural carbohydrates (Chawola, 1996, Deblein and Steinhauser, 2008).

Table 6. Total Biogas production from five treatments

Treatment	Mix ratio	Total Biogas (ml)
A	100%JS	696.32^{Aa}
B	75%JS+25%CD	924.11^{Aa}
C	50%JS+50%CD	764.24^{Aa}
D	25%JS+75%CD	718.87^{Aa}
E	100%CD	648.79^{Aa}

Table 7. It is indicate the cumulative biogas production in different five treatment proportions .each treatments are significantly different at 5% levels. A=*Justicia schimperiana*, B=*Justicia schimperiana*: Cow dung, C=*Justicia schimperiana*: Cow dung, D=*Justicia schimperiana* : Cow dung, E=Cow dung.

There was a significant difference between the substrates in an overall biogas yield (Table.7, $p<0.05$). Compared to CD alone all substrate types resulted in significantly higher cumulative biogas yield with the highest cumulative biogas production observed in 75%JS+75%CD (924.11ml) mix substrate. This might be due to the favorable situation of in 75%JS+25%CD mix to microorganisms as compared to the other substrate mixtures. As the proportion of JS in the mix ratio increased with in 75%JS : 25%CD, the cumulative biogas yield increased (Table 6), suggesting high favorable situation with increasing JS proportion from that of 75%. This observation was in accordance with the results of an experiment done by (Callaghan *et al.*,1999). It can be concluded that co-digestion of JS and CD was more productive with JS proportion of 75%. The higher production from the mixtures could be due to a proper nutrient balance, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of the substrates (Li *et al.*,2009).

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary and Conclusion

Anaerobic digestion tests were carried out to obtain suitable mix ratio for maximum biogas production from co-digestion of JS with CD at 5 different treatments with different ratio. The experiment was carried in 0.5L test batch digester under mesophilic condition (38^0C) at 28 hydraulic retention time. The maximum biogas was produced in a combination of cow dung to *Justicia schimperiana* at the ratio of 75%:25% (924.11ml). Was selected based on high VS, TS and C% reduction. The Volatile solid content of the *Justicia schimperiana* substrate was 89.3% of the TS**.** This shows that a large fraction of the *Justicia schimperiana* is biodegradable. This implies that *Justicia schimperiana* can serve as an important feedstock for biogas production.

Biogas production from 100% CD, 75% JS : 25% CD, 50% JS : 50% CD, 25% JS: 75% CD and 100%CD were statistically significant at 0.05 significance level. Cumulative Biogas production of 75% JS: 25% CD, 33.0% higher , Medium in 50%JS: 50%CD, 27.2% and low in 100% CD 23.17%. Therefore, Co-digestion of cow dung and *Justicia schimperiana* biomass is one way of addressing the problem of lack of enough feedstock for biogas production. If suitable materials for co-digestion, such as manure, are not available, *Justicia schimperiana* can be digested alone and is a good opportunity for poor people who have not livestock as a source of Energy. Environmental, slurry and foreign currency benefit can be obtained from biogas production.

5.2. Recommendation

Based on the findings the following recommendations are forwarded.

From this study, *Justicia schimperiana* leave was found to be potential for generating biogas when mixed with animal excreta. Therefore, the following suggestions should be considered in the future to maximize the yield from *Justicia schimperiana* leaves.

- Efforts should also be made to measure the methane quality of different combinations by Gas chromatography too.
- Experiment to determine Carbon: Nitrogen ratio should be carried out for further analysis.
- This investigation was done at mesophilic temperature (38^0C), but it should also be carried out at room temperature (20^0C) and at thermophilic condition (55^0C).

- Awareness rising on the overall use of *Justicia schimperiana* should be carried out to those who cultivate the plant as they concentrate only on the use of the leaves for food consumption.
- The research result revealed that it is possible to generate biogas from *Justicia schimperiana*. Therefore, researcher recommended that *Justicia schimperiana* should be used either solo or in a mix ratio of 75%JS with 25% CD, and 50%JS with 50%CD in large scale production of biogas in further study.

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7. APPENDIX.

Appendix 1. Daily Biogas production in ml (values are mean ±SE, n=3).

Number	A,100%JS	B,75%JS+25%CD	C,50%JS+50%CD	D,25%JS+75%CD	E,100%CD
1	12.65±0.43	18.60±1.64	16.72±1.34	10.12±2.00	14.16±1.74
2	20.33±2.08	34.12±8.55	30.18±2.00	22.60±4.00	20.69±8.12
3	22.15±2.54	35.25±4.20	31.24±1.55	24.62±2.34	22.75±2.50
4	28.62±11.54	44.00±11.24	35.12±2.2	32.05±8.00	34.17±1.53
5	32.67±10.50	54.16±10.87	39.22±5.58	42.80±16.68	28.11±15.50
6	42.50±2.20	60.24±12.22	42.26±19.53	39.12±8.42	27.24±12.47
7	33.62±2.52	54.65±9.32	36.12±7.54	29.62±15.17	31.18±6.52
8	82.20±2.54	97.72±1.84	89.12±5.56	88.62±4.64	86.11±2.80
9	50.30±1.00	68.62±4.00	50.60±10.33	58.15±6.45	50.16±1.34
10	52.64±3.40	62.92±7.50	62.12±3.2	60.44±10.33	48.26±4.20
11	32.60±11.42	32.70±5.78	34.16±3.84	31.61±5.78	30.15±13..24
12	25.46±1.37	24.28±3.00	30.18±2.88	23.50±0.01	24.22±0.57
13	18.12±1.45	28.12±3.65	31.60±0.60	36.12±1.15	28.52±1.32
14	30.62±2.88	32.60±0.55	28.31±1.66	32.12±2.02	33.10±1.57
15	29.30±0.57	36.90±2.02	30.12±2.60	31.05±4.94	29.20±1.33
16	20.12±1.76	28.61±1.73	26.30±0.57	21.10±1.33	31.18±1.00
17	22.72±0.57	28.52±1.73	24.15±2.73	30.18±3.18	21.20±3.78
18	18.15±0.33	22.16±1.00	20.60±3.72	16.33±0.57	20.42±1.14
19	22.62±0.66	26.26±0.88	22.34±0.57	18.60±0.66	16.20±0.57
20	20.24±0.57	21.34±0.82	18.15±0.60	16.19±0.74	15.20±0.66
21	18.15±4.60	22.62±2.00	20.22±5.00	19.15±2.34	18.24±2.20
22	12.62±0.58	16.72±2.40	11.31±1.50	12.24±2.50	10.35±1.34
23	18.70±1.62	17.32±2.06	10.29±3.20	9.12±2.52	8.10±1.64
24	12.42±0.4	11.60±1.64	12.11±1.34	7.22±2.00	9.30±0.23
25	8.60±10.00	9.25±4.4	6.20±4.6	4.10±1.33	3.62±0.57
26	5.20±1.52	3.62±3.20	2.70±1.65	2.10±0.60	2.16±1.00
27	2.00±0.60	2.06±1.00	1.00±0.20	1.00±0.00	0.00±0.00
28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

8. ANNEX

Figure 2 Batch form of experimental set up

A

B

Figure 4.Fresh cow dung (A) and *Justicia schimperiana* leaves (B) collected from Haramaya University animal farm and Haramaya town respectively