EVALUATION OF BIOGAS PRODUCTION FROM *PROSOPIS JULIFLORA* LEAVES CO- DIGESTED WITH COW MANURE

M.Sc. THESIS

MEKIYA TESFAYE NEGASA

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MEKIYA TESFAYE NEGASA

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POSTGRADUATE PROGRAM DIRECTORATE

We hereby certify that we have read and evaluated this thesis entitled “**Biogas Production from *Prosopis juliflora* Leaves Co-Digested with Cow Manure**” prepared under our guidance by Mekiya Tesfaye Negasa. We recommend that the thesis be accepted as it fulfills the requirements.

Meseret Chimdessa (Ph.D) __________________ ______________
Associate Professor

Major Advisor Signature Date

Ameha Kebede (Ph.D) ________________
____

Co-Advisor Signature Date

As member of the board of examiners of the M.Sc. Thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Mekiya Tesfaye Negasa and examined the Candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Applied Biology.

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DEDICATION

This thesis is dedicated to my beloved parents.

STATEMENT OF THE AUTHOR

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

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Name: Mekiya Tesfaye Negasa Signature ___________

Place:_Haramaya University, Haramaya

Date of submission: ___________

BIOGRAPHICAL SKETCH

The author, Mekiya Tesfaye Negasa, was born in Woliso *Woreda*, Mangura Dildila *Keble*, South West Showa Zone of Oromia Regional State in 1993 G.C. She attended her elementary school at Dajabalcha Abba Nabso Elementary School. She attended her secondary and preparatory school education at Gerasu Dhuki Secondary and Preparatory School. After completing her Secondary and preparatory school, in 2012, she joined Jimma University and graduated with the degree of Bachelor of Science in Biology in June 2014. After graduation, she joined the Haramaya University Postgraduate Program Directorate in 2015 as a regular student to pursue a study leading to the degree of Master of Science in Applied Biology.

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LIST OF ABBREVIATIONS AND ACRONYMS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
C/N	Carbon to Nitrogen Ratio
CM	Cow Manure
GHG	Green House Gas
HRT	Hydraulic Retention Time
MoE	Ministry of Education
NBP	National Biogas Program
OLR	Organic Loading Rate
RF	Rumen Fluid
SPSS	Statistical Package for the Social Sciences
SRT	Solid Retention Time
TS	Total Solids

VFA	Volatile Fatty Acids
VS	Volatile Solid
CSA	Child Support Agency
EPA	Environment Protection Agency

TABLE OF CONTENTS

Continues…

Continues…

LIST OF TABLES

Table
Page

LIST OF FIGURES

LIST OF TABLES IN THE APPENDIX

Appendix Table

Page

LIST OF FIGURES IN THE APPENDIX

Appendix Figure

Page

Biogas Production from *Prosopis juliflora* Leaves Co-digested with Cow Manure

ABSTRACT

Biogas is eco-friendly alternative renewable energy source produced through anaerobic digestion of organic compounds. In this study, biogas production was evaluated from Prosopis juliflora leaves(PJ) and cow manure (CM) co-digestion in five mix ratios under mesophilic conditions (38ºC) using batch digesters in the Botany Laboratory of Haramaya University to evaluate biogas production potential of Prosopis juliflora leaves through anaerobic digestion alone or in combination with cow manure. In 100%PJ, 100% CM, 75%PJ and 25% CM, 50%PJ and 50%CM, and 25%PJ and 75%CM treatments, total solids%, volatile solids%, organic carbon%, moisture content% and pH were measured before and after digestion while carbon to nitrogen ratio was measured before anaerobic digestion only. The daily biogas production was measured by water displacement method. All measured physico-chemical parameters of each substrate showed significant variations.The maximum (81. 6±0.8) and minimum (78.6±0.3), MC% before AD was measured in 50% PJ and 50% CM) and 100% CM respectively.While after AD the maximum (91.1±0.55) MC% was measured in 75%PJ and 25%CM and the minimum (82.2±0.8) MC% was measured in 25% PJ and 75% CM. Likewise, the maximum (44.5±0.42) and minimum (34±0.3) C% before AD was measured in100% PJ, and 100% CM respectively. After AD degradation of organic carbon was highest (19.2 C%) for 75%PJ and 25%CM. The maximum (7.2±0.01) and minimum (6.05±0.01) pH before AD was measured in 100% CM and 100% PJ respectively. After anaerobic digestion, TS% and VS% of all treatments were significantly lower (66.6, 50.9, 64.2, 64.8 and 55.3 and 2.5, 1.2, 34.4, 1.26 and 2) than those of the treatments before AD. In this experiment, the carbon to nitrogen ratio of treatments T1, T2, T3, T4 and T5 before AD were 23.9±00, 22.6±01, 22.7±0.3, 23.75±0.2 and 22.46±00 respectively. Biogas production was detected in both substrate and substrate mix ratios from the second day of digestion and declined to zero in about 28 days of incubation. The maximum (506 ml) cumulative biogas was measured in 25%CM and 75%PJ while the minimum (272 ml) was measured in100% CM. Assessment of cumulative biogas production revealed that the substrate mix ratio of 75% Prosopis juliflora and 25% cow manure was superior to others (506 ml). 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 Prosopis juliflora is co-digested with cow manure in 75%:25% mix ratio.

***Keywords**: Cumulative biogas production, Methane, Rumen fluid, Prosopis juliflora, Substrate mix ratio*

1. INTRODUCTION

Energy is one of the most important factors to global prosperity. In today's energy demanding lifestyle, the need for exploring and exploiting new sources of energy which are renewable, sustainable as well as eco-friendly is demanding. The over-dependence on fossil fuels as primary energy source has led to global climate change, environmental pollution and degradation, thus leading to human health problems. In the year 2040, the world as predicted will have 9 to 10 billion people, which must be provided with energy and materials (Alemayehu, 2015).

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

Access to modern energy services remains an issue for poor people in developing countries. In Ethiopia about 83% of the population does not have access to electricity and 93% uses biomass-based energy for cooking, which surpasses 99% in rural areas Firewood is the main fuel followed by crop residues and cows dung collected from common resource pools or own resources. This situation is typical for many regions in Sub-Sahara Africa. Assessments conducted on availability of wood fuel in the 1970s and 1980s have been published with controversial results ignoring the actual supply and demand gaps The biomass comes from rural resources through purchasing creating a link between urban and rural. For instance, about 84%

of the urban population in Ethiopia uses energy from biomass for cooking, about 45% uses purchased firewood. This indicates a heavy dependence of both rural and urban on common resource pools for both fuel and income. Therefore, common resource pools became scarce and people travel long distances to collect firewood. Ethiopia has a population of about 90 million, of which more than 70 million live in rural areas (Gudina Terefe and Sanderine, N, 2015).
To overcome these problems, alternative energy sources have recently become more and more attractive due to the increasing demand for energy, the limited resource for buying fossil fuel, the environmental concerns, and the strategy to survive post-fossil fuel economy era (Siltan, 2000). Biogas production is one of the technologies that can be used to overcome the problems relating to energy crisis. It is a modern and environmentally friendly technology that is based on the decomposition of organic materials in anaerobic environment at suitable and stable temperature by a mixture of bacterial and archaeal species. Anaerobic digestion consists of several interdependent, complex sequential and parallel biological reactions in the absence of oxygen during which the products from one group of microorganisms serve as the substrates for the next, resulting in transformation of organic matter (biomass) mainly into a mixture of methane and carbon dioxide commonly referred to as biogas (Werner *et al.* ,2007).

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

High levels of methane are produced when manure is stored under anaerobic conditions. During storage and when manure has been applied to the land, nitrous oxide is also produced as a byproduct of the denitrification process. Nitrous oxide (N_2O) is 320 times more aggressive as a greenhouse gas than carbon dioxide and methane 25 times more than carbon dioxide (EPA, 2015). According to Texas (2009), by converting cow manure into methane biogas via anaerobic digestion, the millions of cattle in the United States would be able to produce 100 billion kilowatt hours of electricity, enough to power millions of homes across the United States. In fact, one cow can produce enough manure in one day to generate 3 kilowatt hours of electricity; only 2.4 kilowatt hours of electricity are needed to power a single 100-watt light bulb for one day. Furthermore, by converting cattle manure into methane biogas instead of letting it decompose, green house gases could be reduced by 99 million metric tons or 4% (Webber *et al*., 2008).

In Ethiopia, there are about 22 invasive alien species of plants (McGinley, 2007). Among these invasive alien species, mesquites (*Prosopis juliflora),* parthenium weed (*Parthenium hysterophorus*), water hyacinth (*Eichhornia crassipes*), *Lantana camara,* and *Acacia* species are causing major problems in the country. *Prosopis juliflora* has been identified as Ethiopia's number one priority invasive plant. This plant has got different name in Ethiopia, 'Weyane/Dergi Hara'(Afar), Biscuit'(Dire-Dawa),elsewhere; mesquite, *algarrobo*, *Prosopis.* They came originally from the Americas, there are many species, often confused, but work by the Ethiopian Agricultural Research Organisation (EARO) and Henry Doubleday Research Assosiation (HDRA) has confirmed that *Prosopis juliflora* is the one commonly found in the above mentioned regions of Ethiopia. Its first introduction is believed to have been in the late 1970s at Goro nursery, Dire-Dawa, posibly from India. In Afar, it may have been introduced posibily from Dire-Dawa or independntly from Kenya or Sudan by foreiners working in the middle Awash irrigation project in the late 1970s and early 1980s *Prosopis juliflora* was planted overlarge areas until 1982, continued by the food for work programme from 1986 to1988. Invading *Prosopis juliflora* tends to form dense,impenetrable thickets, associated with unfavorable impacts on human economic activities. In addition, it suppresses the growth of other plant species by denying the plants the most valuable growth factors, light and water (Demise Sertse, 2005).

It was observed that the species has been increasing in density as well as area coverage from year to year and even from month to month. Currently, this noxious tree heavily infests most agricultural as well as potential range lands in our country (Abiyot and Getachew, 2006).

El-Keblawy (2002) indicated that *P. juliflora* shows a great depressive effect on the number, density, and frequency of native vegetations. *P. juliflora* has two main ecological opportunity behaviors: seed dormancy (Hailu *et al*., 2004) and allelophatic effects (Warrage and Al-Humaid, 1998). Warrage and Al-Humaid (1998) reported that *P. juliflora* plants possess allelochemicals that inhibit germination, growth and survival of other species. El-Keblawy and Al-Rawai (2006) also explained that the density of *P. juliflora* seedlings is greater underneath the canopy of the same species than away from them. This indicates that the plant has little or no auto-inhibition effect under field conditions. Removal of *P. juliflora* enhances diversity of other species.

The energy use pattern in rural Ethiopia will bring negative environmental, economic and health impacts. The use of fuel wood and charcoal leads to deforestation, forest degradation, erosion, loss of biodiversity and other environmental problems (Cooke, 2008). In addition, use of crop residues and animal dung when lacking wood fuels deprives the land of essential nutrients, resulting in loss of soil fertility and agricultural output (Gebreegziabher, 2007). Furthermore, smoke from the use of traditional fuels causes indoor air pollution which is damaging to human health, particularly respiratory systems (Duflo, 2008). Apart from this, fossil fuels, which are not renewable, will diminish in availability globally, putting high cost on our foreign exchange in the future (Aklilu, 2008). To solve these problems, it is appropriate to evaluate the potential of biogas production from different plant material and animal wastes. In this regard, biogas production from different organic materials like *Moringa stenopetala* seed cake powder (Eyasu, 2008), poultry litter (Ebrahim, 2006), *Khat* (*Catha edulis*) waste (Tesfaye, 2007) and others were performed in Ethiopia.

In recent decades *P. juliflora* has attracted much attention because of its ability to survive in extremely arid, saline, inhospitable locations and produce excellent firewood, charcoal, and animal fodder. The combination of its long life cycle, ability to survive droughts, high seed production and dormancy of seeds make *P. juliflora* an extremely resilient invader which can quickly take advantage of suitable environments and dominate entire ecosystems. The leaves of *Prosopis juliflora* can be used to produce sufficient amount of biogas through fermentation when supplemented with cow manure (Kathiresan, undated). These invasive species could be controlled or maintained by utilization in biogas production. However, no research has been done so far on biogas production potential of *Prosopis juliflora* in solo or in combination with cow manure. This study was, therefore, designed to evaluate biogas production potential of *Prosopis juliflora* leaves in solo or in combination with cow manure.

Hence the general objective of this study was to:

- evaluate biogas production potential of *Prosopis juliflora* leaves through anaerobic digestion alone or in combination with cow manure.

The specific objectives were to**:**

- characterize *Prosopis juliflora* leaves 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.
- compare the biogas yield from batch fermentation of solo and mixed substrates of *Prosopis juliflora* leaves and cow manure.
- determine the average and daily cumulative biogas production from solo and mixture of *Prosopis juliflora* leaves and cow manure combined in different proportions.

2. LITERATURE REVIEW

2.1. Energy

Our economy depends on the availability of energy most of which has come from the combustion of fossil fuels. Fossil fuels include coal, natural gas, and a variety of liquid fuels, such as gasoline, diesel fuel, and heating oil are derived from petroleum. These are not renewable (at some point they will become depleted) and increasingly costly. Unlike fossil fuel combustion, renewable energy sources provide energy without depleting fossil fuel reserves and with much lower overall carbon dioxide emissions (Smil and Vaclav, 2003).

2.2. Energy from Biomass

The world's energy markets rely heavily on the fossil fuels coal, petroleum crude oil, and natural gas as sources of energy, fuels and chemicals. Biomass is the only other naturally occurring, energy-containing carbon resource known that is large enough to be used as a substitute for fossil fuels. Biomass resources include any organic matter available on a renewable basis, including dedicated energy crops and trees, agricultural food and feed crops, agricultural crop wastes and residues, wood wastes and residues, aquatic plants, animal wastes, municipal wastes, and other waste materials. Energy from biomass (organic matter) is the sun's energy stored through photosynthesis. That energy is released when biomass is used. The technologies of releasing this energy include a variety of thermal and thermo chemical processes for converting biomass by combustion, carbonization, gasification and liquefaction, physical-chemical processes to convert biomass to oil, and the microbial conversion of biomass to obtain gaseous and liquid fuels by fermentative methods (i.e., biomass can substitute all forms of energies; solid, liquid and gas)(Green, 1987).

Solid biomass can be burned directly or after carbonization to produce energy (e.g., heat and steam for electricity production). Biomass can also be used to produce energy in the form of alternative transportation fuels. The two most common bio-fuels (liquid) are Ethanol and Biodiesel. Even gas can be produced from biomass for generating energy. Through the gasification process, biomass can be converted into a combustible gas (producer gas) which can be burned directly for space heat or drying, or it can be burned in a boiler to produce steam. The degradation of biomass in an oxygen-free environment (anaerobic fermentation) also produces a gas (biogas) that can be burned or combusted to produce electricity. All sources of biomass (except lignin) are suited for anaerobic fermentation, but produce different biogas yields depending on their properties (Amon, 2001). Biogas is a colorless, flammable gas produced via anaerobic digestion of animal, plant, human, industrial and municipal wastes amongst others, to give mainly methane (50-70%), carbon dioxide (20-40%) and traces of other gases such as nitrogen, hydrogen, ammonia, hydrogen sulphide, water vapor etc. It is smokeless, hygienic and more convenient to use than other solid fuels (Gerardi, 2003).

2.3. Basics of Biogas Production

Biogas is a mixture of mainly methane gas (CH_4) and carbon dioxide gas (CO_2). Natural gas is about 90-95% methane, but biogas is about 50-65% methane. So biogas is basically low grade natural gas. Biogas is produced when bacteria convert organic matter to methane gas. This process is similar to what takes place in the rumen of a cow, so we often hear biogas plants referred to as anaerobic digesters, or anaerobic fermenters. Four ingredients are needed for biogas production: Organic Matter, Bacteria, Anaerobic Conditions and Heat

Organic matter is the food source for methane producing bacteria. The primary organic matter source for farm-based biogas production is manure. Biogas can be produced using manure as the only organic source, but the gas production can be greatly increased by adding certain types of food wastes with the manure. Energy crops such as corn silage can also be added to increase gas production (Debruyn and Hilborn, 2006).

The second ingredient that is necessary for biogas production is bacteria. Bacteria are necessary to convert the fats, carbohydrates and proteins in the organic matter to simple acids such as acetic and propionic acid. Then, second types of bacteria transform the acids to methane and carbon dioxide. This process takes place simultaneously. The bacteria are commonly present in manure, and under the right conditions they thrive and multiply (Debruyn and Hilborn, 2006).

Two conditions that are necessary for the bacteria are an anaerobic atmosphere (no oxygen) and the right temperature. Most digesters operate in the mesophylic range of 35-40^{o}C, but others are designed to operate in the thermophylic range of 50-60^{o}C, and a few are designed to operate at 15-25^{o}C or the psychrophylic range. Biogas contains moisture and hydrogen sulphide, so before it is used in an engine the moisture must be condensed out, and the hydrogen sulphide removed to reduce maintenance problems. Biogas can be used directly to produce thermal energy, or it can be used to power a gas or diesel engine to run a generator to produce electrical energy (Hilborn, 2006).

2.4. Substrates for Biogas Production

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

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

Anaerobic digestion is a naturally occurring process where biodegradable matter is converted into biogas and 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. It is a series of processes involving micro-organisms to break down biodegradable material successively in a multistep process and parallel reactions (Satoto, 2010).

Related to raw material for biogas production, there is a wide range of raw materials that can be used such as organic residues from agriculture (crop residues), waste from animals (manure), municipal organic waste, industrial waste, sewage sludge, byproducts from production of bio ethanol and biodiesel, energy crops and algae etc. The production technology as well as energy efficiency varies substantially depending on type of feedstock used. Compared to aerobic digestion, anaerobic digestion is a cheaper process, since no need of oxygen supply and less sludge production that needs to be treated further (Lantz *et al.,* 2007). Anaerobic digestion has been considered as waste-to-energy technology, and is widely used in the treatment of different organic wastes, for example: organic fraction of municipal solid waste, sewage sludge, food waste and cow dung (Li et al., 2009). Cow dung has high nitrogen content and due to pre-fermentation in the stomach of ruminant, and has been observed to be most suitable material for high yield of biogas through the study made over the years (Chonkor, 1983).

2.4.1. Cow Manure for Biogas Production

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

2.5. Biogas as Energy Source

Biogas is the mixture of gases, mainly methane (CH_4) and carbon dioxide (CO_2) resulting from the anaerobic fermentation of organic matter. It contains 50-70 % methane and can be used as a fuel for heating or electrical power generation. Production of biogas from agricultural plants offers several environmental benefits including production of renewable energy (CO_2-neutral), and the nutrient rich stabilized liquor (NH_4) is directly available for plants. Further, anaerobic digestion may also assist in reducing and destroying pathogens to acceptable levels, reducing greenhouse gas emissions, and aid in reducing odors often associated with storing and handling liquid wastes. Biogas production, when compared to other biomass energies, has the advantage that it can be produced from specially grown energy crops as well as from organic waste products (Constant, 1989).

2.6. History of Biogas Technology

2.6.1. Biogas Technology in the World

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

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

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

2.6.2. Biogas Technology in Africa

As compared with other world countries, African countries biogas technology shows some limitations. The reasons for these failures are associated with poor design and construction of digesters, wrong operation and lack of maintenance by users, poor dissemination strategies, lack of project monitoring and follow-ups by promoters, and poor ownership responsibility by users. Despite the relative stagnation of biogas programs in Africa, the future prospects are encouraging. Several biogas plants in recent years have been constructed for energy (cooking, lightning and fuel replacement), and for sustainable environmental system in several countries of Africa including Ghana, Kenya, Tanzania, Rwanda, Burundi, and South Africa (Amigun and Blottnitz, 2007). Between 4000 and 5000 digesters are estimated in Tanzania (Marree *et al.,* 2007), while Kenya is said to have disseminated about 2000 digesters as at October 2007. In Ghana, about 200 digesters have been disseminated (Bensah, 2009).

2.6.3. Biogas Technology in Ethiopia

Biogas technology was introduced in Ethiopia in 1979. 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.,*2006b). As (NBP, 2007) reported, around 1000 biogas plants were constructed in various parts of the country. Approximately 40% of these plants are not operational due to lack of effective management and follow-up, technical problems, loss of interest, evacuation of ownership and water problems. Other reasons for the limited success of the technology in Ethiopia include the adoption of a project-based stand-alone approach without follow-up structure in place, variations in design, and the absence of a standardized biogas technology (NBP, 2007).

2.7. Steps Involved in Biogas Production

2.7.1. Hydrolysis

Hydrolysis is the first stage of the organic waste decomposition process involving the breakdown of large organic polymer chains into smaller molecules such as simple sugars, amino acids and fatty acids. Saccharolytic and proteolytic micro-organisms break down sugars and proteins, respectively (Edison, 2014). Proteins, simple sugars and starch hydrolyse easily under anaerobic conditions. Normally the decomposition of organic matter to CH_4 and CO_2 is not absolute and is frequently only about 30 to 60% for animal manure and other substrates that have a high concentration of complex molecules (Peter, 2009).

Different specialized bacteria produce a number of specific enzymes that catalyze the decomposition. The process is extracellular as it takes place outside the bacterial cell in the surrounding liquid (Peter, 2009).

The hydrolysis is often reported as rate limiting in digestion of complex polymers in balanced anaerobic digestion systems, while the methanogenesis is regarded as rate-limiting for more easily degraded substrates. Protein and carbohydrate degrading bacteria grow rapidly, and these kinds of substrates are rapidly fermented, with a retention time of less than a day (Murto *et al.*, 2004).

If the substrate is easily hydrolyzed, the last degradation step is often rate limiting since methanogens grow more slowly than the acidogens upstream in the degradation chain. This is due to a build-up of the metabolic intermediates, mainly volatile fatty acids (VFAs). The acid consuming methanogenic species are more inhibited by a decrease in pH than the acid producing species. This causes further acid accumulation and eventually leads to process failure (Murto *et al.*, 2004).

2.7.2. Acidogenesis

Fermentative bacteria or acidogenic produce an acidic environment in the digestion reactor while creating hydrogen (H_2), carbon dioxide (CO_2), shorter volatile fatty acids (VFA), and organic acids like acetic, propionic, butyric, succinic, lactic acid as well as low alcohols. In a balanced bacterial process approximately 50% of the monomers like glucose, xylose, amino acids and long chain fatty acids (LCFA) are broken down to acetic acid (CH_3COOH). Further 20% is converted to CO_2 and H_2, while the remaining 30% is broken down into short chain volatile fatty acids. If there is an imbalance, the relative level of VFAs will increase with the risk of accumulation and the process "turning sour" because the VFA degrading bacteria have a slow growth rate and cannot keep up. A steady degradation of VFAs is therefore crucial and often a limiting factor for the biogas process (Peter, 2009). However; these resulting organic matter is still very large and unsuitable for methane production (Edison, 2014). They need extra steps for further decomposition. Moreover, at this stage semi-harmful contaminants such as hydrogen sulfide and ammonia are produced in much smaller amounts; <1 % by volume (CIWMB, 2008).

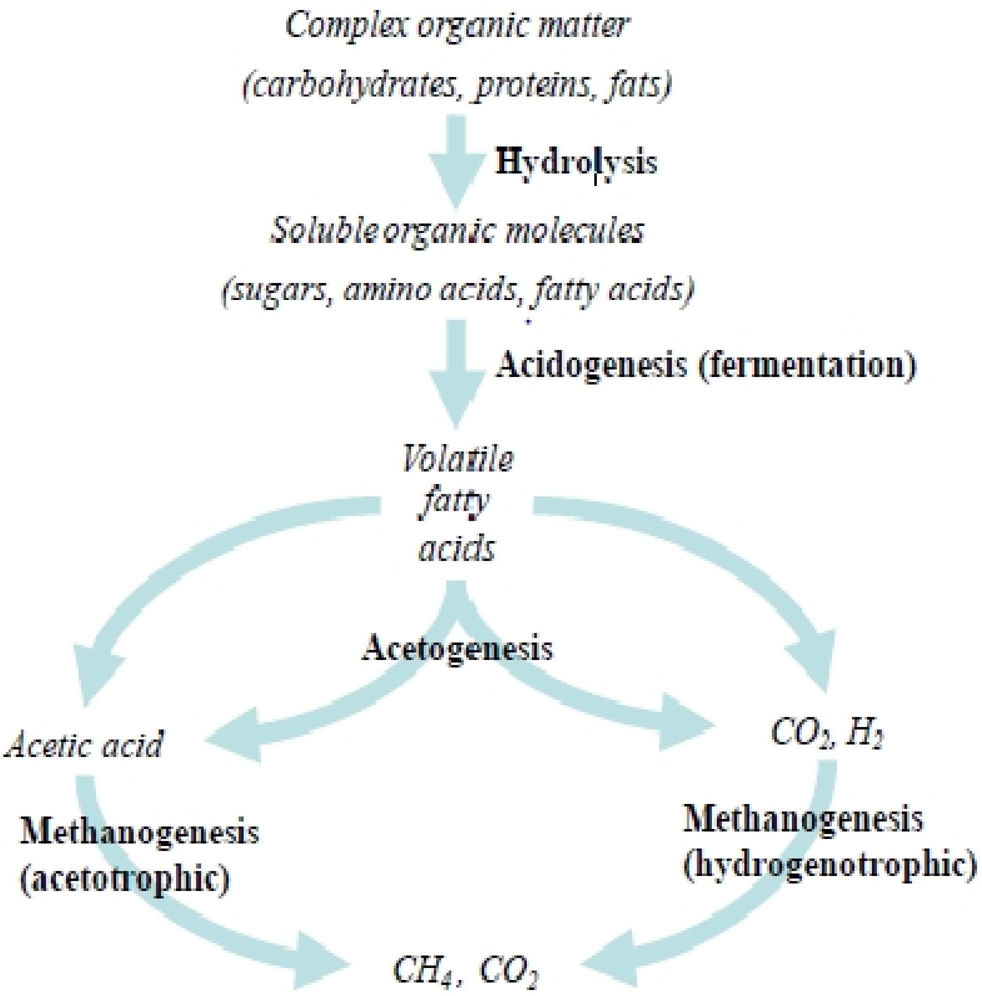


Figure 1: Anaerobic digestion process (Alemu Gizaw, 2016)

2.7.3. Acetogenesis

The third stage of anaerobic digestion phase is acetogenesis. In this process simple molecules which are formed through the acidogenesis phase are further digested by acetogens to produce largely acetic acid, carbon dioxide and hydrogen. Acetogenic organisms are the vital link between hydrolysis/acidogenesis and the methanogenesis in anaerobic digestion. Acetogenesis provides two main substrates for the last step namely hydrogen and acetate. Both the acidogenesis 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 for the disposal of electrons. The acetogenesis is an obligate proton-reducer and can utilize only protons as electron acceptors and only when the H_2 concentration is low. At very low H_2 concentrations, however, methanogenesis from H_2 and CO_2 becomes unfavorable. The chemical reaction is, $2CO_2 + 4H_2 \leftrightarrow CH_3COOH + 2H_2O$ (Elias, 2010).

2.7.4. Methanogenesis

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

The primary route of methane produced (two-thirds of methane gas) 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 Methanothrix. During the thermophilic digestion of lignocellulosic wastes, Methanosarcina species are the dominant acetoclastic bacteria encountered in the bioreactor (Zaher *et al.*, 2007). The remaining methane is generated from H_2 and CO_2 by the hydrogenotrophic methanogens. Mackie and Bryant (1981) reported that acetate synthesis through this pathway accounts for only 1-2% of the total acetate formation at 40°C and 3-4% total solids at 60°C in a cattle waste digester. The chemical reactions of this process are the following.

$$CH_3COOH \leftrightarrow CH_4 + CO_2$$

$$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O$$

2.8. Types of Anaerobic Digestion System

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

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

2.8.1. Continuous versus Batch Process

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

In contrast, in batch digestion all the material is digested at once and the material remains undisturbed in the same place throughout the entire digestion process. No new material is added nor is any digestion residue removed during the process. Methane production is generally highest at the beginning and then decreases over time. When the material is digested, the entire container is emptied of its contents and a new batch of substrate is added. An example of batch digestion, when waste is treated in the same place for a long time, is in landfills. Batch digestion is also common in connection with biogas production for individual households, which is particularly common in Asian countries. Batch digestion is advantageous from a microbiological point of view because the organisms have plenty of time to break down the organic matter. Also, the organisms do not get washed out of the system. However, sometimes it can difficult to achieve a high and even digestion rate, particularly if the substrate has a high content of dry solids (Bjorsson and Bergland 2006; Nordberg, 2007).

2.8.2. Mesophilic versus Thermophilic

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

2.8.3. Single Stage versus Two Stage Digestion

The simplest model for biogas production is to use a single digestion tank for the entire process, so-called one step digestion. With one step digestion, all stages in the microbial breakdown process, i.e. hydrolysis, fermentation, anaerobic oxidation and methane production take place at the same time and in the same place. It is common for one step digestion to take place in total mixed process. A common type of biogas reactor is the Continuously Stirred Tank Reactor (CSTR). The substrate is completely mixed by various mixers. It is often used in one stage process for treating sludge, food waste, manure, etc. sometimes some of the residues/process liquids are returned to the process. This increases the retention time of material and helps more microorganisms to remain in the process (Nordberg *et al*., 2007).

An alternative to a single-stage process is to divide the process into two parts, called two-stage digestion. In two stage digestion, the first step is to load raw material into a digestion tank where the process is focused on hydrolysis and fermentation. This primarily results in acid formation. But a certain amount of biogas is normally also produced, because it is difficult to completely divide the process. Then, the digestate or the process liquid from this process is separated and added to another digestion tank that is specially adapted for methanogenesis (Mshandete *et al*., 2008). This type of process may be appropriate when a substrate contains material that is easy to break down and the hydrolysis stage is fast (Bjornsson, 2002).

2.9. Factors Affecting Biogas Production

The performance of biogas plant can be controlled by studying and monitoring the variation in parameters such as pH, temperature, loading rate, agitation, etc. Any drastic change in these can adversely affect the biogas production (Dioha *et al*., 2013).

2.9.1. Temperature

Temperature inside the digester has a major effect on the biogas production process. There are different temperature ranges during which anaerobic fermentation can be carried out: psychrophilic (<30^{o}C), mesophilic (30–40^{o}C) and thermophilic (50–60^{o}C). However, anaerobes are most active in the mesophilic and thermophilic temperature range. The length of fermentation period is dependent on temperature (Yadvika *et al.,* 2004). The methanogenic bacteria, which facilitate the formation of biogas, are very sensitive to temperature changes and the optimum temperature for the bacteria to operate is between33-38^{0}C.Temperatures below this slow down the biogas production process, while a higher temperature than necessary kills the biogas producing bacteria. This is why the structure for biogas production is generally built underground to keep the temperature as constant as possible (Sibisi and Green, 2005).

2.9.2 .pH

The pH is another important parameter affecting the growth of microbes during anaerobic fermentation. PH of the digester should be kept within a desired range of 6.8–7.2 by feeding it at an optimum loading rate. The amount of carbon dioxide and volatile fatty acids produced during the anaerobic process affects the pH of the digester contents. For an anaerobic fermentation to precede normally, concentration of volatile fatty acids, acetic acid in particular should be below 2000 mg/l (Yadvika *et al.,* 2004). pH values below 6.0-6.5 inhibits the methane bacteria activity. To avoid drops in pH chemicals are added to the organic substrate to supply a buffer capacity. Sodium bicarbonate, sodium hydroxide, sodium carbonate and sodium sulfide are the most used chemicals (Esposito *et al.,* 2012).

Process instability due to ammonia often results in volatile fatty acids (VFAs) accumulation, which leads to decrease in pH and thereby declining concentration of FA. The interaction between FA, VFAs and pH may lead to an ‘‘inhibited steady state’’, a condition where the process is running stably but with a lower methane yield. Control of pH within the growth optimum of microorganisms may reduce ammonia toxicity. Reducing pH from 7.5 to 7.0 during thermophilic anaerobic digestion of cow manure also increased the methane production by four fold. It should also be noted that both methanogenic and acidogenic microorganisms have their optimal pH. Failure to maintain pH within an appropriate range could cause reactor failure although ammonia is at a safe level (Chen *et al.,* 2008).

2.9.3. Moisture

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. However, bioreactors under the 70% moisture regime produced a stronger leachate and consequently a higher methane production rate. At the end of the experiment, 83 ml methane per gram dry matter were produced at the 70% moisture level, while 71 ml methane per gram dry matter were produced with the 80% moisture (Khalid *et al.,* 2011).

2.9.4. Retention Time

The hydraulic retention time (HRT) is the theoretical time that the influent liquid phase stays in the digester, while the solids retention time (SRT) is generally the ratio between solids maintained in the digester and solids wasted in the effluent. The required preservation time for completion of the AD reactions varies with differing technologies, process temperature, and waste composition (Zamudio, 2010).

The conversion of organic matter to gas is more closely related to SRT rather than HRT. The retention time for wastes treated in mesophilic digester ranges from 10 to 40 days. If the retention time is too short, the bacteria in the digester are washed out faster than they can reproduce; so that fermentation practically comes to a standstill. The longer a substrate is kept under proper reaction conditions, the more complete its degradation will become. But the reaction rate will decrease with increasing residence time. The disadvantage of a longer retention time is that a large reactor size is needed for a given amount of substrate to be treated (Hassan, 2003). Although a short retention time is desired for reducing the digester volume, a balance must be made to achieve the desired operational conditions, for example, maximizing either methane production or organic matter removal (Zamudio, 2010).

Digesters operating in the thermophilic range require lower retention times. For instance, a high solids reactor operating in the thermophilic range has been reported to require a retention time of 14 days. The degradability of food waste was approximately 20 – 30% higher than that of bio-waste. This has been attributed to the higher concentration of digestible fat in food waste. To achieve higher biogas amount or conversion efficiency of organics with food waste a relatively long digestion time of around 6 days has been reported; as compared to about 3 days with bio-waste (Nayono, 2010).

2.9.5. Particle Size

The size of the substrate is not to be too large. If so, it would result in the clogging of the digester and difficult for microbes to carry out its digestions but smaller particles on the other hand would provide large surface area for adsorbing the substrate that would result in increased microbial activity and hence increased gas production (Sharma *et al.,* 1988).

2.9.6. Carbon to Nitrogen (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 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. Waste material that is low in C can be combined with materials high in N to attain desired C: N ratio of 30:1. Some studies also suggested that C: N ratio varies with temperature. Use of urine soaked waste materials is particularly advantageous during winter months when gas production is otherwise low (Yadvika *et al*., 2004).

The unbalanced nutrients are regarded as an important factor limiting anaerobic digestion of organic wastes. For the improvement of nutrition and C/N ratios, co-digestion of organic mixtures is employed. Co-digestion of fish waste, abattoir wastewater and waste activated sludge with fruit and vegetable waste facilitates balancing of the C/N ratio. Their greatest advantage lies in the buffering of the organic loading rate, and anaerobic ammonia production from organic nitrogen, which reduces the limitations of fruit and vegetable waste digestion (Khalid *et al.,* 2011) .The C/N ratio of 20–30, may provide sufficient nitrogen for the process. According to Khalid *et al*. (2011) a C/N ratio between 22 and 25 seemed to be best for anaerobic digestion of fruit and vegetable waste, whereas, the optimal C/N ratio for anaerobic degradation of organic waste was 20–35.

2.9.7. Ammonia Concentration

It is generally believed that ammonia concentrations below 200 mg/L are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms. A wide range of inhibiting ammonia concentrations has been reported in the literature, with the inhibitory TAN concentration that caused a 50% reduction in methane production ranging from 1.7 to 14 g/L. The significant difference in inhibiting ammonia concentration can be attributed to the differences in substrates, environmental conditions (temperature, pH), and acclimation periods (Chen *et al.,* 2008).

2.9.8. Agitation

Agitation helps to ensure intimate contact between micro-organisms to each other and with the substrate intimate. Contact between bacteria improves fermentation efficiency (Coppinger, 1979). As investigated by Mahanta *et al.,* (2004a), the recirculation of gas improves the biogas yield. A recent experiment at Indian Institute of Technology Guwahati showed that recirculation of gas increases the biogas production by three times (Mahanta *et al*., 2004a). Mixing is important in anaerobic fermentation to ensure adequate contact between bacteria and substrate and also to help strip gas out of the liquid. Another reason for using mixing is to reduce scum formation which reduces the overall capacity of the digester. Recently Stroot *et al.* (2001) and (Andrew and Mattew, 2009) reported that minimally mixed digesters demonstrated a much more stable operation than digesters that were continuously and vigorously mixed.

2.9.9. Hydraulic Retention Time (HRT)

HRT is the average time spent by the input slurry inside the digester before it comes out. In tropical countries like India, HRT varies from 30–50 days while in countries with colder climate it may go up to 100 days. Shorter retention time is likely to face the risk of washout of active bacterial population while longer retention time requires a large volume of the digester and hence more capital cost. Hence there is a need to reduce HRT for domestic biogas plants based on solid substrates. It is possible to carry out methanogenic fermentation at low HRT's without stressing the fermentation process at mesophilic and thermophilic temperature ranges (Yadvika, 2004).

2.9.10. Toxicity

Toxic materials that inhibit normal growth of microorganisms in the digester include mineral ions, heavy metals and detergents. Small quantity of mineral ions also stimulates the growth of bacteria, while very high concentration of these ions leads to toxic effects. For example, presence of NH_4 from 50 to 200 mg/l stimulates the growth of anaerobic microbes, whereas, its concentration above 1500 mg/l produces toxicity. Similarly, heavy metals in small quantities are essential for the growth of bacteria but their higher concentration has toxic effects. Detergents including also inhibit the activity of methane producing bacteria (Moharao, 1975).

2.9.11. Organic Loading Rate (OLR)

Gas production rate is highly dependent on loading rate. Methane yield is found to increase with reduction in loading rate. As study carried out in Pennsylvania on a 100 m^3 biogas plant operating on manure, when OLR was varied from 346 kg VS/day to 1030 kg VS/day, gas yield increased from 67-202 m^3/day. There is an optimum feed rate for particular sizes of plant, which will produce maximum gas and beyond which further increase in the quantity of substrate will not proportionately produce more gas. According to Yadvika (2004), a daily loading rate of 16kg VS/m3 of digester capacity produced 0.04 - 0.074 m^3 of gas/kg of dung fed. A lab-scale digester operating at different OLRs produced a maximum yield of 0.36 m^3/kg VS at an OLR of 2.91 kg VS/ m^3/day. Based on pilot plant studies (1 m^3 capacity), maximum gas yield was observed for a loading rate of 24 kg dung/m^3 digester/day although percent reduction of VS was only 2/3rd of that with low loading rate (Yadvika, 2004).

2.9.12. Pre-treatment

Pre-treatment of feed was identified as one of the contributing factors for increasing the biogas yield (Mohararo, 1994). For increasing the degradation rate of substrates, a pre- treatment by mechanical, thermal, chemical or enzymatic processes can be applied (Muller, 2007). The barrier to the production and recovery of valuable materials from lignified cellulosic waste (LCW) is the structure of lignocelluloses which has evolved to resist degradation due to cross linking between the polysaccharides (cellulose and hemicelluloses) and the lignin via ester and ether linkage (Yan and Shuya, 2006). Cellulose, hemi-cellulose and lignin form structures called micro fibrils, which are organized into micro fibrils that mediate structural stability in the plant cell (Rubin, 2008).

The main goal of any pre-treatment, therefore, is to alter or remove structural and compositional impediments to hydrolysis and subsequent degradation processing in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products (Mosier *et al.*, 2005; Hendriks and Zeeman, 2009). These methods cause mechanical, physical, chemical or biological changes in the plant biomass in order to achieve the desired products. Treatment by thermal pressure hydrolysis (230^0C, 20-30 bars) results in the splitting of organic polymers by hydrolysis into short chain, biologically good available compounds which increases the biogas yield while the retention time in the digester can be reduced drastically (Mosier *et al.,* 2005).

Pre-Treatment of feed stock can increase biogas production and volatile solids reduction due to increased solubilization. The use of pre-treatment is particularly useful in the digestion of biomass feedstock, as these tend to be high in cellulose or lignin. Pre-treatment can break down these recalcitrant polymers physically, chemically or biologically. These have been shown to be effective in anaerobic digestion of lignocelluloses wastes such as bagasse, maize bran, coconut fibres, water hyacinth, sisal fibre and sisal leaf decortications residues leading to significant increase in methane yield compared to the untreated (Mshandete *et al.*, 2005).

2.10. *Prosopis juliflora*: A noxious invasive weed

Prosopis juliflora is one of the invasive alien plant species that are on threatening the native plant species. The root penetrates to great depth in the soil searching for the required water. It can grow in the variety of soil types including saline and alkaline areas in sandy and rocky soils. The tissue of *P. juliflora* is photosynthetically active throughout the year, presenting a wide spread root system through which fully exploits the available water resources. Its low nutritional requirements and resistance to water deficit give *Prosopis juliflora* a great plasticity of response, which allows its wide distribution in arid and semiarid zones in the tropics (Pacieezuick *et al.*, 2001).

The leaves of *P. juliflora* contain various chemicals including tannins, flavinoids, steroids, hydrocarbons, waxes and alkaloids. These are known to affect palatability to livestock but also have effects on the germination and growth of other trees, shrubs etc. As a result of this, the plant diversity both the number of individual plants of a species and the number of species around *P. juliflora* will be affected by the allelochemicals. These allelochemicals change the microenvironment around. The chemicals will remain in the soil even after *P. juliflora* is removed and hinder the growth of native species from reestablishing again (Pacieezuick *et al*., 2001).

Reduced numbers of seedlings of native species have been recorded under canopy of non-indigenous invaders (Gordon, 1998). This is due to the conversion of more open stands to closed-canopy systems accompanied by low-light, higher humidity, lower temperatures, and other environmental and biological changes (Hobbs and Mooney, 1986).

2.10.1. Allelopathic effects of *Prosopis juliflora*

Allelopathic effects can be positive and negative, depending upon the dose and the organism affected. Allelopathy is the active or passive effects of chemicals released into the environment which influences other organisms. It is the biochemical modification of the environment to enhance *P. juliflora* survival and reproduction (Coder, 1999). The chemicals released inhibit the germination and growth of associated plants. Apart from this, they inhibit nutrient absorption and dry matter accumulation in shoots and roots of target species (Wacker *et al*, .1990).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted at Botany Laboratory of the Department of Biology, Haramaya University. The University is found 525 km away from Addis Ababa to the east. It is located at 9°26’N latitude, 42°03’E longitude and an altitude of 1980m.a.s.l. The university has a total area of about 46 km^2. It has a moderate average temperature of 16°C, and the mean maximum and minimum annual temperatures are 24.02 and 9.73°C, respectively (FAO, 1990).

3.2. Sample Collection and Preparation of Substrate for Anaerobic Digestion

P. juliflora (PJ) leaves and cow manure (CM) were used as feed stocks for AD process of biogas production. *P. juliflora* (PJ) leaves were collected from Dire-Dawa and CM collected from the Haramaya University cattle farm. Both *P. juliflora* (PJ) leaves and cow manure (CM) were dried and crushed using all purpose high speed smashing machine to break into smaller particles to ensure consistency of mix. To facilitate anaerobic digestion, rumen fluid was used as inoculums (Sunarso *et al*., 2012). Fresh rumen fluid was collected from the nearby slaughter house 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 was done in 0.5 L digester. The two substrates were combined in different proportions so as to have five substrate treatments. These were 100% cow manure (CM), 75%:25% mix of CM and *P. juliflora* (PJ) leaves, 50%:50% mix of CM and PJ, 25%:75% mix of CM and PJ and 100% PJ. To have 8% TS in fermenting slurry, appropriate amount of distilled water and rumen fluid (100 ml) were mixed (Tchobanoglous *et al*., 2003). All experiments were carried out at mesophilic temperature (38°C) using five sets of digesters corresponding to the five-substrate mix-ratios. The pH of the slurry was also 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.

3.3. Design of the Experiment

The experiments were arranged in a completely randomized design with three replications of five treatments (5 ×3). The five substrate treatments were 100% CM, 75%:25% mix of CM and *P. juliflora* leaves, 50%:50% mix of CM and *P. juliflora* leaves, 25%:75% mix of CM and *Prosopis juliflora* leaves and 100% *Prosopis juliflora* leaves (Alemayehu, 2015).

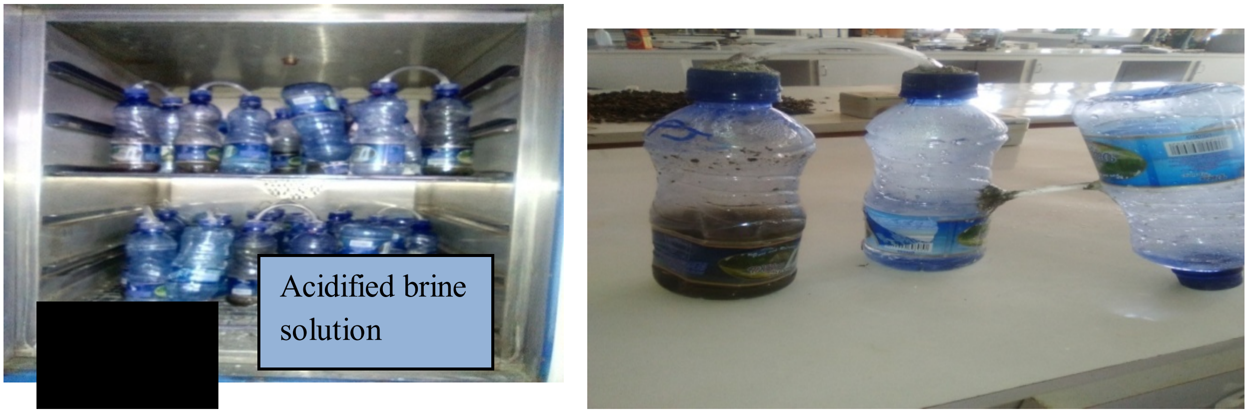
Table 1: The proportion of the two different substrates added into the five digesters

Treatment	Dry wt of *P. juliflora* (gm.)	Dry wt of CM (gm.)	Distilled H_2O added (ml)	RF (ml)	Percentage of *P juliflora*	Percentage of CM
T_1	0	11.1	113.9	100	0.00	100.00
T_2	2.7	8.325	113.98	100	25.00	75.00
T_3	5.43	5.55	114.02	100	50.00	50.00
T_4	8.15	2.8	114.05	100	75.00	25.00
T_5	10.86	0	114	100	100.00	0.00

T1=100% CM, T2=75%:25% mix of CM: PJ, T3= 50%:50% mix of CM: PJ, T4= 25%:75% mix of CM: PJ and T5=100% PJ, RF=Rumen fluid

3.4. Digester Configuration and Setup for Biogas Production

Digesters were setup in order where the digester containing the slurry was connected to the 0.5L plastic bottle with acidified brine solution that was again linked to an empty bottle meant 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 was sealed tightly using super glue in order to control the entry of oxygen and loss of biogas.



[redacted]

Figure 2: Batch form of experimental setup

3.5. Analysis of the Physicochemical 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 PJ 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:

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).

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

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

%VS = percentage of Volatile Solid

The total nitrogen in the sample was determined using the Kjeldahl method. This method has three main steps. These are digestion, distillation and titration. One gram of sample and 6 ml of concentrated H_2SO_4 were added into a tecator tube and mixed carefully. Then 3.5 ml of H_2O_2 was added step by step. Violet color due to reaction was observed. As soon as the violent reaction has ceased the tube was shaken by hand. After adding 3g catalyst mixture the sample was allowed to stand for 5 to 15 minutes in the tecator rack before digestion. Then the digester was switched on and allowed to wait until its temperature reached 370°C. As the digester gained this temperature the rack was placed in it and the digestion continued for about 4 hours until a clear solution was observed.

The tube in the rack was transferred to the fume hood for cooling. About 50 ml of distilled water was added and shaken by hand to avoid 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 were placed under the condenser of the distiller with its tip immersed into the solution and the distillation continued for about 8 minutes until the total volume became between 200 ml to 250 ml. Finally the solution was titrated using 0.1N HCl to a reddish color and %Nitrogen was calculated using the following formula:

Where,

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

Wo = Sample weight on dry matter basis and

14 = The molecular weight of nitrogen

0.1= Normality of HCl

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

3.5.4. Moisture Content Determination

The most commonly used method to determine moisture content is 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^{O}C for 24 hours and weighed. Moisture content was then calculated using the following formula (Elias, 2010).

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

3.5.5. Determination of pH

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

3.6. Digester Composition

3.6.1. Feed Stocks

The amount of TS in the digester was fixed to be 10g and for the purpose of this research a set of five batch reactors were used as digesters. Each digester contained cow manure and *Prosopis juliflora* leaves of varying proportions with the objective of investigating the level of mixing that yields the highest biogas through anaerobic co-digestion. In general, five treatment levels varying in substrate composition and proportion were used and labeled as: treatment 1 (T1) = 100% *Prosopis juliflora* leaves, treatment 2 (T2) =100% cow manure, treatment 3 (T3) = 75% *Prosopis juliflora* leaves and 25% cow manure, treatment 4 (T4) = 50% *Prosopis juliflora* leaves and 50% cow manure, and treatment 5 (T5) = 25% *Prosopis juliflora* leaves and 75% cow manure.

3.6.2. Water Content

The water content for each digester was determined according to the recommendation of (Ituen *et al.*, 2007). Feed stocks were mixed with distilled water to get about 8% of TS suspension. The amount of water to be added was then determined using the following formula:

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

3.7. 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 is 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 next day of inoculation.

3.8. Data Analysis

Experimental data were subjected to analysis of variance (one-way ANOVA) using SPSS for windows version 16) to investigate statistical significance between the different treatments, whereas paired samples T-test was used to investigate statistical significance within a treatment. Differences between means were considered statistically significant at $P< 0.05$.

4. RESULTS AND DISCUSSION

4.1. Physicochemical Properties of the Substrates Used in Co-digestion

4.1.1. Analysis of Moisture Content, Organic Carbon and pH of Substrates within and Between Different Treatments

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

Table 2: Comparison of % MC%, Organic carbon% and pH between before and after AD of the various treatments (values are mean ± SE, n=3)

		Parameters				
Treatment	Initial MC%	Final MC%	Initial C%	Final C%	Initial pH	Final PH
T1	80.9 ± 0.47^{Bb}	91.1 ± 0.55^{Aa}	44.5 ± 0.42^{Aa}	42 ± 0.66^{Ab}	6.05 ± 0.01^{Eb}	6.4 ± 0.3^{Ea}
T2	78.6 ± 0.3^{Db}	86.5 ± 1.5^{Ba}	34 ± 0.3^{Ca}	33.6 ± 0.2^{Db}	7.2 ± 0.01^{Ab}	7.5 ± 0.05^{Aa}
T3	78.8 ± 0.44^{Cb}	91 ± 0.57^{Aa}	40 ± 0.3^{Ba}	20.8 ± 0.19^{Eb}	6.25 ± 0.1^{Db}	6.6 ± 0.3^{Da}
T4	81.6 ± 0.8^{Ab}	85 ± 1.1^{Ca}	39.9 ± 0.35^{Ba}	37.3 ± 0.42^{Bb}	6.69 ± 0.00^{Cb}	6.9 ± 0.3^{Ca}
T5	78.8 ± 0.18^{Cb}	82.2 ± 0.8^{Da}	35.5 ± 0.2^{Ca}	34.7 ± 0.7^{Ca}	6.9 ± 0.2^{Bb}	7.25 ± 0.4^{Ba}

T1 = 100%PJ, T2 =100% CM, T3 = 75% PJ and 25% CM, T4 = 50% PJ and 50% CM, and T5 = 25% PJ and 75% CM.

Means followed by the same small letters in row are not significant at $P< 0.05$ probability levels for paired samples T-test within treatment while means followed by the same capital letter in column are not significantly different at 5% level of significance between treatments for one way ANOVA.

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

There were also significant differences at (P<0.05) within treatments in both the initial and final C%. The C% generally showed a significant decrease as a result of AD in all treatments (Table 2). The decrease in C% reflects the occurrence of degradation during anaerobic digestion (Abdel-Hadi *et al.*, 2008). The degradation is meant for conversion of the substrates to cellular materials for growth and reproduction of bacteria or as a result of biogas production (Gerardi, 2003). However, degradation of organic carbon was highest (19.2 C%) for T3 (i.e. 75%PJ and 25%CM). This mix ratio was also found to yield more biogas than the rest of the treatments as shown in Fig. 6. Likewise, significant variations were shown between the initial and final pH values of all treatments (Table 2). As can be seen from Table 2, the initial mean pH values of 100%PJ and 100% CM were 6.05±0.01 and 7.2±0.01, respectively. However, after mixing the substrates in different proportions, the initial mean pH values of the mixed treatment groups (75% PJ and 25%CM, 50%PJ and 50% CM, 25%PJ and 75%CM) showed significant increase and gave values of 6.25±0.1, 6.69±0.00 and 6.9±0.2, respectively. This result showed that the pH values of the substrates in the digester increases with the increase in the CM content and a simultaneous decrease in the PJ content of the mix ratios. Mixing substrate is a good way of adjusting the pH value to the optimum (Hills and Roberts, 1981). In this study, significant differences between pH value before and after AD were observed in all substrate mix ratios (P<0.05). As shown from the table 2, the final mean pH values of all treatments T1, T2, T3, T4 and T5 (i.e. 6.4±0.3, 7.5±0.05, 6.6±0.3, 6.9±0.3 and 7.25±0.4, respectively) were higher than the initial pH values for the same treatments (i.e. 6.05±0.01, 7.2±0.01, 6.25±0.1, 6.69±0.00, 6.9±0.2, respectively) . The reason for the increment of the pH values after AD may be attributed to production of alkali compounds, such as ammonium ions during the degradation of organic compounds in the digester (Gerardi, 2003).

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

The total solids and volatile solids of substrates were analyzed for all treatments both before and after AD (Table 3 and Figure 3). The percentage TS and VS showed significant difference between before and after AD at $P<0.05$. The maximum TS% and VS% before AD were measured in 100%PJ, whereas the minimum TS% and VS% were measured from the digester with 100% cow manure. The observed maximum values of TS% and VS% in 100%PJ may suggest the presence of more biodegradable substrates for biogas production.

Table 3: Mean values of %TS of substrates before and after digestion

Treatment	Initial TS%	Final TS%	TS% Removed
T1	92^{Aa}	25.4^{Db}	66.6
T2	89^{Ca}	38.1^{Ab}	50.9
T3	91^{Ba}	26.8^{Cb}	64.2
T4	90.8^{Ba}	26^{Cb}	64.8
T5	90.2^{Ba}	34.9^{Bb}	55.3

Means followed by the same small letters in row are not significant at $P< 0.05$ probability levels for paired samples T-test within treatment while means followed by the same capital letter in column are not significantly different at 5% level of significance between treatments for one way ANOVA. T1 = 100%PJ, T2 =100% CM, T3 = 75%PJand 25% CM, T4 =50%PJ and 50%CM, and T5 = 25%PJ and 75%CM.

Figure 3: Mean values of VS% of substrates before and after digestion

Capital letters represent significant differences between VS% of the various substrates before digestion while small letters represent those of the after digestion. Bar graphs with the same capital or small letters are not significantly different, whereas those with different capital or small letters are significantly different. Asterisk (*) shows there was significant difference in %VS between before and after anaerobic digestion; T1 = 100%PJ*,* T2 =100% CM, T3 = 75%PJ and 25% CM, T4 =50%PJ and 50%CM, and T5 = 25%PJ and 75%CM.

After anaerobic digestion, TS% and VS% of all substrate types were significantly lower than those of the substrates before AD (Table 3 and Figures 3). But more reduction was observed in T3 (75%PJ and 25%CM), T4 (50%PJ and 50%CM), and T1 (100%PJ) than in T2 (100%CM and T5 (25%PJ and 75%CM). In T1, there was a relatively higher percentage of TS and VS removal but less amount of biogas. Less biogas production observed from PJ can be attributed to its toxicity or conversion of the substrates to cellular materials for growth and reproduction of bacteria (Gerardi, 2003; Tamrat *et al.*, 2013). Thus, total solids and volatile solids destruction is a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012).

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

Carbon to nitrogen ratio is a major factor affecting the anaerobic process which in turn affects methane yield and production rates. Therefore, the balance of carbon and nitrogen in a feed material is important. It is often suggested that an optimum C: N ratio is between 20:1 and 30:1(Marchaim, 1992; Fulford, 1988). If the C:N ratio is very high, the nitrogen will be consumed rapidly by methanogens to meet their protein requirements and will no longer act on the left over carbon content of the material. As a result, gas production will be low. On the other hand, if the C: N ratio is very low; nitrogen will be liberated and accumulated in the form of ammonia (NH_3). The increased concentration of NH_3 will increase the pH value of the slurry in the digester and ultimately lead to the inhibition of the growth of bacteria (Braun, 1982).

Table 4: The carbon to nitrogen ratio of PJ and cow manure when used singly and in different combinations (values are shown as Mean+ SE, n=3)

	Parameters		
Treatment	%C	%N	C:N ratio
T1	44.5±0.42	1.86±0.00	23.9:1
T2	34±0.3	1.5±0.01	22.6:1
T3	40±0.3	1.76±0.00	22.7:1
T4	39.9±0.35	1.68±0.02	23.75:1
T5	35.5±0.2	1.58±0.01	22.46:1

T1 = 100%PJ, T2 =100% CM, T3 = 75%PJ and 25% CM, T4 =50%PJ and 50% CM and T5 = 25%PJ and 75% CM.

In this experiment, the carbon to nitrogen ratio of treatments T1, T2, T3, T4 and T5 before AD were 23.9±00, 22.6±01, 22.7±0.3, 23.75±0.2 and 22.46±00 respectively (Table 4).

This result was in agreement with the optimum C: N ratio (20:1 to 30:1) reported by Braun (1982). It is also comparable with the carbon to nitrogen ratio of paddy straw (26:1) and groundnut shell (27:1) mixed with cow dung in pre-digested slurry reported by Anonymous (1981) and also comparable with the carbon to nitrogen ratio of cattle manure (22.22:1) mixed with rumen content (26.17:1) reported by Solomon (2014). For good biogas production the adjusting of C/N ratio is desirable and this can be achieved by mixing wastes of high ratio with those of low ratio (FAO/CMS, 1996).

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

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

Of the three mix ratios, digester with 75%PJ + 25% CM (T3) produced biogas 506ml which is larger than the other two mix ratios. The rapid biogas production in the digester containing 75% PJ + 25% CM might be also due to the shorter lag phase in the growth of bacteria, the availability of readily biodegradable organic matter in the substrate. The highest biogas production was also observed from this digester probably due to the availability of balanced nutrient composition for microorganism and the existence of stable pH which was attained as a result of the addition of inoculum and the particular mix ratio.

Figure 4: Daily mean biogas yield of the five treatments in 28 days.

There was a significant difference between the substrates in the overall biogas yield (Figure 4, $p<0.05$). However, there is no biogas production observed in 1st day, this may be due to the fact that the microorganisms were in their lag phase (the period of apparent inactivity in which the cells are adapting to a new environment and preparing for reproductive growth). Cells are usually synthesizing new components (Cundiff and Mankin, 2003).

On the other hand, from the 2nd day up to the 5th day biogas production sharply increased as the microorganisms entered into their log phase (high growth or exponential phase, the period in which the organisms are growing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing) (Cundiff and Mankin, 2003).

After 15 days, more or less biogas production showed a decrease presumably when the microorganisms entered the decline phase (Cundiff and Mankin 2003). At the end of the 27th and 28th day biogas production ended and stopped. This may be due to the fact that the microorganisms entered the death phase, the period in which the cells are dying at an exponential rate. Some of the reasons for entering this phase could be continued accumulation of wastes, loss of cell's ability to detoxify toxins, the depletion of the necessary nutrients from the digesters and the increase in ammonium concentration that resulted in an increased pH values (Hansen *et al*., 1998).

Figure 5: Mean cumulative biogas yield of the five treatments

Bar graphs with different letters indicate significant differences between means while those with same letters show no significant difference between means. T1 = 100%PJ, T2 =100% CM, T3 = 75%PJ and 25% CM, T4 =50%PJ and 50%CM, and T5 = 25%PJ and 75%CM.

The cumulative biogas productions of the five treatments are summarized in Figure 5. The results showed that T3 produced the highest mean cumulative biogas, which was 506 ml within 28 days of incubation period. This was followed by T4 which produced 379 ml gas. The 3rd and 4th highest biogas production, yielding 318 and 317ml, were obtained in T5 and T1, respectively. Cow Manure alone (T2) yielded less biogas, which was 272 ml. As it is a product of a substrate that has already undergone partial fermentation in the intestinal tract of the animal and as it contained less degradable material (Chawala, 1986; Deublein and Steinhauser, 2008).

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Production of biogas through anaerobic digestion of organic waste materials provides an alternative energy. The experiment was conducted under mesophilic conditions (38ºC) using five batch digesters of triplicate. 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 28 days.

The pH was found to increase significantly with increasing of cow manure proportion in the mix, suggesting that cow manure helps to maintain the pH to meet the optimum required. The comparison of pH values between before and after AD showed that pH values are significantly increase for all the treatment. It showed the pH ranges between 6.4 for 100% *PJ* and 7.5 for 100% CM.

The %initial highest moisture content (80.9±0.47) was observed in 100% *P. juliflora* and the %initial lowest moisture content (78.6±0.3) in 100% Cow manure. Moreover mixing substrates with *P. juliflora* increase the moisture content compared with cow manure alone.

The results also revealed that there are 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% significantly decreased AD in all substrate types. In this study, C: N ratio of all treatments was found in between 20:1-30:1 which is a suitable condition for methanogenic bacteria to reproduce and produce optimum biogas. High reduction of VS was measured in 25%CM + 75% *P. juliflora* mix substrates compared to the rest of substrates after AD (Figure 3). The biogas in T3 (75% *P. juliflora* + 25% CM produce higher (54.23 ml) amount of biogas than others at 5th day.

5.2. Conclusion

The results showed that there is a strong possibility to enhance the biogas production. Use of certain organic additives seems to be promising for enhancing biogas production. Among different types of biomass (plant and crop residues) used as additives, *Prosopis juliflora* leaves have been found to enhance the gas production significantly. The general outcome of this study suggested that the *Prosopis juliflora* leaves co-digested with cow manure improved the biogas potential compared to cow manure alone. The experimental data also showed that the highest gas production was obtained in the mix ratio of 75% *PJ*: 25% CM (506 ml). Thus, compared to the mono-digestion of pure cow manure and pure *Prosopis juliflora,* anaerobic co-digestion of 75% *Prosopis juliflora* 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°C), it is recommended that further study be carried out at room temperature (20°C) and at thermophilic conditions (55°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 *P. juliflora* (PJ) leaves and cow manure further similar studies should also be made on the co- digestion of *P. juliflora* (PJ) leaves with other substrates.

- The percentage of methane in the biogas produced needs to be qualified for better efficiency of biogas production from *P. juliflora* co- digested with cow manure.

6. REFERENCE

Abdel-Hadi, M.A. and S.A.M. Abd El-Azeem. 2008. Effect of heating, mixing and digester type on biogas production from buffalo dung. *Misr Journal of Agricultural Engineering*, 25(4): 1454-1477.

Abiyot Berhanu and Getachew Tesfaye. 2006. The *P. juliflora* Dilemma, Impact on Dry land Biodiversity and Some Controlling Methods. *Journal of the Dry Lands*, 1:158-164.

Abubaker Umar and Ismail Nasir. 2012. Anaerobic digestion of cow dung for biogas production. *ARPN Journal of Engineering and Applied Sciences,* 7(2):169-172.

Aggarwal, D. 2003. Biogas plants based on night soil. *The energy and resources institute (TERI),* 21(4): 1154-1177.

Alemayehu Gashaw. 2015. Co-digestion of municipal organic wastes with night soil and cow dung for biogas production. *African Journal of Biotechnology*, 5(2): 32-44.

Alemu Gizaw. 2016. Comparative Study on Biogas Production Potential of Sewage, Slaughterhouse, Fruit-Vegetable Wastes and their Co-digestion. M.Sc. Thesis. Addis Ababa University, Addis Ababa, Ethiopia.

Amigun, B. and Von Blottnitz H. 2007. Investigation of economies for African Bio-gas Installation. *Energy Conversion and management*. 48(12): 3090-3094.

Amon, T., Hackl, E., Jeremic, D., Amon, B. and Boxberger, J. 2001. Biogas production from animal wastes, energy plants and organic wastes, in 9th World Congress, Anaerobic Digestion, Anaerobic Conversion for Sustainability. Proceedings part 1. Antwerpen Belgium.

Andrew Trivett and Matthew Hall. 2009. Feasibility of Biogas Production on Small Livestock Farms UPEI (University of Prince Island) Department of Engineering. pp, 27-48.

Anonymous. 1981. Biogas technology and utilization. A status Report. Department of Science and Technology. *Joural of coffee research*. 19: 17-18.

Bensah, E.C. 2009.Technical evaluation and standardization of biogas plants in Ghana.MSc. Thesis, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. 9: 117-128.

Biety, E.M .,Musibono.E.D., and Verstrate,W. 2003. Start-up of the multi stage system for biogas production and solid waste treatment in low-tech country. *Water science and technology*, 48(4):239-243.

Borjesson, P, and Bergland Maria. 2006. Enviromental systems analysis of biogas system part 1: fuel cycle emissions. *Biomass and bioenergy*, 30:469-485.

Braun, R. 1982. Biogas-MethangarungorganischerAbfallstoffe:Grundlagenund

Chawala, O.P. 1986. Methane fermentation technology. In: advances in biogas technology. Indian council of agricultural research, New Delhi, India, 19-57.

Chen .X, Ba. Y, Ma. L, Cia. X, Yin Y, Wang K, Guo J, and Li X. 2008. Methane and protein production from animal feedlot wastes. *Journal of Soil and water Conservation*, 34:16-19.

Chonkor, P.K., 1983.Microbial Ecology during Anarodic Fermentation of cellulose waste Materials in S.K.Vyas, Grewal, N.S (Ed), Biogas Technology USG Publisher, Luthiana, India. 21-26.

Coder, K. D. 1999. Allelopathy in Trees. University of Georgia Daniel B. Warnell School of Forest Resources. Extension publication FOR99-004.

Constant, M., Naveau, H., Ferrero, G.-L. and Nyns, E.J. 1989. Biogas end-use in the European community. Commission of the European Communities Directorate-General for Energy. Elsevier applied science, London, New York.79-88.

Cooke, P. 2008. Fuel wood, forests and community management - Evidence from household studies. *Environment and Development Economics*, 13(1):103-135.

Coppinger, E.R. 1979. The Operation of a 50,000 Gallon Anaerobic Digester at the Monroe State Dairy Farm, Ecotope Group, 2332, East Madison, Seattle, WA. 12(1):113-125.

Cundiff, S and Mankin, R. 2003. Dynamic of biological systems. *International Journal of*

Chemical and Biomolecular Engeneering, 3(1): 39-44.

Danqi, Z. 2010. Co-digestion of Different Wastes for Enhanced Methane Production. Msc thesis, The Ohio State University, Ohio, USA.

Debruyn, J. and Hilborn, D. 2006. Anaerobic Digestion and Associated Technologies Resource and Contact Information. InfoSheet. Ontario Ministry of Agriculture, Food, and Rural Affairs. pp,123-140.

Demetriade. 2008. Thermal Pre-treatment of cellulose rich biomass for biogas production. Swedish University of Agricultural Sciences, Uppsala. *Water Research*, 28:2383-2392.

Demissew Sertse. 2005. Controlling the spread of *Prosopis* in Ethiopia by its utilisation. *Journal of Arid Environment*, 58: 124-153.

Deublein, D. and A. Steinhauser. 2008. *Biogas from Waste and Renewable Resources*. Wiley VCH Verlag GmbH and Co. pp, 128-136.

Deublein, D. and Steinhauser,A. 2008. *Biogas from waste and renewable source*: An introduction. Wiley pp, 20-29

Dioha,J., Ikeme, C.H., Nafi'u, T.; Soba, N. I., Yusuf, M.B.S. 2013. Effect of carbon to nitrogen ratio on biogas production. *International Research Journal of Natural Sciences*, 1: 1-10.

Duflo, E. 2008. Indoor air pollution, health and economic well-being. SAPIENS: Surveys and Perspectives *Integrating Environment and Society*. 1(1): 124-165.

Ebrahim Ali. 2006. Evaluation of economic and Technical feasiblty of biogas production from poulitry litter in elfora agro industry debreziet. *Water Resources research journal*, 30:17-28.

Edison, M. 2014. Bio-methane generation from organic waste. A review, Proceedings of the World Congress on Engineering and Computer Science (WCECS). San Francisco, USA.pp, 256-270.

Elias Jigra. 2010. Renewable biogas energy production from Cladodes of opuntiaficus- indica. *Bioresource Technology Journal,* 55:91–101.

Elijah T, Ibifuro A, Yahaya S. 2009.The Study of Cow Dung as Co-Substrate with Rice Husk in Biogas Production. *Scientific Research and Essays*, 4(9):861-866.

El-Keblawy, A. 2002. Causes and Consequence of the Invasion of the Exotic *P. juliflora* to the Environment of the UAE In: Third Annual Conference for Research Funded by UAE University. pp,1024-1036.

El-Mashad, H.M., Zeeman, G.K.P. Wilko, P.A. Gerard and G. Lettinga, 2010. Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure. *Bioresource Technology Journal,* 95:191–201.

EPA (Environmental Protection Agency). 2015. Climate Change, United States Environmental Protection Agency.

Eshete, D.G., Sonder, D. K. and Heegde, M. F. T. 2006b. Report on the Feasibility Study of a National Programme for Domestic Biogas in Ethiopia.

Esposito,G., Frunzo, L., Liotta, F., Panico, A., Pirozzi, F. 2012. Bio-Methane Potential Tests to Measure the Biogas Production from The Digestion and Co-Digestion of Complex organic. *The Open Environmental Engineering Journal,* 5: 1-8.

Eyasu Mekete. 2008. *Moringa stenopetala* seed cake powder: a potential for biogas production. M.Sc. Thesis. Addis Ababa University, Addis Ababa, Ethiopia.

FAO (Food and Agricultural Organization). 1990. Food and Agricultural Organization of the United Nations Agro meteorology group, Remote Sensing Center Research and Technology Division. Rome, Italy.pp 234-248.

FAO/CMS. 1996. *A system Approach to Biogas Technology. Biogas Technology*: a training manual for extension. Available at website:-http://www.fao.org.

Fulford, D. 1988. Running a biogas program, a hand book. Intermediate technology Publications, London.

Gebreegziabher, Z. 2007. Household fuel consumption and resource use in rural-urban Ethiopia. Wageningen University.pp, 563-578.

Gerardi M.H. 2003. *The Microbiology of Anaerobic Digesters.* Hoboken, *N.J. John Wiley and Sons*. Verjinia USA.pp, 134-140.

Gordon, D. R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. *Ecological Application*, 8:975-89.

Green, M. B. 1987. Energy in pesticide manufacture, distribution and use. In: Stout, B.A., Energy in world agriculture. Band 2: Helsel, Z.R (Ed), and Energy in plant nutrition and pest control. Elsevier, Amsterdam.

Gudina Terefe and Sanderine N. 2015. Bio-Wastes as an Alternative Household Cooking Energy Source in Ethiopia. *Energies*, 8: 9565-9583.

Hailu Shiferaw, Demel Teketay, Sileshi Nemomissa and Fassil Assefa. 2004. Some biological characteristics that foster the invasion of *P. juliflora* (Sw.) DC. At Middle Awash Rift Valley, North-eastern Ethiopia. *Journal of Arid Environment*, 58: 34-153.

Hansen, K., I. Angelidaki and B.K. Ahring.1998. Anaerobic digestion of swine manure: inhibition by ammonia. *Water Resources research journal*, 32:5-2.

Haug R T. 1993. The practical handbook of compost engineering, Lewis Publishers,Florida, USA.

Hendriks ATWM and Zeeman G. 2009. Pre-treatment to enhance the digestibility of lignocelluloses biomass. *Bioresource Technology Journal*, 100(1) 10-18.

Hilborn, D. 2006. "Calculations and Information for Sizing Anaerobic Digestion Systems". InfoSheet. Ontario Ministry of Agriculture, Food, and Rural Affairs.

Hills D, J, and D.W. Roberts. 1981. Anaerobic digestion of dairy manure and field crop residues. *Agricultural Wastes*, 3:179–189.

Hobbs, R. J. and Mooney, H. A.1986. Community changes following shrub invasion of grassland. Oecologica 70:508-513.

Hssan M.2003. Co-digestion of Ethiopian food waste with cow dung for biogas production. *An international journal of research*, 7(2):123-135.

http://www.iges.or.jp/APEIS/RISPO/inventory/db/pdf/0005.pdf (access date: 22/10/11).

http://www.mnproject.org/pdf/Haubyrptupdated.

Ituen E, John NM, Bassey. 2007. Biogas production from organic waste in AkwaIbom State of Nigeria.Appropriate Technologies for Environmental Protection in the Developing World. Selected Papers from ERTEP 2007, July 17-19, *Ghana, Journal of Engineering*, 7:169-172.

Jianzheng L, Ajay K, Junguo H, Qiaoying B, Sheng C, Peng W. 2011. Assessment of the effects of dry anaerobic co-digestion of cow dung with waste water sludge on biogas yield and biodegradability. International *Journal of* Physical Sciences, 6(15):3679-3688.

Kathiresan, R. M. 2004. Invasive weeds in agro-ecosystems of South India [Abstracts]. In: National Workshop on Invasive Alien species and Biodiversity in India, Banaras Hindu

University, Vanarasi, India. 149.

Khalid, A., Arshad, M., Anjum, M.,Mahmood, T., Dawson, L. 2011.The anaerobic digestion of solid organic waste. *Waste Management*, 3: 1737–1744.

Lantz, M. Svensson, M. Björnsson, L. Börjesson, P. 2007.The prospects for an expansion of biogas systems in Sweden Incentives, barriers and potentials. *Energy policy*, 35:839-1843.

Lettinga G. 2005. The anaerobic treatment approach towards a more sustainable and robust environmental protection. *Water science and technology,* 55:1-11.

Li R, S. Chen, and X. Li. 2009. Anaerobic co-digestion of kitchen waste and cattle manure for methane production.Energy Sources. 31: 1848-1856.

Li X, Li L, Zheng M, Fu G, Lar J. 2009. Anaerobic co-digestion of cattle manure with corn Stover pretreated by sodium hydroxide for efficient biogas production. *Energy Fuel* 23:4635-4639.

Mackie,R.L. and M.P.Bryant. 1981. Metabolic activity of fatty acid oxidizing bacteria and the contribution of acetate, propionate, butyrate and CO_2 to methanogenesis in cattle waste at 40-60 ºC. *Applied Environmental Microbiology,* 40: 1363-1373.

Mahanta, P., Dewan, A., Saha, U.K. and Kalita, P. 2004a. Influence of Temperature and Total Solid Concentration on the Gas Production Rate of Biogas Digester, *Journal of Energy in Southern Africa*, 15 (4): 112-117.

Marchaim,U. 1992. Biogas process for sustainable development. MIGAL Galilee Technological Center.Kiryatghmona Israel, FAO.

Marree F., Nijboer M., Kellner C. 2007. Report on the feasibility study for a biogas support program in the northern zones of Tanzania.SNV publication, Nairobi, Kenya.

McGinley. 2007. Effects of the biological invasion of algaroba:*P. juliflora* (Sw.) DC. On composition and structure of the shrub-tree stratum of the caatinga in Monteiro

Moharao, G.J. 1974. Scientific Aspects of cow Dung Digestion. *Khadi Gramodyog*, 20:340 -347.

Moharao, G.J. 1975. Aspects of Night Soil Digestion, Sewage Farming and Fish Culture, A Working Paper, All India Institute of Hygiene and Public Health.

Mosier N., Wyman C., dale B., Elander R., Lee Y.Y., Holtzapple M.. and Ladisch M. 2005.

Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology Journal*, 96(6): 673-686.

Mshandete, A. Kivaisi, M. Rubindamayugi and B. Mattisaaon. 2004. Anaerobic batch co-digestion of sisal pilp and fish wastes. *Bioresource Technology Journal,* 95:19-24.

Mshandete,A ., Bjornsson , L ., Kivasi, A.K., Rubindamayugi, M.S.T.,and Mattiasson ,B. 2008. Effect of particle size on biogas yield from sisal fiber waste. *Renewable Energy,* 34:234-260.

Muller, C. 2007. Anaerobic Digestion of Biodegradabale solid waste in Law and Middle income Countries. Overview over existing technologies and relevant case studies.

Murto, M., Bjornsson, L., and Mattiasson B. 2004. Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. *Journal of Environmental Management,* 70:101-107.

Myles R. 2008. Practical pictorial field guide on Grameen Bandhu biogas plant. Second and revised edition.INSEDA,NewDelhi,India,36,Availableathttp://www.inseda.org/Manuals/Grameen%20BandhuBiogas%20plant%20-%20A%20Pictorial%20Field%20Guide.pdf (access date: 08/10/11).

Nayono J. 2010. Anaerobic co-digestion of biodegradable municipal solid waste with human excretes for biogas production. *American journal,* 3(2)134-143.

NBP (National Biogas Programme) Ethiopia. 2007. Biogas for Better Life.

Nordberg U and Nordberg, A. 2007. Anaerobic digestion of alfalfa silage with recirculation of process liquid. *Biorecourse Technology Journal*, 98:104-111.

Nordberg, A., Jarvis, A., Stenberg, B., Mathisen, B and Svenson, B. 2007. Anaerobic digestion of alfalfa silage with recirculation of process liquid. *Bioresourse Technology*, 98: 104-111.

Pasiecznik, N. M., Felker, P., Harris, P. J. C., Harsh, L. N., Cruz, G., Tewari, J. C., Cadorer, K., and Maldonado, L.J. (2001). The *Prosopis juliflora* Complex: A Monograph. 172. HADRA, Coventry.

Peter, J.J. 2009. Biogas green energy, Plan Energy and Researcher for a Day. Faculty of Agricultural Sciences: Aarhus University, 2nd edition.

Rubin, E.M. 2008. Genomics of cellulosic bio fuels. *National Journal*, 454(14):841-845.

Sakar, S.,Yetilmezsoy, K. and Kocak, E. 2009. Anaerobic digestion technology in poultry and livestock treatment a literature review. *Waste management and Research,* 27:3-18.

Satoto, E.N. 2010. Anaerobic digestion of organic solid waste for energy production. KIT Scientific Publishing, German.Service on Appropriate Technology (ISAT).

Sharma, S.K., Mishra, I.M., Sharma, M.P., and Saini, J.S. 1988. Effect of particle size on biogas generation. *Biomass,* 17:251-263.

Sibisi, N.T.; Green, J.M. 2005. A floating dome biogas digester: perceptions of energizing a rural school in Maphephetheni. KwaZulu-Natal, *Journal of Energy in Southern Africa*, 16(3), 45-55.

Siltan Abrha. 2000. a brief note on bio gas technology, ministry of mine and energy. Ethiopia national energy committee. 29-31.

Smil and Vaclav. 2003. Energy at the Crossroads: Global Perspectives and Uncertainties, MIT Press, Cambridge.

Solomon Legesse. 2014. Biogas Production from Co- Digestion of Slaughterhouse Waste and Cow Dung. *International Journal of Advanced Research,* 2(7): 920-925.

State Energy Conservation Office (Texas). 2009. "Biomass Energy: Manure for Fuel."State EnergyConservation Office (Texas). State of Texas.

Stroot P. G., McMahon K. D., Mackie R. I. and Raskin L. 2001. Anaerobic co digestion of municipal solid waste and biosolids under various mixing conditions: I. Digester performance. *Water Research*. 35: 1804-1816.

Sunarso, S.Johari, Z., Widiasa, I.N.and Budiyono. 2012. The effect of Feeds Inoculums Ratio on Biogas production Rate from Cattle Manure using Rumen Fluid as Inoculums. *International Journal of Waste Resources,* 2(1):1-4.

Suzie, C. 2013. Anaerobic Digestion across the UK and Europe, Research and Information Service Research Paper. Paper 52/13 1NIAR 944-12. Northern Ireland.

Tamrat Aragaw, Mebeaselassie Andargie and Amare Gessesse. 2013. from the co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. *International Journal of Physical science,* 8(11): 444-450.

Tchobanoglous, G., Burton, F.L., Stensel, H.D.2003. Waste water Engineering: Treatment and Reuse, 4th ed., Metcalf and Edy, Inc., Tata Mcgraw- Hill publishing Company Ltd.: New Delhi, India.

Tesfaye Nigussie.2007.study on anaerobic digestion of Chat wastes. MSc. Thesis,Addis Ababa

University,AdisAbaba,Ethiopia.

Wacker, T.L., Safir, G.R., and Stephens, C.T. 1990. Effects of ferulic acid on Glomus faculatumand associated effects on phosphorous uptake and growth of asparagus (Asparagus officinalis). *Journal of Chemical Ecology* 16:901-909.

Warrage, M.O.A. and Al-Humaid, A.I. 1998. Allelopathic effects of *Prosopis juliflora* foliage on seed germination and seedling growth of Bermuda grass (Cyanodon dactylon). *Journal of AridEnvironment*, 38: 237-243.

Webber,Michael E and Amanda Cuellar D. 2008. Cow Power. In the News: Short News Items of Interest to the Scientific Community." Science and Children os. 46(1): 13-27.

WEC (World Energy Council). 1994. New Renewable Energy Resources: a guide to the future.

Werner Kossmann, U.P., 2007. Biogas Digest-Volume-I.Eschborn: Information and Advisory

www.envitec-biogas.com/fileadmin/.../EnviTecForumBiogasSpezial1En.

Yadvika.,Santosh., Sreekrishnan, T.R., Kohli,S., VineetRana. 2004. Enhancement of biogas production from solid substrates using different techniques. *Review of Biotechnology*. 95:1-10.

Yan L, Shuya, T.2006. Ethanol fermentation from biomass resources: Current state and prospects. *Applied Microbial Biotechnology.* 69(6):627-642.

Zaher, U., D. Cheong, B. Wu, and Chen, S. 2007. Producing Energy and Fertilizer from Organic Municipal Solid Waste. Washington State University. Washington.

Zamudio Canas. EM. 2010. Technical Feasibility of Anaerobic co-digestion of Dairy Manure with chicken Litter and other Wastes. Master's Thesis, University of Tennessee. 10-12.

7. APPENDIX

Appendix Table 1: Daily biogas yield in 28 days fermentation time

Day	Treatment				
	T1	T2	T3	T4	T5
D1	0	0	0	0	0
D2	6.93	4.433	16.433	7.573	9.933
D3	9.93	13.8	33.266	13.226	36.53
D4	20.2	20	44.23	40	23.03
D5	25.93	21.5	54.23	31.76	36.88

D6	30	21	43.73	30.03	27.5
D7	24.86	28.16	45.43	28.8	32
D8	17.13	20.26	45.5	26.53	21
D9	18.56	18.13	40.83	28.04	25
D10	15.56	11.23	20.43	19	19
D11	23.8	10	26.8	12.86	12.55
D12	10.5	10.466	19.76	20	10.5
D13	12	11	17.83	18.5	8.29
D14	11.5	10	17.93	14.06	10.34
D15	13	11.166	16	13.03	8
D16	11.9	10.16	12	12.766	7.43
D17	10.5	9.33	9	10.5	6
D18	10.16	8.06	10	11.83	5
D19	7.83	6.33	10.16	11	5.1
D20	8.83	5.66	4.5	10.83	3.3
D21	6.93	6.95	5.9	6.83	3.73
D22	7.66	4.166	4	2.5	2.86
D23	6	3.833	3	4.4	2.36
D24	3.16	2.7	2	2.2	2.1
D25	2.76	2.06	2	1.523	0
D26	1.86	1.933	1.6	1.466	0
D27	0	0	0	0	0
D28	0	0	0	0	0

Appendix Table 2: Total Biogas production from five treatments

Treatments	Mix ratio	Total biogas(ml)
T1	100%*P.juliflora*	317
T2	100%CM	272
T3	75%*P.juliflora*+25%CM	506

T4	50%*P.juliflora*+50% CM	379
T5	25%*P.juliflora*+75%CM	318

Appendix Table 3: Average percentage composition of VS and TS Values of Substrate before and after AD

Treatment	Initial VS%	Final VS%	VS% Removed	Initial TS%	Final TS%	TS% Removed
T1	76.5	74	2.5	92	25.4	66.6
T2	61.2	60	1.2	89	38.1	50.9
T3	72	37.6	34.4	91	26.8	64.2
T4	68.26	67	1.26	90.8	26	64.8
T5	63	61	2	90.2	34.9	55.3

Appendix figure 1: Daily biogas yield in ml from T1

Appendix figure 2: Daily biogas yield in ml from T2

Appendix figure 3: Daily biogas yield in ml from T3

Appendix figure 4: Daily biogas yield in ml from T4

Appendix figure 5: Daily biogas yield in ml from T5