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Biogas Production by Anaerobic Co-Digestion of Cactus Cladodes with Cow Dung and Goat Manure

A Thesis Submitted to the School of Graduate Studies of Jimma University in Partial Fulfillment of the Requirements for the Master of Science Degree in Sustainable Energy Engineering

BY

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Jimma University

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School of Graduate Studies

Faculty of Mechanical Engineering

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ABSTRACT

Production of biogas through anaerobic digestion of organic waste materials provides an alternative environmentally friendly renewable energy. In this study, biogas production from co-digestion of cladodes of *Opuntia ficus-indica* with cow dung and goat manure in the same mix ratios was evaluated under mesophilic conditions (38ºC) using batch digester in bioenergy laboratory of Addis Ababa institute of technology . In all substrates, TS and VS, organic carbon, percent moisture and pH were measured before and after digestion. The daily biogas production was subsequently measured by using 100ml calibrated gas syringe and methane content of the gas was measured by using gas analyzer for 14 days. All measured physico-chemical parameters of each substrate were significantly varied between before and after AD, and also the rate of variation of these parameters between before and after AD was varied between substrates. Gas production was noticed in all of the substrates types from the first day of digestion experiment and went to minimum at about 14 days in all substrates. Assessment of cumulative biogas production revealed that substrate in a mix ratio of 66.4% GM and 33.6% CC showed the highest production, suggesting this mixture of the two substrates is an optimal mix to yield better biogas. Overall results indicate that the biogas yield and VS and TS reduction can be significantly enhanced when CD, GM and CC are co-digested. The mean for average biogas yield and methane percentage of the four treatments, respectively was carried out using simple mean and average mean calculation equations. The percentage of methane gas obtained from the experiment for treatments CC, CC+CD+GM, CC+CD, and CC+GM were 56.02%, 72.6%, 56.65%, and 67.95% respectively. Among all treatments CC+CD+GM was found to produce high methane percent of the biogas. Treatments (CC+CD+GM and CC+GM) that have C: N ratio within the range of 20-30 found to perform better in biogas yield and methane production than those are not. The experimental findings further showed that the composition of methane for all treatments were within the range of 50 to 72.6%. As determined in laboratory the Physico-chemical characteristics of the Cladodes further revealed the suitability of the substrate for biogas production and if suitable materials for co-digestion, such as manure, are not available, Cladodes can be digested alone.

Keywords: Biogas, co-digestion, Cladodes, cow dung, goat manure, percentage of methane.

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DECLARATION

I, the undersigned, declared that this research work entitled **"**Biogas Production by Anaerobic co-digestion of cactus cladodes with cow dung and goat manure" is my original work and has not previously submitted by any other person for an award of a degree to this or any other university.

Name: -Mathewos Melore

Signature ____________

Date --06/08/2020--------

As thesis research advisors, we hereby certify that we have read and evaluated this thesis entitled "Biogas Production by Anaerobic co-digestion of cactus cladodes with cow dung and goat manure" prepared under our guidance by Mathewos Melore. We recommend that it be submitted as fulfilling the thesis requirement.

Professor Venkata Ramaya Major Advisor	Signature	06/08/2020 Date
Mr. Fikadu Kifle (Msc) Co-advisor	Signature	06/08/2020 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 Mathewos Melore and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of **Master of Science in Sustainable Energy Engineering.**

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DEDICATION

This thesis manuscript is dedicated to my dear wife Wubalem Tamirat, my dear kid Hasset Mathewos and my dear friend Mekonnin Lemma, and everyone who have supported me throughout the process. Your love, support and encouragement have immeasurably guided me throughout the process.

ACRONYMS

AD	Anaerobic Digestion
BOD	Biological oxygen demand
C/N	Carbon/Nitrogen
CAM	Crassulacean acid metabolism
CD	Cow dung
CC	Cactus Cladodes
COD	Chemical Oxygen Demand
GM	Goat manure
HRT	Hydraulic Retention Time
MSW	Municipal Solid Waste
NBP	National Biogas Program
OLR	Organic Loading Rate
SRT	Solid Retention Time
TS	Total Solid
V	Volume
VFA	Volatile Fatty Acids
VS	Volatile Solid

1. INTRODUCTION

1.1. Background

In developing countries the availability of adequate energy to satisfy basic needs, improving social welfare and achieving economic development is one requirement for sustainable development (Rogner ,2004) .Some of the issues associated with the current major sources of fuel include the fact that they are non-renewable and therefore can be exhausted, and that they contribute to generation of greenhouse gases leading to global warming, and consequently climate change and its negative environmental impacts (Moshi, 2015).

To date, corn and sugarcane are the main feedstock used in the biofuel production (Cruz, 2018) ; this is attributed to their relatively simple conversion to biofuel as well as availability of infrastructure for planting, harvesting, and processing which are already in place. Nevertheless, their large scale cultivation for biofuel production is associated with issues such as decrease in food availability and dramatically increases in food prices worldwide (). Therefore, there is a need to find alternative sources in order to reduce competition of these natural materials which are also used as human food and animal feed. Presently, with the reality of global warming, crassulacean acid metabolism (CAM) plants that can withstand and resist drought have become more attractive as feedstock for anaerobic digestion(Yang,2015).Among these plants is the fast-growing *Opuntia ficus indica*, which is known to have high w**a**ter use efficiency. *Opuntia ficus indica*is the most widely distributed species of the cactus family (Nobel, 2002). This plant have been reported to pose a great potential as source of lingo cellulosic biomass with a yield of 10to50 tone dry mass/(year·ha) (Calabr, 2017).

These are desert plants that can survive where most of the plants cannot grow (Tarisse, 2008),Hence suitable plant resource for climate change adaptation. Using spineless cacti as a potential energy generating crop may offer serious perspectives to countries prone to drought and relying on imports for their energy consumption (Nobel, 2008). Moreover, the ability to grow on unfertile land will make use of the land that currently is not occupied with agricultural crops and hence improve land utilization. The fact that spineless cacti are not used as food in most areas would reduce the competition of food versus fuel use (Calabr, 2017).There is an increasingly interest in evaluating the potential of *Opuntia ficus indica* feedstock for anaerobic digestion and biogas production (Jigar, 2011) (M, 2014)(Calabr, 2017); (Ramos, 2014), (Yang, 2015). Nevertheless, limited studies have dealt with anaerobic co-digestion the plant cladodes prior to anaerobic digestion and the effect they could have on both methane production and yield

*Opuntiaficus-indica*grows abundantly in northern part of Ethiopia. It is considered to be important energy crop for biogas production because of its high organic matter yield per hectare and high availability to supplement cow dung for biogas production. In addition, this plant can easily be propagated and tolerate drought and poor soil fertility.
Cattle dung has been used as the major feed material for anaerobic digesters which is not likely to have significant impact. This calls for widening the scope of this technology by tapping other organic materials like energy crops. Using spineless cacti as an energy crop is offering serious perspectives to countries prone to drought and relying on imports for their energy consumption (Tarisse, 2008). (Jemal, 2018)Reported that cactus biomass is highly organic that has less nitrogen, therefore it needs feed stocks which are rich in nitrogen, if used as substrate for biogas production. Further investigation will be needed to confirm which nitrogen reach substrate is suitable for co-digestion with cactus for optimum biogas production .The main objective of this paper is to maximize the yield of methane production from cladodes of Opuntia ficus indica through anaerobic co- digestion with cow dung and goat manure.

1.2. Statement of the problem

Availability of suitable energy source to sustain the needs of rural communities in African countries remains one of the greatest obstacles for development. The continual use of fossil fuels and its effect of greenhouse gases (GHGs) on the environment necessitate more efforts in the production of alternative fuels from bio resources. Global energy demand have led to the increase in the use of fossil fuels making up to approximately 88% of the energy produced presently, this in turn drastically increases the amount of GHG emission into the atmosphere. Reliability of fossil fuels should be reduced for the security of energy supply and because most of natural energy resources including oil are non-renewable.

The prospect of an increasingly hotter and drier climate has led many researchers to reevaluate heat and drought tolerant CAM species for use as feed stocks for bioenergy production on semi-arid and arid lands. Of these groups of CAM plants Agave species have been most studied and the potential of Opuntia ficus indica, which is one of the species under cacti group have been for many years overlooked.

Studies have been carried out to evaluate the potentials of Opuntia in anaerobic digestion for biogas production but limited studies have dealt with co-digestion of the plant cladodes prior to anaerobic digestion and the effect they could have on both methane production and yield. Currently, there are limited scientific reports on the anaerobic digestion of Opuntia plant with regard to biogas production and methane yield. There is scarce documentation on the enhancement of biogas production and methane yield using Opuntia as feedstock by anaerobic co-digestion with cow dung and goat manure. It is therefore important to assess the impact that co-digestion have on the anaerobic digestion of Opuntia.

pointed out that to maintain the C/N level of the digester material at optimum levels, substrates with high C/N ratio can be co-digested with nutrient rich plant waste (low C/N ratio) like Opuntiaficus-indica waste.

Opuntiaficus-indica has been reported as a good substrate for co-digestion studies and the literature has encouraging reports regarding the use of fresh cow dung for co-digestion purpose .

The total nitrogen (TN) contents of fresh GM (1.01%).TN content is beneficial to co-digestion with cactus cladodes because it decreases the carbon-to-nitrogen (C/N) ratios of single cactus cladodes substrate.GM is also insensitive to acidification during anaerobic fermentation. Hence, GM is an excellent raw material for AD. Although various raw materials, such as agricultural waste, animal manures, sewage sludge and food waste have been reported as potentially feasible for co-digestion the suitable mixing ratios of multi-component substrates between GM and various Cactus are largely unknown. Therefore we can maximize the biogas production from cactus cladodes by co-digesting with low C/N substrates such as goat manure and cow dung. This study will improve the yield of biogas production.

1.3. Significant of the research

The need for clean energy and phasing out the fossil fuels which have high amount of GHG emissions in the atmosphere is continuously rising. This necessitates turning to 'greener alternatives' which will have sustainable clean energy production, the use of Opuntia as feedstock being one of them.*Opuntia ficus indica* is one of the heat and drought-durable CAM species suitable for use as bioenergy feed stocks on semi-arid and arid lands ((Consoli., 2013). The plant is found in abundance in these parts and therefore the feedstock for biogas production is not limited. In addition, the plant may not be significantly affected by climate change and its cultivation requires low agronomic input (Nobel and Bobich, 2002). These plants are recognized as ideal crops for arid regimes because they are extremely efficient at converting water into biomass (Cushman, 2015). The fact that spineless cacti is not used as food in most areas would reduce the competition of food Versus fuel use and represent an inexpensive renewable energy source, which, through anaerobic digestion and biogas production, has a very good potential to contribute to sustainable energy supply.

The conversion of lingo-cellulosic biomass in methane production usually requires some form of pretreatment prior to anaerobic digestion to facilitate enzymatic hydrolysis (HahnHa, 2006). Biological pre-treatment reduces the problems caused by other forms of pre-treatment such as chemical, thermal and mechanical methods which have high financial or environmental cost, while increasing the hydrolysis of the feedstock during anaerobic digestion and increase the overall methane yield (Carlsson, 2012). This study provides detailed information on the effect of anaerobic co-digestion on methane yield during anaerobic digestion of Opuntia ficus indica.

Using these species as feed stocks would inform future biofuel production plans on waste or bare land that is currently not used for the production of C3 and C4 crops and provide the possibility of targeted cultivation, harvesting, and utilization strategies of the species as feed stock in biogas production. This in turn will provide or increase employment to the growing young generation who can take part and participate in the whole production process.

Producing biogas from cactus is significantly preventing soil erosion in tropical and semi-arid region of Ethiopia. To prevent deforestation by using biogas for cooking and baking activities, this energy also minimize the health problem that are related to burning of fire wood and minimize the problem of children absence from school. The energy of the cactus is clean, inexhaustible, creates bonds of carbon, permanent jobs and solves the energy problem in a sustainable manner in the short, medium and long term. The production of biogas from cactus helps the people by supplying gas for lightning and cooking purposes.

1.4 Objectives

1.4.1. General objective

The general objective of this study was to maximize methane yield of cactus cladodes of *Opuntiaficus indica*to produce biogas through anaerobic digestion alone and in combination with fresh goat manure and cow dung.

1.4.2Specific objectives

- ✓ To characterize the cactus cladodes, cow dung and goat manure biomass in terms of total solids (TS) and volatile Solids (VS), fixed solids, organic carbon, and moisture content.
- ✓ To find out the quantity of biogas production with different combination of feed stocks.
- ✓ To determine the quality of biogas production from cactus cladodes and its combination with cow dung and goat manure in terms of methane percentage.

1.5 Scope of the study

The scope of the study was to characterize the cactus cladodes, cow dung and goat manure biomass in terms of total solids (TS) and volatile Solids (VS), fixed solids, organic carbon, and moisture content and to find out the optimal cactus cladodes, cow dung and fresh goat manure mix for high biogas production.

2. LITERATURE REVIEW

2.1 Biogas Production

Biogas is a methane rich gas produced by anaerobic breakdown of organic wastes with the help of archaebacteria under oxygen free environment and it comprises 60% of methane, 40% of carbon dioxide and 0.2 - 0.4% of hydrogen sulfide (Molina, 2007) The natural generation of biogas is an important part of the biogeochemical carbon cycle. Archaebacteria are the last link in a chain of micro-organisms which degrade organic material and return the decomposition products to the environment. In this process biogas is generated, as a source ofrenewable energy (Werner, 2000).

It is a flammable gas made of a mixture of gases produced by methanogenic bacteria while acting upon biodegradable materials in an anaerobic condition. Biogas is a colorless gas that burns with clear blue flame. It is about 20% lighter than air and has an ignition temperature in the range of 650° to 750° (Claude,2009).

Biogas consists of mainly methane, carbon dioxide, hydrogen sulfide and traces of other gases (Table 2.1). Methane is produced by the anaerobic breakdown of organic materials including agricultural wastes, organic kitchen wastes etc., (Werner, 2000).Biogas producing microorganisms include organic material splitting bacteria and archaebacteria that degrade complex organic materials to produce methane under anaerobic conditions (Claude, , 2009). The resulting bio-slurry, which is used as fertilizer has a reduced load of parasitic diseases and pathogenic bacteria for crop production (Environment, 2010).

Biogas production is a simple technology that helps to reduce the use of forest resource for house hold energy consumption, and hence prevents deforestation (Dagnachew, 2003).

Table 2.1: Chemical compositions of biogas

Constituents	%Composition
Methane	55-75
Carbon dioxide	30-45
Hydrogen sulphide	1-2
Nitrogen	0-1
Hydrogen	0-1
Carbon monoxide	Traces
Oxygen	Traces

Source: *(Madu and Sodeinde, 2001)*

2.2.Biogas Production for Sustainable Environments

Replacing biomass energy with biogas could help to solve a lot of problems in the environment that are typically associated with using biomass fuels. The indoor air quality of homes will be dramatically improved as a result of employing biogas instead of burning biomass directly(Li, 2005). Substituting biogas for firewood also helps to reduce the pressure on forests for energy demand. This in turn has important implications for watershed management, slowing down deforestation and soil erosion. This in turn maintains water cycle and avoids recurrent drought (Dagnachew, 2003).

The use of slurry improves nutrient recycling in agriculture and can substitute chemical fertilizers, thus reducing the related environmental problems (Shrestha, 2010).

2.3. Biogas Technology in Ethiopia

Biogas technology is a promising option for the most efficient utilization of organic waste in a fermentation tank. It involves organic matter, microorganisms, an environment that lacks air (oxygen) and optimum temperature to produce biogas. Biogas technology also has various benefits for human beings, such as socio-economic and environmental benefits. The environmental benefits of biogas technology include improvement of indoor air quality, better management of animal manure and human excreta, thus improving sanitary conditions in the immediate vicinity of the rural homes. Through reduction of deforestation, the technology also helps in a better watershed and soil management. The use of slurry helps in improving the soil nutrient and use of biogas for energy also helps in reserving the expenditure of imported petroleum products. At a global perspective, reduction in the use of fuel wood, dung cakes and kerosene reduces greenhouse gas emission (Charushre, 2009).

In Ethiopia, biogas technology was introduced in 1979 and the first batch type of biogas digester was constructed at Ambo Agricultural College. However, the technology was less disseminated until the National Biogas Program (NBP) was launched in 2008 (Eshete, 2006). Much of the energy derived from biogas technology had been allocated for household energy consumption since its introduction to Ethiopia in 1979 (Siltan, 1989) Currently around 40 % of these biogas plants are not operational due to lack of effective management, technical problems, loss of interest, reduced animal holdings, evacuation of ownership and water problems (NBP, 2007).

After the establishment of national biogas program, close to 859 biogas plants have been constructed and in a regular use. Out of these 206 were in Tigray region, 143 in Amhara region, 330 in Oromia region and 180 are found in SNNP regional state (Yitayal, 2011). Pointed out that at least over one million household in Amhara, Oromia, Tigray and Southern Nations, Nationality and People's Regional States have the potential for the installation of a domestic biogas plant. The domestic biogas technology attracted interest mainly due to consideration of animal dung, which is available in many rural households of the country.

2.4. Feedstock for Biogas Production

Though there can be variation in biogas production potential among feedstock, all organic materials can serve as substrate for biogas production in sole or in combination. Plant materials and animal manure have recently been used for production of biogas by co-digestion under anaerobic condition. The co-digestion of plant material and animal manure increases the rate of biogas production as compared to the sole digestion of feedstock. Because mixing substrates was found to balance between carbon and nitrogen ratio(Mashad, 2004).In this study, co-digestion of goat manure, cow dung and cactus cladodes have been considered for biogas production under anaerobic condition in sole and mixed in different ratio.Opuntia ficus-indica grows abundantly in northern part of Ethiopia. It is considered to be important energy crop for biogas production because of its high organic matter yield per hectare and high availability to supplement cow dung for biogas production. In addition, this plant can easily be propagated and tolerate drought and poor soil fertility. Given the large production of this species in northern parts of Ethiopia

2.4.1 Description of cactus (Opuntia ficus indica)

Anaerobic digesters can be fed with various organic biomasses such as purpose grown energy crops like maize but due to food insecurity the focus is moving mostly toward nonfood crops including CAM plants (Mata, 2000). *Opuntia ficus indica* (L)Mill (Plate 2.1) is found under genus Opuntia, which belongs to the subfamily Opuntioideae, family cactaceae which is xerophytic family consisting of about 200 to 300 species (Stintzing and Carle, 2005).

Figure 2.1:-*Opuntia ficus-indica***Sources; renewable energy world.com**

Opuntia ficus indica is one of the species found under CAM group of plants which is widely distributed in the arid and semi-arid regions throughout the world (Borland, 2009). Opuntia ficus indica is the most widely distributed species of the cactus family and at the same time the most economically important (Nobel and, 2002). The plant, which has succulent and thick stems called cladodes, grows up to 3-5m in height (Borland,2009). It normally produces flowers when they are 1-2 years old, and later on form fruits (Stintzing and Carle, 2005). Natural hybridization, associated with polyploidy and geographic isolation, has led to a great genotypic variability of Opuntia, displaying at the same time high levels of phenotypic plasticity (Wallace and Gibson, 2002).

Opuntia ficus indica is native to Mexico, but it is widely distributed and adapted to the arid and semi-arid regions of South and Central America, Africa and the Mediterranean area (Mohamed, 1995). Due to the trend of Mediterranean area moving towards global desertification and decline of water resources, *Opuntia ficus-indica* has a great potential as feedstock in anaerobic digestion (Calabr ., 2017). Using spineless cacti as an energy crop is offering serious perspectives to countries prone to drought and relying on imports for their energy consumption (Tarisse, 2008).

There are studies which have been done to evaluate the potential of *Opuntia ficus indica* in anaerobic digestion for biogas production. During an experiment in a semi-continuous 1m3 mesophilic digester, the biogas potential of Opuntia with methane yield equal to around 500 mLCH4/gVS was reported (Obach and Lemus, 2006). Other studies have reported as low as 244 NmLCH4/gVS in the production of methane from Opuntia (OrtizLaure, 2014).

Cactus pear was introduced to Ethiopia between 1848 and 1920 (Habtu, 2005). The plant is widely distributed in the arid and semi-arid regions of the country; especially in eastern and southern zones of Tigray Region of Ethiopia.

2.4.2 Cow Dung

Cow manure has a medium biogas potential. It should be kept in mind that cow manure is made up of two fractions: a rapidly biodegradable one (which is soluble in water) and a slowly biodegradable part, which is mainly lignocellulosic fiber.

Due to the low C/N ratio, anaerobic co-digestion of manure with lignocellulosic residues, with high C/N ratios, is a convenient alternative (Neshat, 2017). Manure has been co-digested with diverse residues. Cow manure and sewage sludge were used as primary waste along with kitchen waste, yard waste, floral waste, and dairy wastewater as co-substrates (Kumari, 2018).

2.4.3 Goat Manure

The total nitrogen (TN) contents of fresh GM (1.01%) and chicken manure (1.03%) are significantly higher than those of dairy manure (0.35%) and swine manure (0.24%). High TN content is beneficial in co-digestion with crop residues because it decreases the carbon-to-nitrogen (C/N) ratios of single Crop residues. GM is also insensitive to acidification during anaerobic digestion (AD). Hence, GM is an excellent raw material for AD and biofuel production (Zhang, 2013.) .

2.5. Anaerobic Digestion for Biogas Production

Anaerobic digestion is a process of controlled decomposition of biodegradable materials under managed conditions where oxygen is absent. It is a complex process that requires specific environmental conditions and different bacterial populations. The bacterial populations degrade organic compounds so as to produce a valuable high energy mixture of gases (mainlyCH_4 and CO_2) and a nutrient rich fertilizer(Sandars, 2003).The process usually prefers mesophilic or thermophilic condition for anaerobic archaebacteria to convert the inputs into biogas (Steinmetz, 2013).

According to (Li., 2009)anaerobic digestion is considered as waste-to-energy technology and it is widely used in the treatment of different organic wastes. It consists of mixed biological systems in which organic materials such as carbohydrate, lipids and proteins are utilized by microorganisms to produce methane and carbon dioxide-rich in their normal metabolic activities. The anaerobic digestion involves a large number of microorganisms including hydrolytic bacteria, acetic acid-forming bacteria and methanogenic bacteria, which convert the feedstock to the methane and carbon dioxide rich biogas through a number of different processes (Ciborowski, 2004).

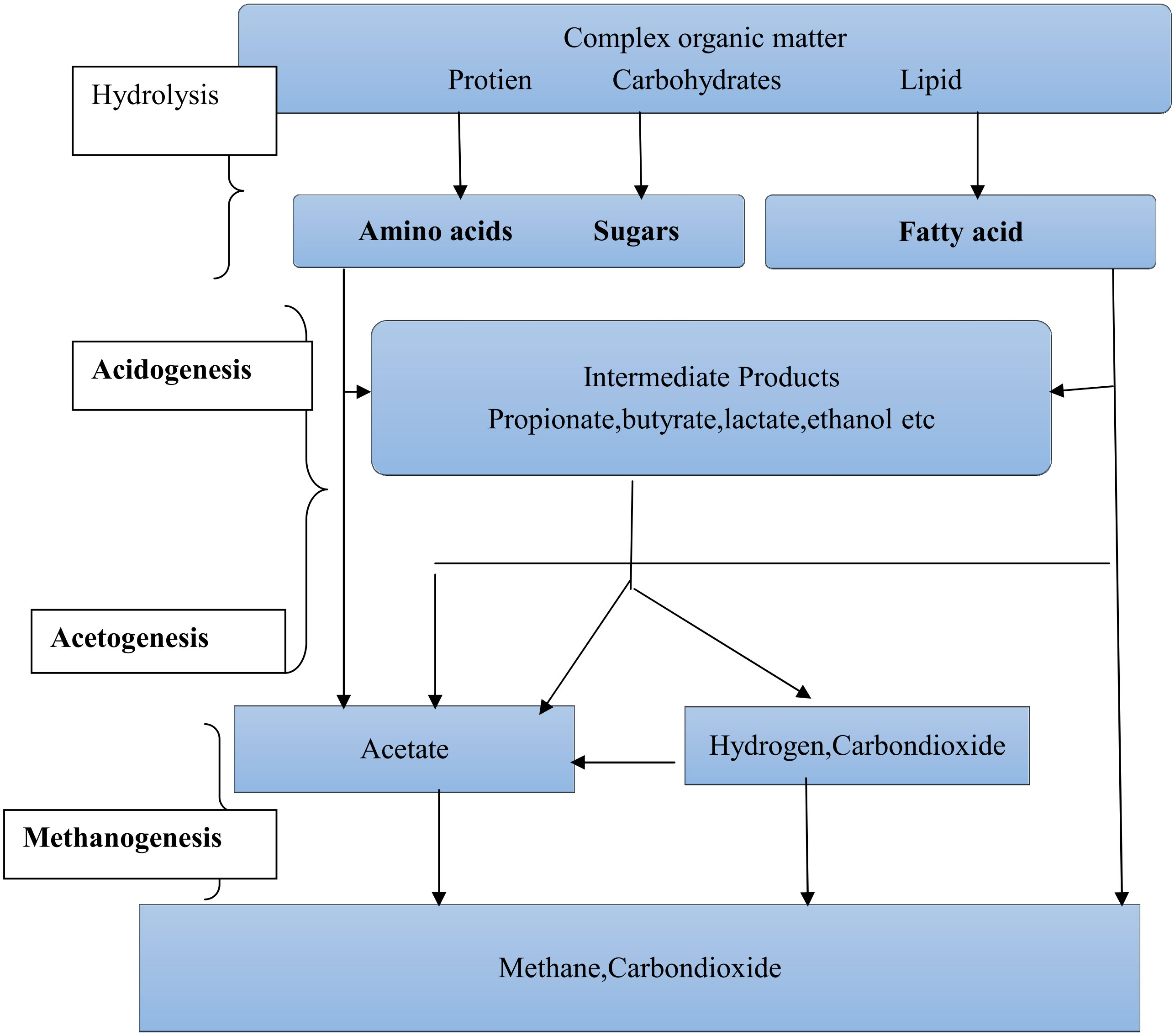

Figure 2.2: Biochemical stages of anaerobic digestion/biogas product ((Jewitt, 2009)

2.5.1. Hydrolysis

Hydrolysis is the first step of anaerobic digestion in the degradation of large organic matter like polysaccharide, protein and fat into their monomers, such as sugars, amino acids and fatty acids using water as a medium of reaction (Parawira, 2008). This is formed by extra hydrolytic enzymes. The hydrolytic enzymes include cellulase, hemi cellulose amylase, lipase and protease (Parawira, 2008) .Many cellulose degrading organisms have their enzymes attached to the cell wall and simultaneously they attach to the substrate for more effective degradation. The hydrolysis of complicated structure such as lingo-cellulose requires weeks (Gerardi, 2003).As such hydrolysis is time limiting step, while the methanogenesis is considered rate-limiting step for already available substrate (Vavilin.V.A, 2008).

In hydrolysis, complex organic substances are converted to simple ones. For example, carbohydrate to sugar, fats to fatty acids and protein to amino acids by hydrolytic bacteria. This step takes longer time due to limiting accessibility of the extra cellular enzymes to intra cellular polymeric materials which are protected by cell covering (Navia, 2002).

2.5.2. Acidogenesis

During acidogenesis, the monomers formed in the hydrolysis stage are taken up by anaerobic bacteria and degraded in the acidogenic stage. The aim of this stage is that to degrade the results in hydrolysis stage into shorter chain and convert into alcohol, hydrogen, ammonia, carbon dioxide and organic acid such as butyric acid, propionic acid, acetic acid. An organic acid produced in this stage is called intermediate products (Garedi, 2003).

2.5.3. Acetogenesis

Products from acidogenesis are converted to CH_3COOH, H_2 and CO_2 by acetogens. These products are formed from organic acids. In the acetogenesis process simple molecules are created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen. Acetogenic organisms are the vital link between hydrolysis-acidogenesis and the methanogenesis in anaerobic digestion. Acetogenesis provides the two main substrates for the last step in the methanogenic process material, namely hydrogen and acetate (Buswell, 1948)

2.5.4. Methanogenesis

The production of methane and carbon dioxide from intermediate products is carried out by methanogenic archaebacteria. Formic acid, acetic acid, methanol and hydrogen can be used as energy sources by the various methanogenesis (Dhadse, 2012). The formation of methane is the ultimate product of anaerobic treatment. The acetoclastic group comprises two main methanogenic bacteria (Zaher, 2007).

High concentration of hydrogen disables acetogens to act and that is why it is of importance that methanogenic bacteria use the hydrogen (Bie, 2002). The vital functions of these archaea bacteria are that to consume hydrogen in stable temperature modes (Zorg, 2009)

The methane former works slower than that acid former, therefore, the pH has to stay constant consistently, slightly basic, to optimize the creation of methane. One needs to constantly feed in sodium bicarbonate to keep pH slightly basic (Prebeheim,2010). This compound involves in the conversion of simple compounds (acids) into methane and CO_2 by CO_2utilization anaerobic methanogenic bacteria (Itodo, 2001).

Three biochemical pathways are used by methanogens to produce methane gas. The pathways along with the stoichiometries of the overall chemical reactions are:

Acetotrophic methanogenesis: $4\ CH_3COOH \rightarrow 4\ CO_2 + 4CH_4$ (2.1)

Hydrogenotrophic methanogenesis: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$ (2.2)

Methylotrophic methanogenesis: $4CH_3OH + 6H_2 \rightarrow 3\ CH_4 + 2\ H_2O$ (2.3)

The first and second reaction above involves aceticlastic methanogenesis and reductive methanogenesis, respectively (Satoto, 2010). Methanol is shown as the substrate for the methylotrophic pathway, although other methylated substrates can be converted. Sugars and sugar-containing polymers such as starch and cellulose yield one mole of acetate per mole of sugar degraded. Since acetotrophic methanogenesis is the primary pathway used, theoretical yield calculations are often made using this pathway alone (Edison, 2014).

According to the solid content of the material digested and the temperature at which the process operates, the various bio gasification processes can be classified as under:

a. Wet anaerobic digestion:

Wet digestion means a process where the substrate contains less than 12% TS and is possible to pump (Catarina, 2011). Wet anaerobic digestion is suitable for treatment of wastes with low solid concentration such as sewage waste, industrial wastewaters, slaughterhouse waste and etc. Digestion takes place in a stirred tank. Stirring is required to maintain an even temperature and prevent foaming and sedimentation. To avoid problems with mixing, the material needs to be fine. In drier substrates liquid might need to be added in order to obtain a pumpable consistency. Various types of stirring in the digestion tank occur. The most common method is the propeller stirring (Catarina, 2011). Wet anaerobic digestion has relatively lower retention time as the movement of the micro-organisms in the solution is not impeded by solids in the digester. The main challenge of this method is that the digester volume is not effectively used and also it incurs cost due stirring.

b. Dry anaerobic digestion:

Dry digestion is a process where the substrate contains 20 to 40% TS. This process is mainly used for stackable substrates such as organic waste, solid manure and crop residues. No mixing equipment is necessary, and crust formation is not possible due to the relatively solid nature of the digester contents (Catarina, 2011). Dry anaerobic digestion relatively helps to effectively use the digester volume in waste treatment. In dry or high-solids systems, handling material at high solids concentration requires different pre-treatment and transfer equipment like conveyor belts, screws, and special pumps for the highly viscous streams. Research in the 1980"s indicated that biogas yields and production rates for single-stage dry systems were as high as or greater than that of wet systems. The challenge of dry systems is handling, mixing, and pumping the high-solids streams rather than maintaining the biochemical reactions (California Integrated Waste Management Board, 2008).

2.6. Factors That Affect the Rate of Anaerobic Digestion

Environmental factors which influence the process of biological reaction amenable to the external control in the anaerobic process. Any drastic change in these factors can adversely affect the biogas production (Chatterjee, 2007).The performance of anaerobic digestion plants can be controlled by studying and monitoring the various parameters (Yadvika, 2004)

2.6.1 Moisture Content

High moisture contents usually facilitate the anaerobic digestion; however, it is difficult to maintain the same availability of water throughout the digestion cycle. Initially water added at a high rate is dropped to a certain lower level as the process of anaerobic digestion proceeds. High water contents are likely to affect the process performance by dissolving readily degradable organic matter. It has been reported that the highest methane production rates occur at 60–80% of humidity. Methanogenesis processes during anaerobic digestion at different moisture levels i.e., 70% and 80% (Khalid, 2011).

According to (Sadaka, 2003)water content is one of the very important parameters affecting anaerobic digestion of solid wastes. There are two reasons viz.; (a) water make possible the movement and growth of archaea facilitating the dissolution and transport of nutrient and (b) water reduces the limitation of mass transfer of non-homogenous or particulate substrate.

2.6.2. Temperature

Anaerobic digestion may be carried out under psychrophilic, mesophilic or thermophilic conditions. In the sewage sludge mesophilic anaerobic digestion is more widely used compared to thermophilic digestion, because of the lower energy requirements and higher stability of the process. However, thermophilic digestion is more efficient in terms of organic matter removal and methane production (Ahring, 2001)

Methane production has been documented under a wide range of temperatures but archaebacteria are most productive in either mesophilic conditions 25-45°C, or in the thermophilic conditions at 50-65°C (Ostrem, 2004).A thermophilic temperature reduces the required retention time. The microbial growth, digestion capacity and biogas production could be enhanced by thermophilic digestion, since the specific growth rate of thermophilic bacteria is higher than that of mesophilic bacteria (Kim, 2002).

2.6.3. pH

The acidity of substrate is measured by pH meter, which is an important parameter affecting the growth of microbes during anaerobic digestion. The acid concentration in aqueous systems is expressed by the pH value, i.e., the concentration of hydrogen ions (Yadvika, 2004).

pH values below 6.8 inhibit the archaebacteria activity. To avoid drops in pH chemicals are added to the organic substrate to producing a buffer capacity. Sodium bicarbonate, sodium hydroxide, sodium carbonate and sodium sulphide are the most used chemicals (Esposito, 2012).pH value of 7 is neutral, less than 7 is acidic and more than 7 is alkaline. During anaerobic fermentation, microorganisms require a neutral or mildly alkaline environment for efficient gas production. Biogas production needs an optimum pH value of 6.8 to 7.2 (Mahanta, 2004).The rate of methanogenesis may decrease if the pH is lower than 6.8 or higher than 7.8.The pH of the digester is created by concentration of volatile fatty acids produced, bicarbonate alkalinity of the system and the amount of carbon dioxide produced (Gomec, 2003).

2.6.4. Particle size

The production of biogas is also affected by particle size of the substrate. Too big particle size is problematic for microbes to digest and it can also result in blockage in the biodigester. However, small particle size gives a large surface area for substrate adsorption and thus allows the increased microbial activity followed by increase in the production of gas (Yadvika., 2004).According to the reports of (Asnake., 2008)out of the five particle sizes (0.088, 0.40, 1.0, 6.0 and 30.0 mm), maximum quantity of biogas was produced from raw materials of 0.088 and 0.40 mm particle size. Large particles could be used for succulent materials such as leaves. However, for other materials such as straw, large particles could decrease the gas production. The results suggested that a physical pretreatment such as grinding could significantly reduce the volume of digester required, without decreasing biogas production (Gollakota, 1988).

2.6.5. Retention Time

The number of days the organic material stays in the digester is called the retention time. There are two significant retention times in an anaerobic digester: solids retention time (SRT) and hydraulic retention time (HRT). The SRT is the average time the bacteria (solids) are in the anaerobic digester. The HRT is the time the liquid is in the anaerobic digester. The process of degradation requires at least 10-30 days in mesophilic condition, while in thermophilic environment HRT is usually shorter (Demetriades, 2008). (Salminen, 2002), reported even a longer retention time of 50 – 100 days for a digester treating solid waste from

Opuntiaficus-indica. The advantages of high SRT values in anaerobic digesters include maximizing the gas recovery capacity and the buffering capacity to protect against the effects of shock loadings and toxic compounds in feedstock, as well as permitting the bacteria to acclimate to toxic compounds (Arogo, 2009).The same author pointed out that SRT is the more important retention time, and should be determined correctly because it indicates the potential of bacteria washout. If a significant washout of bacteria occurs, the digester can fail.

2.6.6. Toxicity Effects

Mineral ions, heavy metals and detergents are some toxic materials that inhibit the normal growth of pathogens in the digester. Small quantity of mineral ions (e.g., sodium, potassium, calcium, magnesium, ammonium and sulphur) also stimulates the growth of bacteria, while very heavy 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 such as copper, nickel, chromium, zinc, lead etc., in small quantities are essential for the growth of bacteria, but their higher concentrations has toxic effects (Moharao, 1975).Detergents including soap, antibiotics, organic solvents etc. also inhibit the activity of methane producing bacteria and hence addition of these substances in the digester should be avoided (Perez, 2002)

2.6.7. Organic Loading Rate (OLR)

The rate at which substrate is supplied to the digester is referred to as organic loading rate and usually expressed in terms of Kg volatile solids per m^3 and day. In general, materials with high volatile-matter content produce more biogas if digested properly (Spencer, 1991).The same author reported that the potential danger of a rapid increase in the organic loading would be that the hydrolysis and acidogenic bacteria would produce intermediary products rapidly. Since the multiplication time of methanogenic bacteria is slower, they would not be able to consume the fatty acids at the same rate. The accumulation of fatty acids will lead to a pH drop and hampering the activity of methanogenic bacteria, causing a system failure (Agunwamba, 2001)

2.6.8. Free Ammonia

A number of studies have cited the inhibitory effects of free ammonia (NH_3) on the metabolism of methanogens (Braun, 1981).As ammonia is added to a digester, the pH increases until a chemical equilibrium is reached (Georgacakis, 1982). However, as ammonia inhibits methanogen metabolism, VFAs accumulate, resulting in a lower pH and a lower concentration of free ammonia. (Sterling, 2001) Concluded that total biogas production was unaffected by small increases in ammonia nitrogen while higher increases reduced biogas production to 50% of the original rate. However, the underlying reason of this effect is still unknown. It also was found that the free ammonia concentration not only affects the acetate-utilizing bacteria but also the hydrolysis and acidification process (ElMashad, 2004).

2.6.9. Seeding

To start up a new anaerobic process, it is critical to use inoculums of microorganisms to commence the fermentation process. The common seeding materials include digested sludge from a running biogas plant or material from sewage (Wen and Chen, 2007).

(Forster, 2007)Indicated that digested sludge is best inoculums source for anaerobic thermophilic digestion of the treatment of organic fraction of municipal solid waste at dry conditions (30% TS). (Holm, 2009) state that inoculums caused biogas production rate and efficiency increase more than two times as compared to substrate without inoculums. (Rojas, 2010), stated that the addition of fresh cow dung to the batch reactor as part of the starter improves the biogas production.

2.6.10. Carbon/Nitrogen Ratio

(Kayhanian, 1994), reported that C/N ratio between 25 and 30 as being optimal for anaerobic digestion. However, some investigators argue that the C/N of approximately from 16.8 to 18 is optimal for methanogenic performance if poorly degradable compounds such as lignin are taken into account (Kivaisi, 1998).The digestion of plant waste containing high nitrogen to carbon ratio is more likely to result in toxic conditions for bacteria arising from the concentration of free ammonia (Arogo, 2009)

(Hartmann, 2006) Reported that a solid waste with high C/N ratio is not suitable for bacterial growth because deficiency of nitrogen. As a result, the gas production rate and solids degradability will be low. On the other hand, if the C/N ratio is very low, the degradation process leads to ammonia accumulation which is toxic to the bacteria.

(Zheng, 2009) pointed out that to maintain the C/N level of the digester material at optimum levels, substrates with high C/N ratio can be co-digested with nutrient rich plant waste (low C/N ratio) like Opuntiaficus-indica waste (Cui, 2006).

2.6.11. Agitation

The close contact between micro-organisms and the substrate material is important for an efficient digestion process. The agitation of the digester contents has a number of benefits, one of the most obvious being that it helps to mix up material, evening out any localized concentrations, thus also helping to stop the formation of 'dead zones' or scum. In addition, it increases the waste's availability to the bacteria, helps remove and disperse metabolic products and also acts to ensure a more uniform temperature within the digester. There have been some suggestions that efficient mixing enhances methane production, but the evidence is inconclusive, so it seems likely that this may only be of noticeable benefit for some systems or operational regimes (Gareth, 2003).Mixing also promotes heat transfer, particle size reduction as digestion progresses and release of produced gas from the digester contents. (Rojas, 2010)showed that there is significant stirring effect on the anaerobic digestion only when seed sludge from a biogas plant was used as a starter. In this case, the experiments without stirring yielded, without starter, only about 50% of the expected biogas for the investigated substrates.

2.6.12. Quality and Characteristics of Substrates

(Lorimor, 2000), reported that substrate properties may depend on several factors: plant species; digestibility, protein and fiber content; and environment, and stage of production. Opuntiaficus-indica can be characterized in several ways such as the solid content (the percentage of solids per unit of liquid) and the size and makeup of *Opuntiaficus-indica* (fixed and VS, suspended solids, and dissolved solids).

2.6.13. Dilution and consistency of input

All waste materials fed to a biogas plant consist of solid substance volatile organic matter and non-volatile matter (fixed solids) and water (Braun, 1981). During anaerobic fermentation process, volatile solids undergo digestion and non-volatile solids remain unaffected. According to a finding by The Energy and Resources Institute, fresh cattle waste consists of approximately 20% total solid (TS) and 80% water. TS, in turn, consist of 70%Volatile solids and 30% fixed solid. For optimum gas yield through anaerobic fermentation, normally, 8-10% TS in feed is required. This is achieved by making slurry of fresh cattle dung in water in the ratio of 1:1. However, if the dung is in dry form, the quantity of water has to be increased accordingly to arrive at the desired consistency of the input (i.e., ratio could vary from 1:1.25 to even 1:2). If the dung is too diluted, the solid particles will settle down into the digester and if it is too thick, the particles impede the flow of the gas formed at the lower part of the digester. In both cases, gas production will be less than optimum (Anonymous, 1981).It is also necessary to remove inert materials such as stones from the inlet before feeding the slurry into the digester. Otherwise, the effective volume of digester will decrease.

2.7. Co-digestion

(Fernández, 2005), described co-digestion as the term used to describe the combined treatment of several wastes with complementary characteristics, being one of the main advantages of the anaerobic technology. Recent works on co-digestion have been showed that there is synergism or antagonisms effect among the co-digested substrates. For instance, the work of (Sosnowski, 2003) showed that optimization of the carbon to nitrogen ratio was found when municipal wastes and sewage sludge are co-digested. The improvement of the buffer capacity is also reported as a positive effect in the co-digestion process by (Mshandete, 2004). On the other hand, some authors have shown negative results in co-digestion processes, which are attributed to the specific characteristics of the digested wastes. Co-digestions of cattle slurry with poultry litter (7.5% and 15% TS) gave higher cumulative productions of methane, and the system with the lower concentration of poultry litter gave a higher specific methane yield. However, there was some evidence of ammonia inhibition.

Comparing the single waste digestions with co-digestion of combined wastes, it was shown that co-digestion resulted in higher methane gas yields. In addition, co-digestion of MSW promotes synergistic effects resulting in higher mass conversion and lower weight and volume of digested residual (Macias-Corral, 2008)Opuntiaficus-indica has been reported as a good substrate for co-digestion studies and purpose (Anand, 1991).

2.8. Environmental, economic and social benefits of biogas

2.8.1. Energy and Climate Concerns

Biogas is a renewable source of energy. The carbon dioxide that is released when biogas is combusted and mixed with the oxygen in the air does not contribute to the greenhouse effect. The carbon in the methane molecule produced by the biogas process originates from carbon dioxide in the air that growing plants have previously taken up by photosynthesis.

The use of biogas is thus an important step in climate change mitigation. The development of biogas represents a strategically important step away from oil dependency that will contribute to a sustainable energy supply in the long term. Renewable means that there will be no “peak biogas”; rather biogas will be continuously available and thus offers improved energy security. Biogas is also produced locally meaning that it is not dependent on trade relationships. This also contributes to improved energy security (Lars, 2012) .

2.8.2. Increase Agricultural Productivity

Anaerobic digestion increase nitrogen fixation. The digestate is used as fertilizer. Anaerobic digestion kills certain bacteria, parasites and weed seeds that otherwise might have had negative effects on crop production. Organic biogas production helps to ensure food security (Florian, 2013) .

In Ethiopia, it leads to a reduction in agricultural productivity as a result of using dung and crop residue as fuel instead of using these as soil nutrients. Due to the use of dung as a source of domestic energy it is estimated that 10% of the annual grain production is lost for the Tigray region. Through the biogas programme the utilization of slurry is promoted, thus contributing to increased crop production (NBPE, 2008).

2.8.3. Sustainable Development

Production of biogas offers many benefits to society and is an important contribution to a sustainable development. One of the most important tasks we face today is to reduce our exploitation of the earth"s finite resources and to develop systems for re-cycling of nutrients and energy that are sustainable in the long term. In the biogas process, waste is converted into energy and nutrients and hence, the exploitation of finite resources is reduced. The biogas process has many advantages from the point of view of the environment, especially since it results in two environmentally-friendly final products: biogas and bio fertilizer (Lars, 2012).

2.8.4. Carbon Revenues

A biogas installation results in greenhouse gas (GHG) abatement. This abatement is denoted as "carbon offsets" and has a value under the clean development mechanism (CDM) or the voluntary carbon market. These offsets can be sold as carbon credits and utilized for policies to stimulate biodigester adoption, by, for instance, providing subsidies or soft loans.

Consequently, these carbon revenues can cover a part of the required capital investments to tackle the impact of the low ambient temperature on biogas production (Buysman, 2009). All the CDM certified and biogas projects under validation are studied to determine the claimed carbon reduction per digester to estimate carbon income. On average around 4.01 tCO_2eq per year per digester is claimed, higher if methane from manure management is included and less without (Buysman, 2009).

2.8.5. Biogas and Recycling of Nutrients

When biogas is produced from organic waste, manure or food waste, the residue, digestate, contains all the nutrients in the original substrate. These nutrients are retained in soluble and plant-available forms in the residue, and cannot be lost by leaching, since the digestion takes place in closed containers. Using the digestion residue as a bio fertilizer reduces the need for mineral fertilizers. The return of the bio fertilizer to arable land constitutes an excellent case of recycling of a natural resource.

2.8.6. Biogas as a Bio-fuel

Biogas is a high quality bio-fuel. As any fuel it can be used to produce electricity and heat, or both in CHP equipment. As it consists of methane it is easily adaptable to existing processes where natural gas, also methane, is used. Methane is a fuel in demand by industry, partly because it is a gas, which gives a high quality combustion that can be precisely controlled. Methane burns with a clean and pure flame, which means that boilers and other equipment are not clogged by soot and cinders.

This leads to a cleaner workplace environment and less wear and tear on the plant. Biogas is the most environmentally friendly vehicle fuel on the market today (Lars, 2012). Biogas gives the smallest emissions of carbon dioxide and particulate matter of all vehicle fuels on the market. Emissions of carbon monoxide, hydrocarbons, sulphur compounds and nitrogen oxides are less than when petrol or diesel is used as fuel. A gas engine is quieter and vibrates less than a diesel engine, which means a better working environment for professional drivers. Biogas is lighter than air. If a leakage occurs, methane rises through the surrounding air. Biogas has a higher temperature of ignition than petrol and diesel, which reduces the risk of fires and explosions at accidents (Lars, 2012)

2.8.7. Decrease Eutrophication

If manure is just unloaded in the environment, it will leak and be carried by water to the nearest water course. Leaking manure is a main cause of Eutrophication of surface waters in the region. Besides from this, anaerobic digestion also to a great extent reduces the pathogenic contents of the manure. Also, the process greatly reduces the smell of the manure (Lars, 2012)

2.8.8. Used as Waste Management Option

Biogas is also produced with organic waste as substrate. This is a great advantage in waste management; the waste does not need to be land filled or just incinerated for recovery of its heat content. When fermenting organic waste the two important resources are recovered, the biogas and the nutrients in the residue (Lars, 2012)

2.8.9. **Woman Empowerment and Health**

Biogas is widely accepted in Ethiopia as a cooking fuel and will mainly benefit women and children (National Biogas Programme Ethiopia, 2008). Cooking on biogas has also a significant health advantage over traditional cooking with an open fire. The major point is the fact that cooking is smokeless and that will diminish the number of eye infections and respiratory problems among in particular women usually in charge of cooking and small children being near their mothers. Moreover, in rural area collecting fire wood takes time. Always this activity is done by women. It is expected that biogas will reduce the overall workload of women by providing the daily energy demand and increase women empowerment (National Biogas Programme Ethiopia, 2008).

Also the danger that children burn themselves while cooking is less when using a biogas stove (Jan and Felix, 2010).

2.8.10. Reduce Deforestation

The energy saving aspect and thus saving on cost for firewood is from the point of view of the farmer household an important aspect. Moreover it is one of the major considerations of a government to promote this technology because it reduces the burden on the environment. It saves trees and helps thereby to combat erosion and to store carbon (Jan and Felix, 2010). In Ethiopia, more than 90% of the energy demand of the country provided by biomass, a dire energy situation exists due to a high rate of depletion of the country's forest cover (National Biogas Programme Ethiopia, 2008).

2.8.11. Reduce Greenhouse Gas (GHG)

The conversion of animal wastes and manure to methane/biogas can yield significant health and environmental benefits. Methane is a GHG that has 21 times more global warming potential than carbon dioxide in trapping heat in the atmosphere. By trapping and utilizing the methane, GHG impacts are avoided (Sergio, 2010).

2.9. The millennium development goals (MDG) and biogas

Of the eight Millennium Development goals, domestic biogas has a very direct relation with four the main goals as discussed in (Jan and Felix, 2010).

MDG 1: Eradicate extreme poverty and hunger

Target 1: To halve extreme poverty

Biogas plants reduce financial and economic costs expended on fuel for cooking and to a lesser extent also lighting. The produced bio-slurry is a potent organic fertilizer and may reduce the use of chemical fertilizer. In general, biogas households are not typically the ones in developing countries that suffer from extreme poverty, although many of them are poor.

However, the biogas dissemination process and the resulting reduced claim on common ecosystem services do affect the livelihood conditions of very poor non-biogas households as well through:

- Construction and installation of biogas creates employment for landless rural people.
- Biogas saving on the use of traditional cooking fuels increases the availability of these fuels for (very) poor members of the community.

MDG 3: Promote gender equality and empower women

Target 4: Eliminate gender disparity in education

Women and girls predominantly spend time and energy on providing traditional energy services. Housekeeping and absence of proper illumination creates barriers for women and girls in accessing education and information as well as their mobility and participation in „public" activities:

- Domestic biogas reduces the workload: collection of firewood, tending the fire, cleaning soot of cooking utensils with 2 to 3 hours per household per day.
- Biogas illumination is highly appreciated for lighting, facilitating reading / education economic activities during the evening.

MDG 6: Combat HIV/AIDS, malaria and other diseases.

Target 8: Halt / reverse the incidence of malaria and other major diseases Half of the world's population cooks with traditional (mostly biomass based) energy fuels of which the collection becomes increasingly cumbersome. Indoor air pollution from burning of these fuels kills over 1.6 million people each year, out of which indoor smoke claims nearly one million children's (<5) lives per year. Diseases that result from a lack of basic sanitation, and the consequential water contamination, cause an even greater death toll, particularly under small children (<5 mortality caused by diarrhea is approximately 1.5 million persons per year):

- Biogas stoves substitute conventional cook stoves and energy sources, virtually eliminating indoor smoke pollution and, hence, the related health risks (e.g. respiratory diseases, eye ailments, burning accidents).
- Biogas greatly reduces the workload involved in the collection of traditional cooking fuels like wood.
- Biogas significantly improves the sanitary condition of farm yard and its immediate surroundings, lowering the exposure of household members to harmful infections generally related with polluted water and poor sanitation.
- Proper application of bio-slurry will improve agricultural production (e.g. vegetable gardening), thus contributing to food security for the community.

MDG 7: Ensure environmental sustainability.

Domestic biogas can help to achieve sustainable use of natural resources, as well as reducing (GHG) emissions, which protects the local and the need for application of chemical fertilizer.

Target9: Integrate the principles of sustainable development into country policies and program and reverse the loss of environmental resources. Particularly larger biogas dissemination programmes have a considerable governance component. As such, they positively influence national policies on sustainable development.

Target10: Halve the proportion of people without sustainable access to safe drinking water and basic sanitation. Biogas reduces fresh water pollution as a result of improved management of dung. Connection of the toilet to the biogas plant significantly improves the farmyard sanitary condition(Jan and Felix, 2010).

3 .MATERIALS AND METHODS

3.1 Methods

In laboratory different analysis were carried out to characterize the samples and the biogas product. General experimental workflow is given Figure 3.1.

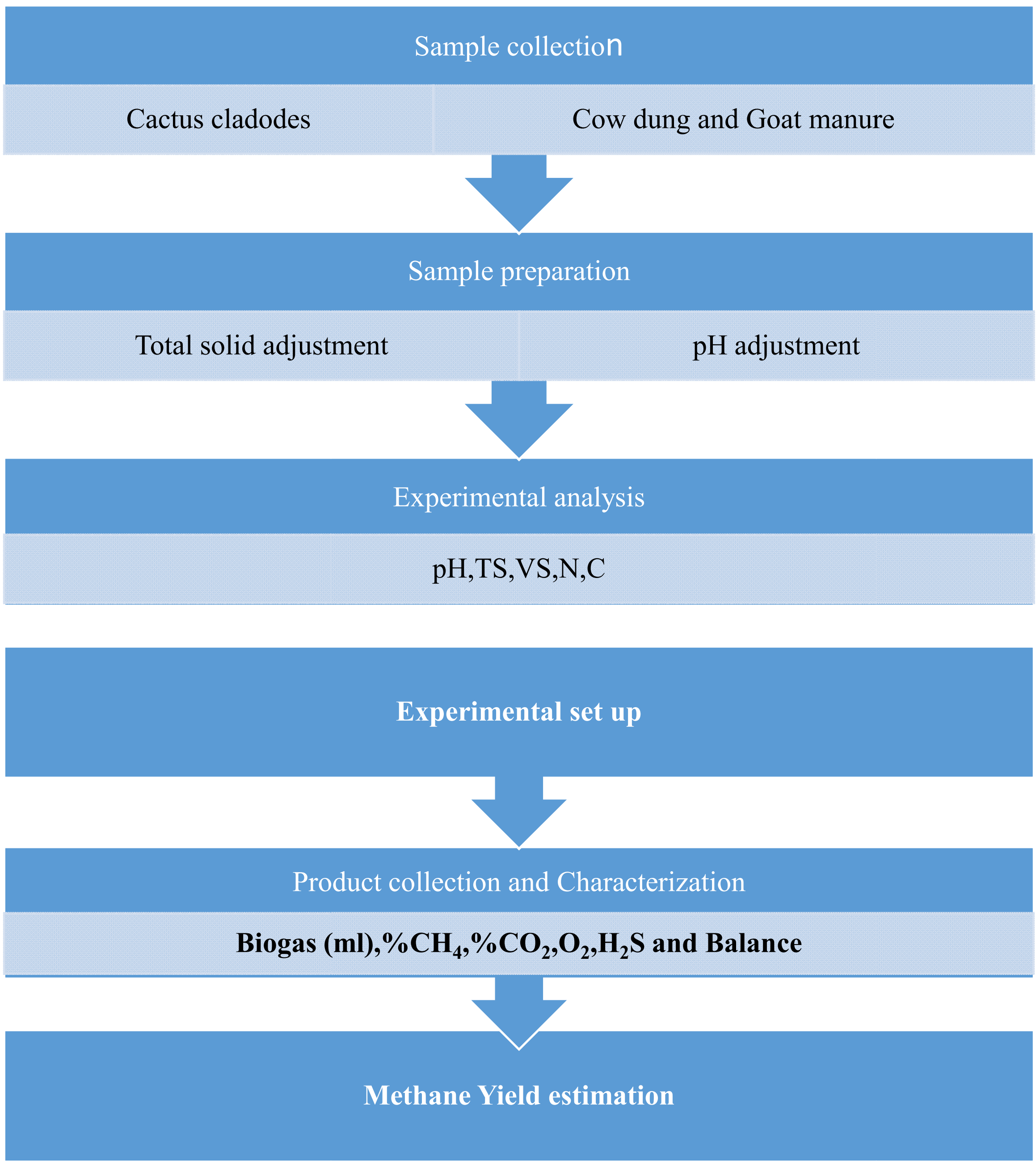

Figure 3.1: Experimental workflow

3.2. Raw Materials

The raw materials needed for the experiment were:-

- ✓ Cactus cladodes
- ✓ Cow dung
- ✓ Goat manure
- ✓ Digestate

3.3. Equipment and Chemical

The equipment and chemicals to be used were:-

- ✓ Digital pH meter
- ✓ Electrical balance (digital weight measuring device)
- ✓ Crucible
- ✓ Furnace
- ✓ Tractor tube
- ✓ Sulfuric acid
- ✓ Hydrogen peroxide
- ✓ Sodium hydroxide solution
- ✓ Hydrochloric acid
- ✓ Plastic bottle digesters
- ✓ Knife and 2mm sized crusher
- ✓ Water bath
- ✓ calibrated gas syringe
- ✓ gas analyzer (Geotech)

3.4. Substrate Preparation

The substrates used as feed stock materials for the generation of biogas in the laboratory were samples of Cladodes (flat green, plate-like sections of *Opuntia ficus-indica* called cladodes (pads), cow dung and goat manure and different combinations of substrate with cow dung and goat manure was set as different treatments. The Cladodes was obtained from O. ficus indica producing community in Mekele (Tigray region) whereas, the required quantity of manure was obtained from a private farm (YehaBiofarm) in Addis Ababa. Cladode was cut manually into small pieces and used for digestion (physical pretreatment, particle size reduction) as reported by Badger. (1979). Inoculum to start anaerobic digestion was obtained from existing biogas plant.

3.5 General Procedures

The total solid (TS), volatile solid (VS), fixed solid (FS) and the C: N ratio of the feed stock was determined before the anaerobic digestion process began, and the sample of the plant was cut, purified from spin and homogenized each sample. The process of anaerobic digestion for the generation of biogas was then conducted by varying the mixing ratio of substrates of cactus cladodes, cow dung and goat manure, In the laboratory each the water content for each sample was determined using the recommendation for better biogas production as reported by Nijaguna (2002). That is, a total solid (TS) of 9% in the fermentation slurry.

3.6 Methods to determine physico-chemical properties of the feedstock

3.6.1. Total Solid

First the crucibles was cleaned, dried in the oven and weighted. Then the sample was added to the crucible and weighted again. According to the Standard Methods for the Examination of Water and Wastewater 2540 B (APHA, 1999) oven was switched on and allowed to reach 105 °C. Crucible with each sample type was placed in the oven and allow to dry overnight to ensure constant weight on sample dry. The dried sample was weighted immediately to avoid absorption of moisture due to its nature. Finally the following calculation was computed to determine total solids in the sample.

$$\%Ts = \frac{WDS}{WWS} \times 1003.1$$

Where: % Ts = percentage of total solid

W_{DS} = Weight of dry sample

W_{WS} = Weight of wet sample

3.6.2. Volatile and Fixed Solids

Sample dried at 105°C was further heated in the muffle furnace at 550°C for 15 to 20 minutes 2540 E (APHA, 1999). The crucible was cooled in the desiccators and weighted. The percent volatile solid was determined according to the Equation 3.2.

$$\%Vs=\frac{W_{VS}}{W_{DS}}\times 100 \qquad 3.2$$

Where: % Vs = percentage of volatile solid (in gram)

W_{VS} = weight of volatile solids (in gram)

W_{DS}= Weight of dry sample

3.6.3. Total nitrogen

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 sampled and 6 ml of concentrated H_2SO_4 was added in tecator tube and mixed carefully. Then 3.5 ml of H_2O_2 was added step by step. Violent color due to reaction was observed. As soon as the violent reaction has ceased the tube was shaked by hand. After adding 3g catalyst mixture the sample was stand for 5 to 15 minutes in the tecator rack before digestion. Then the digester switched on and waits until it reaches 370°C. As the digester gain this temperature place the rack in it and continue digestion for about 4 hours until clear solution was observed.

The tube in the rack was transferred to the fume hood for cooling. About 50 ml distill water was added and shaked by hand to avoid sulphate precipitation in the solution. At this time 25 ml 40% NAOH solution was added into digested and diluted solution. Then 250 ml conical flask containing 25 ml of boric acid, 25 ml of distilled water and indicator solution was placed under the condenser of the distiller with its tip immersed into the solution and the distillation continued for about 8 minutes until a total volume become between 200 ml to 250 ml. Finally the solution was titrated using 0.1N HCl to a reddish color. Calculation:

$$\%Nitrogen=\frac{V\times 0.1\times 14\times 100}{Wo} \qquad 3.3$$

Where,

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

W_OSample weight on dry matter basis and
14The molecular weight of nitrogen
0.1Normality of HCL

3.5.4. C: N ratio

Once the values of total carbon which is equal to chemical oxygen demand in the sample and total nitrogen were determined in the laboratory, C: N ratio of the substrate was calculated according to the following relation:

$$C{:}\ N\ Ratio = \frac{\%C}{\%N} \qquad 3.4$$

Where,

COD Chemical oxygen demand (mg/l)

N Total nitrogen (mg/l)

The mixture was designed based on the C: N ratio value of each substrate. Fixing the value of mixture C: N ratio at 20:1, the amount of each substrate to be added was determined iteratively using the following Equation:

$$Mixture\ C{:}\ N\ ratio = \frac{CC(ml)\times C{:}N + CD(ml)\times C{:}N + GM(ml)\times C{:}N}{Digester\ effective\ volume} \qquad 3.5$$

Where,

Digester effective volume = 58mil liters anaerobic digester used in this experiment.

3.7. Batch digestion composition

3.7.1. Feed Stock Content

The amount of TS in the bottle is fixed to be 58g and for the purpose of this research a set of four batch reactors were used as digesters. Each digester contains Cow dung: Cactus Cladodes, Goat manure: cladodes, Cow dung: cactus cladodes and Cactus cladodes: proportions and aimed at investigating the biogas production from cactus Cladodes co digested with animal manure. The treatment combinations were as follows: T1; 100%CC, T2; 33.6%CC+33.2%CD+33.2%GM, T3; 33.6%CC+66.4%CD, T4; 33.6%CC+66.4%GM:,

Cow dung: Cactus Cladodes: goat manure on a weight percent weight basis.

The water content for each sample was determined using the recommendation for better biogas production as reported by Ituen et al. (2007), that is, a total solid (TS) of 8% in the fermentation slurry.

3.7.2. Water Content

According to Nijaguna (2002), a wet anaerobic digestion process has an optimal total solid (TS) content of 5 to 10%. When the TS values were above the optimal value water was add to obtain the optimum concentration of 9% TS, according to; Nijaguna (2002). For this study the water content will be adjusted according to the indicated optimal condition. The amount of water added was then determined by the formula and added after determining the moisture content and the total solid of cactus.

$$\frac{\text{mTs}}{\text{Ms+Mw}} \times 100 = 9\% \qquad 3.5$$

Where mTs = mass of total solid (in gram)

Ms= mass of sample (in gram)

Mw= mass of water (in gram)

In this case the %TS was determined 9.09 which is in between optimum range, therefore, no need of adding water.

3.7.3. Inoculation

300ml of inoculums was added for all treatments to start up the digestion process. The inoculum was obtained from Addis Ababa zenebework area kebele 04 raey trade union restaurant cow dung digester. The Digestate contain methanogenic bacteria.

Table 3.1. Mixing ratio of substrates

Treatment	Proportion	Fresh Cactus	Fresh Cow	Fresh Goat	Inoculum(ml)	Total mass(g)
CC	1:0	58	0	0	300	358
CC+CD+GM	I:1:1	19.4	19.3	19.3	300	358
CC+CD	1:2	19.4	38.6	0	300	358
CC+GM	1:2	19.4	0	38.6	300	358

Figure 3.2: Experimental setup

3.9. Controlling Conditions

The digesters' internal working temperature was maintained at 38^{o}_{C} and pH was adjusted at optimum range for methanogenic bacteria for all treatments.

3.10. Temperature

38^{0}C was used as an optimum temperature for mesophilic condition. This temperature was constant throughout the process time. This temperature was controlled by using the water bath. The actual inside temperature of the digester was not being measured directly but it was determined by the outside temperature (temperature of water bath).

3.11. pH

The pH values were determined using digital pH meter before and after anaerobic digestion. Before anaerobic digestion, the samples were diluted using distilled water before inoculation with cow dung biogas plant and an electrode was inserted into samples of substrate to measure the pH values. However, pH measurements after anaerobic digestion were done using pH electrode which was inserted into samples of substrate that is digested at the end of the experiment. During anaerobic fermentation, microorganisms require a neutral or mildly alkaline environment for efficient gas production. Biogas production needs an optimum pH value of 6.8 to 7.2 (Mahanta; 2004

Figure 3.3 Adjusting of pH values

3.12 Measuring yield and quality of biogas

The amount of biogas generated and its %CH_4 measurement was started after second days of anaerobic digestion and then always by allowing a two day gap between successive measurements to collect sufficient biogas for analysis. The amount of biogas produced and collected in air bag was measured using graduated syringe of 100ml capacity. Percent methane in biogas was analyzed using Geotech gas analyzer.

This gas analyzer helps to quantify the percent composition of CH_4, CO_2, O_2, H_2S and balance in the biogas. Biogas produced was collected from four digesters using syringe was directly connected to the gas analyzer for its composition analysis.

3.13. Data Analysis

After the completion of the whole laboratory process the data were subjected to analysis of variance (one-way ANOVA) using MS excel Toolpak to investigate statistical significance between digesters, where as paired samples T-test was used to investigate statistical significance within a digester. Differences between means were considered statistically significant at $P<0.05$.

4. RESULTS AND DISCUSSION

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

Table 4. 1: Comparison of pH and % moisture content between before and after AD of the various substrates

Treatment	Parameters			
	Initial pH	Final pH	Initial MC%	Final MC%
100%CC	5.2	6.3	77.3	78.5
CC+CD+GM	6.62	6.9	77.8	80.9
CC+Cd	6.38	6.7	78.1	81.5
CC+GM	6.65	7.14	76.6	78.3

Means are significant at 0.05 probability levels for Paired samples T-test within treatment and significantly different at 5% level of significance between treatments. CD= Cow dung, CC=cactus cladodes, GM=goat manure

The pH of 100% CD and100% GM slurry before anaerobic digestion was about 7.29 and 6.94 whereas that of 100% CC was 5.2. The pH value of 100% CD and GM was optimum for biogas production, whereas that of 100% CC was less optimal (Yadvika, 2004) (Table4.1).This might be due to the presence of relatively high ammonia content in cow dung and goat manure. Mixing the substrates resulted in the rise of pH compared to that of CC alone, but decreased pH from that the pH to meet the optimum required. Mixing substrates is a good way of adjusting the pH of CD and GM alone. The pH after AD was found to increase with increasing of CD and GM proportion in the mix, suggesting that CD and GM helps to maintain value to the optimum (Hills and Roberts,1981).Significant differences were seen in pH values between before and after AD (Paired samples T-test, $P<0.05$). Increase in pH value of the substrates 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).The high pH value recorded after AD for 66.4%CD+33.6%CC, 66.4% GM+33.6 % CC, 33.6 % CC+33.2 % CD+33.2%Gm and 100 % CC in this study may be attributed to increased production of ammonia resulting from less organic C of cactus cladodes than cow dung (Gray , 1971). The pH value increases by ammonia accumulation during degradation of protein while accumulation of VFA (volatile fatty acid) resulting from degradation of organic matter decreases the pH value (Gray, 1971).

The mean moisture content of 66.4% CD+33.6% CC, 66.4% GM+33.6% CC, 33.6% CC+33.2% CD+33.2GM and 100% CC were 79.8± 0.23%, 78.1± 0.14%, 77.45% and 77.9%, respectively. This result shows that the moisture content of CD and GM was higher than CC ($P<0.05$), but after AD the mean moisture content of 100%CD and 100%CC were 82.4 and 78.5%, respectively. According to Buysman (2010), CD tends to have more water content than CC, thus increasing the degree of digestion as bacteria can easily access liquid substrate for relevant reactions to take place easily. There was significant difference in moisture content values between before and after AD (Paired samples T-test, $P<0.05$). Since studies on the most favorable percentage of total solids for biogas productions suggest 8% as the optimum TS, the initial moisture content of substrates used for this study was not optimal for wet anaerobic digestion process (Tchobanoglous, 1993). Therefore, dilution is required to bring the total solids percentage to 8%.

Table 4. 2: Comparison of, % organic carbon between before and after AD of the various substrates

Treatments	Parameters	
	% initial C	% final C
100%CC	43.2	28.08
33.6%CC+66.4%CD	54.24	29.83
33.6%CC+33.2%CD+33.2GM	33.68	15.5
33.6%CC+66.4%GM	46.58	20.5

Means are significant at 0.05 probability levels for paired samples T-test within treatment and significantly different at 5% level of significance between treatments. CD= Cow dung, CC= cactus cladodes, GM=goat manure

The percent degradation of organic carbon for 66.4% Gm+33.6% CC was higher than all (56% reduction) (Table 4.2). 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 %C showed that %C significantly decreased AD in all substrate types. Organic carbon can be removed in anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or biogas production (Gerardi, 2003). Therefore, the decrease in C reflects the degradation process during anaerobic digestion (Devlin*,* 2011). This suggests that mixing can enhance degradation and biogas production. Similar result has also been reported by Animut (2013) in his experiment of co digestion of cow dung and poultry litter.

4.2. **Values of total solid and volatile solid of Substrate Co-digestion**

The total solids and volatile solids were determined for all substrates both before and after AD (Table 4.3).When determining TS and VS, it is important to understand that high content of volatile fatty acids (VFAs) in the substrates can produce misleading results since they may volatilize from the substrate when they are first heated and thus give total solids and volatile solids values that are too low. This in turn can produce incorrect estimates of biogas production, which depend on volatile solids (Anna, 2010). The maximum initial TS (TS before AD) were measured in cactus cladodes, whereas the minimum TS were measured from cow dung alone (Table 4.3). This may show that cactus cladodes of *Opuntia ficus-indica* contain more biodegradable substrates for biogas production. The total solid content of all mix before AD was between 9.09%   and 28.45%.

After AD, TS and VS of all substrate types were significantly decreased, but more decrease was observed in mixed substrates than in sole substrates (Table 4.3). This might be because balanced acidogenesis and methanogenesis in mixed substrates than sole substrates.

Removal of VS after AD suggests its conversion to biogas. Similar results were reported by Joung.(2008) The TS content of 9.09% of CC used for this experiment is in the range of 21 to 23% TS reported by (Sadaka,2003). The TS obtained (19.6%) in this experiment for cow dung is in the range of 19 to 22% reported by (Devlin, 2011) for cow dung. For cow dung, the VS as % of TS was 83.7% , whereas for cactus cladodes, 77.78 % (Table 4.3). This is in accordance with Fulford (1988) who reported that the composition of animals and human wastes typically consist of 15-48 percent of TS and VS is 77-90 percent of TS.

The TS and VS values before digestion was found to vary significantly ($P<0.05$, Table 4.3) with increasing of CC proportion in the mix, suggesting that mixing helps to adjust the TS and VS. Although CC alone has the highest volatile solid for biodegradation than all mix, the mixture with 66.4% Gm and 33.6% CC resulted in a high reduction of amount of VS and TS. The TS and VS values between before and after AD there was significant differences (Paired samples T-test, $P<0.05$). As shown in the Table 4.3, addition of 33.6%CC to goat manure alone and cow dung resulted in an increase of the amount of volatile solids and total solid reduction, from 20%VS, (i.e. from 1.53±0.00 to 1.22±0.01) and 19.7%TS, (i.e. from 2.18±0.00 to 1.75±0.01) Compared to the values measured before digestion, TS and VS significantly ($P<0.05$) decreased after digestion for all mix. Thus total solids and volatile solids destruction is a good parameter for evaluating the efficiency of anaerobic digestion (Abubaker and Ismail, 2012.

Table 4. 3. Values of TS for substrates before and after digestion.

Biodigester	%TS		%VS	
	Initial	Final	Initial	Final
CC	9.09	4	77.78	50.56
CC+CD+GM	25.62	15	60.63	27.9
CC+CD	28.45	13	97.64	53.7
CC+GM	22.75	12	83.85	36.9

There was significant difference in %TS and %VS between before and after digestion. CD=cow dung, CC=cactus cladodes, Gm=Goat manure, TS=Total solids, VS=Volatile solids,

Co- digestion of several combined wastes can utilize the nutrients and bacterial diversities that could provide buffering capacity (Macias-Corral, 2008). According to Malik . (1988), there was high content of lignocellulose in the CD. This demonstrated that the addition of a small amount of CC as co-substrate improved C/N ratio, thereby decreasing the risk of ammonia inhibition to the digestion process in the anaerobic digestion of CD can highly increase VS and TS reduction compared with CD alone. It can be seen that the VS and TS reductions for CD alone were 7.7% and 11.7%, respectively. Most of the volatile solids contained in 100% CD remained unaffected after the anaerobic treatment indicating the low bioavailability of organic material in the samples (Hobson, 1981).

4.3. Average Daily and Cumulative Biogas Production of Substrates Co-digestion in each Treatment

Gas production was noticed from day one of the experiment in all substrate types. However, the amount of biogas measured varied with substrate type; highest for substrate mix of 66.4%GM+33.6%CC and lowest for 100%CC (Figure 4.1). The fact that gas production occurred on the first day of the experiment suggests the existence of microbes in the added inoculum to act on readily degradable materials of the substrates (Animut 2013). In the course of measurement, all substrate types appeared to yield more biogas than CC alone. This may be due to more availability of biodegradable material in CC than CD and GM to serve as a source of energy for microbes (Yeole and Ranande, 1992). Thus, biogas production is a function of the feedstock's organic content and its biodegradability (Macias*,* 2008).

Biogas production showed fluctuating decline after the first day of measurement and eventually reached minimum values 14day of the experiment (Figure 4.2). This might be due to the depletion of readily decomposable substrate after the first day (Ahn, 2009) and/or an increase in ammonium concentration that resulted in an increased pH values (Hansenl., 1998). It is also possible that accumulation of toxic wastes due to increasing microbial population in the digester might have inhibited gas production. However, this explanation needs further testing of the level of some toxic metabolites (e.g., secondary compounds).

There was a significant difference between the substrates in an overall biogas yield ($p<0.05$).

This figure shows that cumulative gas production from CC digested alone was the smallest of the rest of the substrates followed by the co-digestion of CC and CD. Larger cumulative biogas yield was produced when CC, CD and GM were co-digested and the largest cumulative yield was found when CC was co-digested with GM.

Compared to CC alone all substrate types resulted in significantly higher cumulative biogas yield with the highest cumulative biogas production observed in 66.4%GM+33.6%CC mix substrate. Though its %VS was higher, the 100%CC did not result more biogas than the three CD to CC, GM to CC, CC: CD: GM substrate mixtures. This might be due to the less favorable situation of 100%CC to microorganisms as compared to the substrate mixtures. As the proportion of CC in the mix ratio increased from 25% to 75%, the cumulative biogas yield decreased, suggesting less favorable situation with increasing CC proportion from that of .This observation is in accordance with the results of an experiment done by (Callaghan, 1999) using cactus cladodes and cow dung, where higher cumulative production was produced in the system with the lower concentration of cactus cladodes. This may be due to the high concentration of total nitrogen (ammonia) resulting from anaerobic breakdown of proteins to inhibit anaerobic digestion (Angelidaki and Ahring, 1993).

Recently, Costa et al. (2012) reported the less productivity of the digestion of substrates having 7.6% TS. Thus, it can be concluded that co-digestion of CD and CC is more productive with CC proportion not exceeding 25%. The higher production from the mixtures could be due to a proper nutrient balance, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of the substrates (lema, 2013).

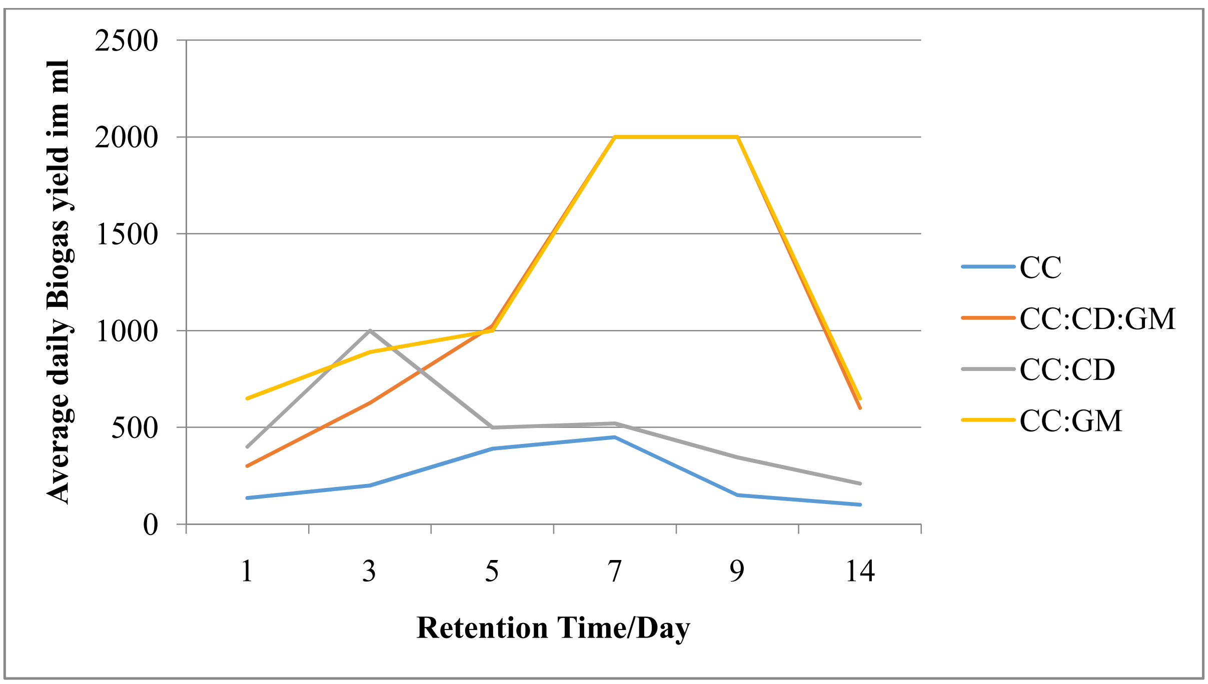

Figure 4.1: Daily mean Biogas yield of the different substrate proportion

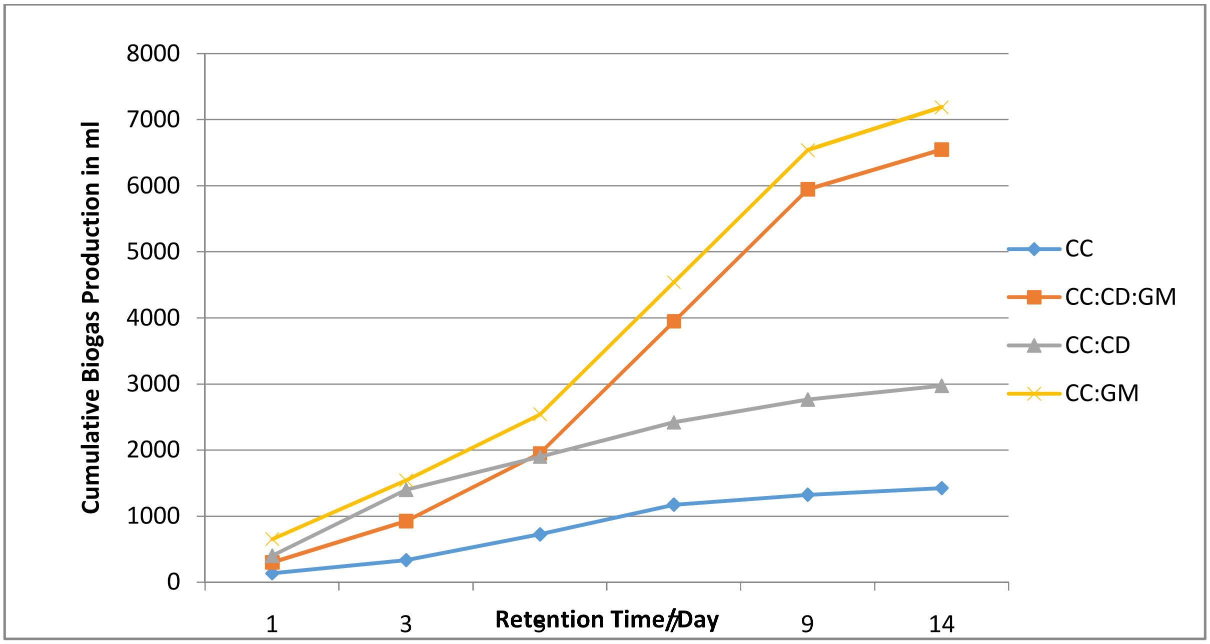

Figure 4.2: Cumulative biogas yields of the different substrate combinations

CD=Cow dung, CC=cactus cladodes, GM=goat manure

In general, studies on possible uses of cactus cladodes have indicated its potential use in biogas Production, followed by fertilizer application as reported (Jemal, 2018). Similarly, in this experiment it would appear that cactus cladodes have potential for biogas production. In addition to this, Volatile solid from TS content of the Cactus cladodes substrate was 77.78%.This shows that a large fraction of the Cactus is biodegradable. This implies that Cactus cladodes can serve as an important feedstock for biogas production and if suitable materials for co-digestion, such as manure, are not available, Cactus cladodes can be digested alone. Co-digestion is the main factor like pre-treatment and type of digester to affect the biogas production. During the co digestion, two or more organic materials should be managed properly to increase biogas production as compared to mono-digestion of these substrates. Co-digestion can enhance biogas production from 25% to 400% over the mono-digestion of the same substrates [(Cavinato, 2010), (Shah , 2015)].

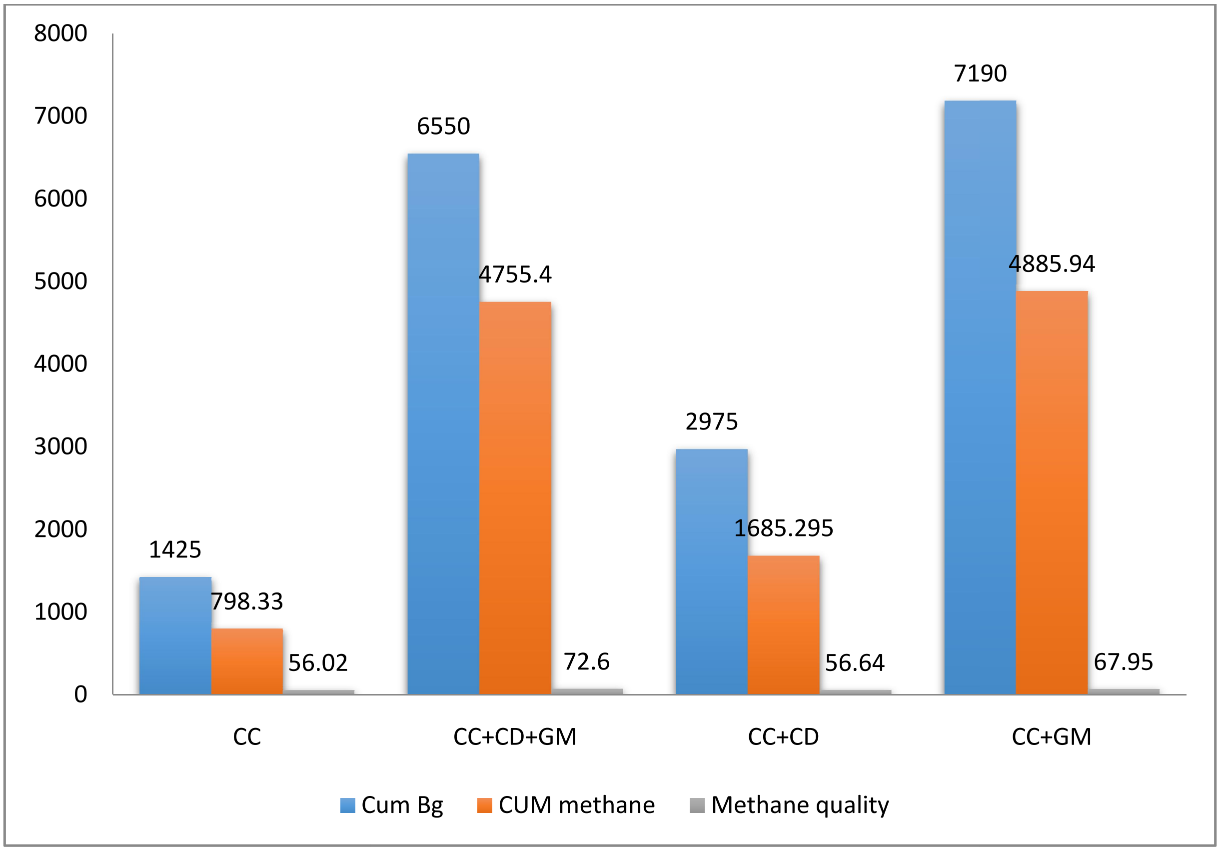

Figure 4.3: Amount of biogas and methane produced from the four treatments

The total biogas from each treatment was measured until it stopped to produce any more gas. It can be seen from Figure 4.3 that a relatively high volume of total biogas production was recorded in the T_4 (33.6%CC: 66.4%GM). The volume of biogas produced increases when the goat manure was mixed with Cladodes at 33.6 to 66.4% whereas it decreases to some extent when the Cladodes were mixed with 66.4% cow dung. The percentage of methane in the biogas was analyzed by gas analyzer (GEOTECH). As shown in Figure 4.3, the percentage of methane forT_1, T_2, T_3 and T_4 were 56.02, 72.6, 56.64 and 67.95respectively. Value of the percentage of methane in this experiment is almost similar to the value (40 to 80) suggested by Stewart. (1984).Results further indicate that T_2 was found to produce methane rich biogas with 6550 ml: the next is T_4 by providing 7190 ml followed by T_3 with 2975 ml. Again, the lower performance is from digester T_1 by an amount of 1425ml.Treatment Two (T_2)stands first in terms of quality of the biogas that is the proportion of methane from the produced biogas (72.6%).Treatment four (T_4) and Treatment three(T_3)

follows 2nd and 3rd by 67.95 and 56.65% respectively, while treatment (T_1)is the least in the quality of biogas.

To sum up, T_4 has the best performance relative to other digesters of treatments because it gives highest amount of biogas with quality. The other treatments such as T_1, T_2 and T_3 were comparable with the typical quality of biogas from animal manure mostly from 50% to 60 % as reported by Marchaim (1992). Statistical test for the mean difference of dependable variable biogas and methane content of the gas produced by T_4 vary significantly at 0.05 levels except with T_2 which means the biogas and methane content of the gas produced by T_4 was comparable with that of T_2.

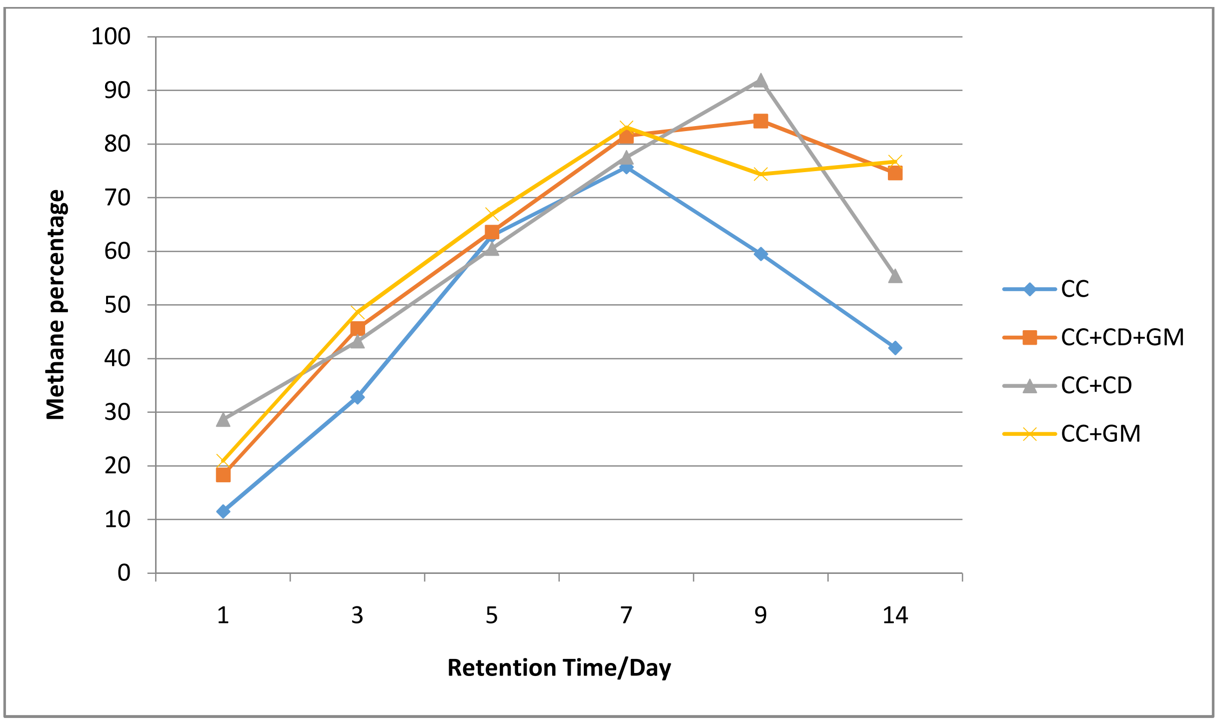

Figure 4.4: Daily methane quality

The percentage methane was between 56.02% and 72.6% (Figure 4.4). T_2 (CC+CD+ GM) scored total highest overall average percentage of methane (72.6%) the other treatments were 56.02%, 56,65%, and 67.95%.From this result the three treatments shows that highest methane percentage when compared to treatment one which is mono digestion(cactus alone).The overall methane percentage was between 56.02% to 72.6% during the whole digestion period. The Value of the percentage of methane of four treatments (T_1, T_2, T_3 and

T_4) in this experiment is almost similar to the value (40 to 80) suggested by Stewart. (1984) Cited in Jigar(2011).

Graphs of methane and carbon dioxide against retention time (RT) were generated as shown in Figures 4-4 and 4-5. Generally methane production increased with time. From Figure 4-4, maximum methane production was harnessed between the 5th to the 14th days of digestion. Treatment T_3 produced more methane than the rest of the treatments and its proportion by volume was 91.19% and it occurred on the 9th day of digestion. It was followed by treatment T_2 and T_4 with84.3%and 83.1 respectively and it occurred on the 9thand 7thday of digestion. Lastly was treatment T_1 with 11.5% methane by volume on the 1st day of digestion.

Though T_1 (cactus cladodes alone) produced the maximum in the first week of digestion, its average methane content especially in the first three days was very low (mean 22.15 %) (Figure 4.4) which means that about 45 % of the gas constituents in this period was CO_2. The gas therefore cannot be used as an energy source directly during this period of digestion. The fact that no lag phase was observed at the beginning of the experiment, but only low methane content suggests a higher hydrolytic-acidogenic than methanogenic activity in the reactors of this treatment.

In such cases two mechanisms are used to improve the quality of biogas. The first mechanism is absorbing (scrubbing) the CO_2 by basic substances: lime, sodium hydroxide or potassium hydroxide so that the percentage of methane could be maximized and the gas could burn easily.

The other one is removing the total gas produced in the first three days of the first week through the water drainage of the biogas plant installation and using the gas produced after these periods as currently practiced by the household biogas users of Ethiopia.

After day 5,the methane content of the treatment, T_1, increased and remained in the range 59.5 to 75.7 which agrees with the literature value of 50 to 75 (EEMBPM, 2002) and 55 to 80 (Jemmett, 2006). Therefore, it could be important to use cactus cladodes alone after fifth day of digestion.

Table 4.4: Potential increase in methane yield from all treatments with reference to control

Substrate	Reactor volume	Methane yield (m^3CH_4/kg VS added)	Reference
Opuntia ficus indica	250ml	0.702±0.053	Myovela,2018
Opuntia spp	200ml	0.600	Calabr ,2017
Sisal waste	350ml	0.301	Muthangya,2013
Maize grains	1000ml	0.72	Hutňan , 2010
Corn silage	600ml	0.872	Li , 2018
Cactus Cladodes	1.5l	0.07	Jemal ,2018
Cactus Cladodes	1.8l	0.022	Jigar ,2011
Cactus Cladodes	500ml	0.445	This study

4.6. Percentage of CO_2

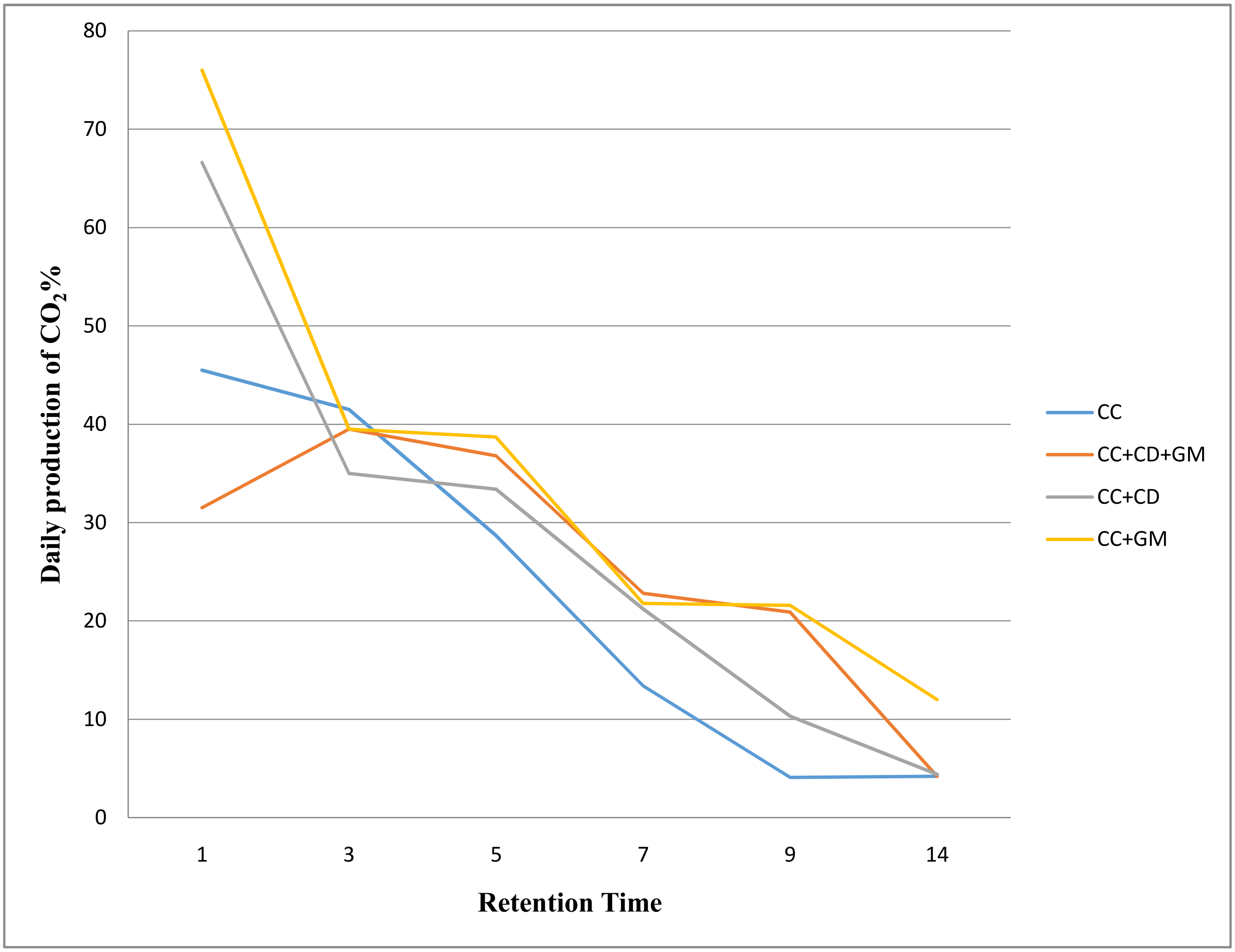

Figure 4.5: CO_2 composition of biogas produced from Treatments

The yield and composition of biogas from different substrates were evaluated and the cumulative curves for the four treatments were estimated as shown in Figure 4.5. Carbon dioxide production increased for the first days of digestion and observed to be at its peak on the 1st day of digestion, there after it reduced significantly. Treatment CC+GM produced the highest emission of carbon dioxide with 76% by volume and it occurred on the 1st day of digestion. It was followed by Treatment CC+CD with 66.6% which was noticed on the 1st day of digestion and least was CC with 4.1% which happened on the14th day.

4.7. Percentage of Oxygen (% O_2)

Oxygen is not usually present in biogas since the facultative aerobic microorganisms should consume it in the digester. But, if there is air present in the digester nitrogen will still be existing in the gas when leaving the digester. Oxygen and nitrogen can be existing in landfill gas if the gas is collected using an under pressure. Those gases can be reduced by adsorption with activated carbon, molecular sieves or membranes. At some extent, these gases can also be removed in desulphurization processes or some of the biogas upgrading processes. Both compounds are challenging (i.e. expensive) to remove. Hence, their presence should be avoided unless the biogas is used for CHPs or boilers.

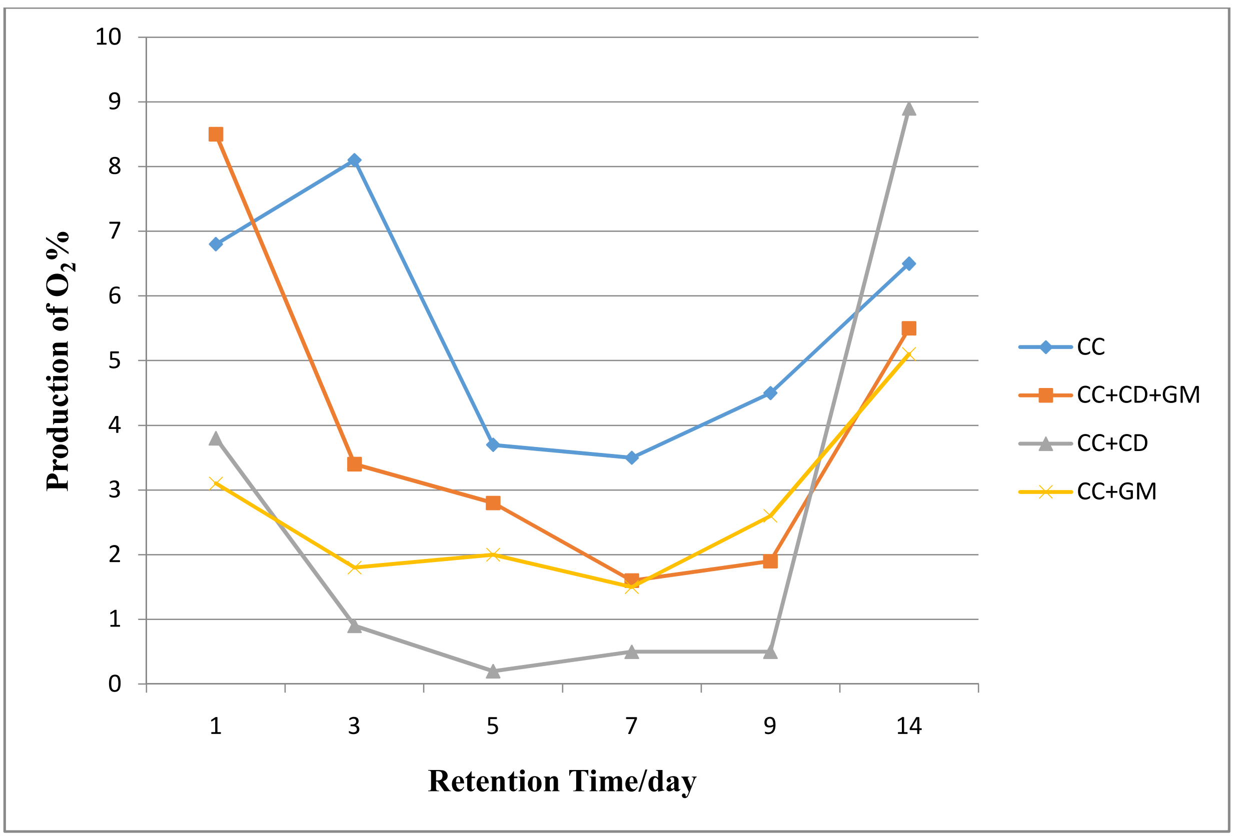

Figure 4.6:O_2 composition of biogas produced from Treatments

The content of oxygen rarely exceeded 0.5% by volume. In the few instances in which the oxygen content was above 0.5% by volume, there is a risk of the oxygen coming from contamination with ambient air during sampling, which will also affect the nitrogen gas content, while the quantities of methane and carbon dioxide are affected to a moderate extent.

This was why we chose not to present all the results for oxygen and nitrogen gas. The nitrogen gas content was between 0.6 and 3% by volume in the samples analyzed.

4.8. Daily Percentage of H_2S

More H_2S production was observed in the digester which contains cactus cladodes only. This is because of unbalanced fermentation could occur due to prolonged exposure to this temperatures, thus favoring the sulphur-reducing bacteria, resulting in the formation of more H_2S (Sue, 2009).Hydrogen sulfide is produced during microbiological reduction of sulfur-containing compounds (sulfates, peptides, amino acids). The concentrations of hydrogen sulfide in the biogas can be lowered either by precipitating in the biogas digester liquid or by treating the gas either in a stand-alone vessel or while removing carbon dioxide. Precipitation Addition of Fe^{2+} ions or Fe^{3+} ions in the form of $FeCl_2$, $FeCl_3$ or $FeSO_4$, to the digester precipitates the relatively insoluble iron sulfide that is removed together with the digested. The method is mainly used in digesters with high sulfur concentration as a first measure or in cases where H_2S in the biogas is allowed to be high (e.g. greater than 1.000 ppm).

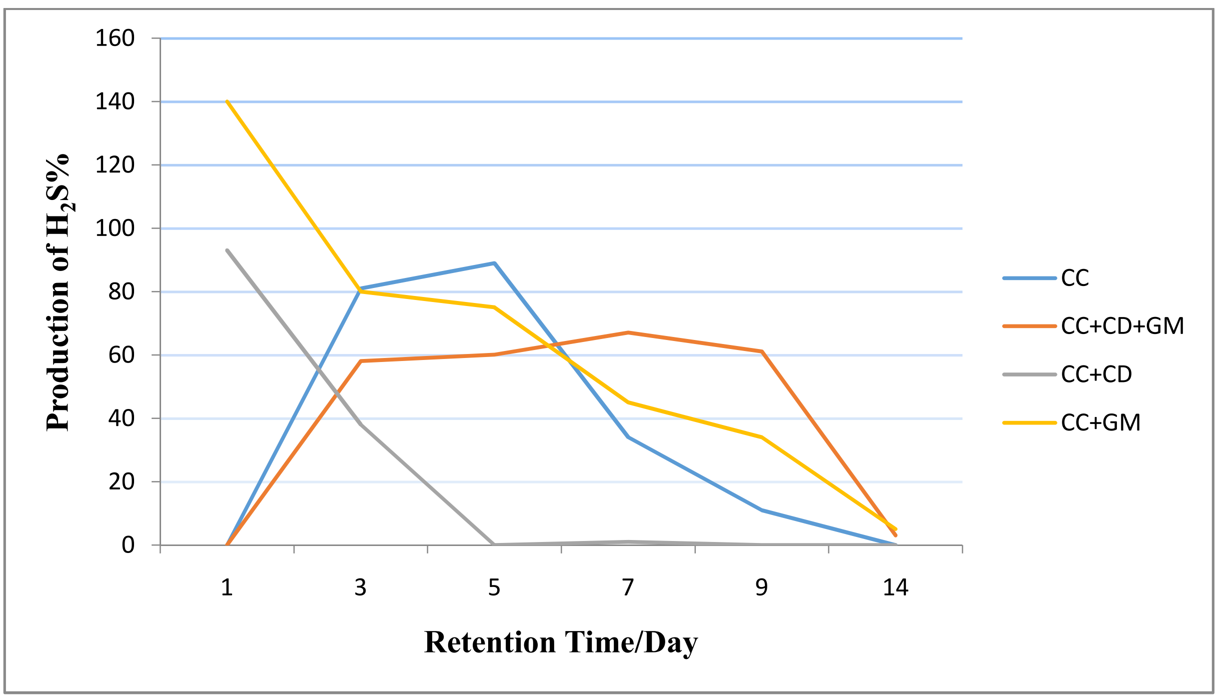

Figure 4.7: H_2S composition of biogas produced for the Treatments

From Figure 4.9, hydrogen sulphide increases with time up to day 7 and thereafter starts to reduce significantly. Treatment T_4 generated a lot of hydrogen sulphide followedby T_1 and the least being T_3. Measuring hydrogen sulfide levels makes it possible to keep the concentration of this toxic and corrosive gas as low as possible by taking appropriate action (The Biogas Technology in China, 1989). Substrate CC has a high hydrogen sulphide emission and this resulted in low methane yield since the gas adversely affect both the generation of biogas and downstream processes. Also high levels of hydrogen sulphide wear down the anaerobic digester and high concentration of it has a toxic effect which hinders bacteria growth.

5. SUMMARY, CONCULSIONS AND RECOMMENDATIONS

5.1. Summary and Conclusions

Anaerobic digestion tests were carried out to obtain suitable substrate mixture for maximum biogas production from co-digestion of CC with at 4 different treatments with different substrates. The experiment was carried in 500ml test batch digester under mesophilic condition (38℃) at 14 hydraulic retention times. The maximum biogas was produced in a combination of goat manure to cactus cladodes at the ratio of 1:2(66.4%:33.6%)was selected based on high VS, TS and %C reduction. Again from the laboratory result, the Volatile solid content of the Cactus cladodes substrate was 77.78% of the TS. This shows that a large fraction of the cactus cladodes is biodegradable. This implies that cactus cladodes can serve as an important feedstock for biogas production.

Biogas production from 100% CC, 66.4% CD: 33.6% CC, 66.4% GM: 33.6% CC and 33.2% CD: 33.2%GM: 33.6%CC were statistically significant at 0.05 significance levels. Cumulative Biogas production of 66.4% GM: 33.6% CC, 404.56% higher than that of 100%, CC 141.68% higher than that of 66.4% CD: 33.6% CCand 9.77% than 33.6% CC: 33.2%CD: 33.2%GM.

The percentage of methane for T_1, T_2, T_3 and T_4 were 56.02, 72.6, 56.64 and 67.95respectively. From the two co-substrates goat manure is the best substrate for co-digestion with cactus cladodes because the mixture of cactus cladodes and goat manure gave highest biogas and methane in volume. The next option is co-digesting the three substrates because it produced highest percentage of methane quality biogas.

Therefore, Co-digestion of cow dung and Cactus cladodes or goat manure and cactus cladodes biomass is one way of addressing the problem of lack of enough feedstock for biogas production. If suitable materials for co-digestion, such as manure, are not available, Cactus cladodes can be digested alone and is a good opportunity for poor people who have not livestock as a source of Energy. Environmental, slurry and foreign currency benefit can be obtained from biogas production.

5.2. Recommendations

Based on the finding of this study, scope for future research studies and development activities need to consider the following recommendations:

- Further work is again necessary to look at composition of organic matter (carbohydrates, proteins, lipids) and process state indicators (VFA, Ammonia level).
- Awareness and skill development training on the sustainable use of cactus as additional substrate for biogas production for users and organizations is essential.
- There is need to evaluate the applicability of Opuntia ficus indica on an industrial scale for the production of biogas by leveling up these experiments to pilot scale.
- This investigation was done only at mesophilic temperature of 38℃, but further similar studies should also be carried out at 40℃ or above temperature conditions to increase the rate of digestion.

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7. APPENDIXES

Appendix A: Sample Characterization: Total solids and volatile solids data sheet

After overnight drying sample at 105°C to get constant mass and cooling in desiccators			
Weights(g)	Substrates		
	CD	GM	CC
W_{DC}	22.3	34	41.4
$W_{DC} + W_{WS}$	26	49.7	61.2
$W_{WS} = (W_{DC} + W_{WS}) - W_{DC}$	3.7	15.7	19.8
$W_{DC} + W_{DS}$	23.7	38.6	43.2
$W_{DS} = (W_{DC} + W_{DS}) - W_{DC}$	1.4	4.6	1.8
$\%TS = W_{DS}/W_{WS}x100$	37.83	29.3	9.09
VS at 550°C			
W_{DC} + ash	23.3	34.7	41.8
Ash =(W_{DC} + ash) - W_{DC}	0	0.7	0.4
$W_{VS} = W_{DS}$– Ash	1.4	3.9	1.4
$\%VS = W_{VS}/W_{DS}x100$	97.2	84.78	77.78
Carbon to nitrogen ratio	24	17	48

%TS percent of total solids

%VS percent of volatile solids

CD Cow dung

GM Goat manure

CC Cactus Cladodes

V_{WS} Volume of wet sample

W_{DC} Weight of dry crucible

W_{DS} Weight of dry sample

W_{VS} Weight of volatile solids

W_{WS} Weight of wet sample

Appendix B: Measured data from the digesters

Date	Sample	CH4	CO_2	O_2	H_2S	BAL	volume
24/12/2019	CC	11.5	45.8	6.8	0	0	135
	CC+CD+GM	18.3	31.5	8.5	0	44.8	300
	CC+CD	28.6	66.6	3.8	93	0	400
	CC+GM	20.9	76	3.1	140	0	650
26/12/2019	CC	32.8	41.5	8.1	81	0	200
	CC+CD+GM	45.6	39.5	3.4	58	0	625
	CC+CD	43.2	35	0.9	38	0	1000
	CC+GM	48.6	39.5	1.8	80	0	890
28/12/2019	CC	62.9	28.7	3.7	89	4.6	390
	CC+CD+GM	63.6	36.8	2.8	60	0	1025
	CC+GM	60.5	33.4	0.2	0	5.9	500
	CC+GM	66.9	38.7	2	75	0	1000
30/12/2019	CC	75.7	13.4	3.5	34	7.5	450
	CC+CD+GM	81.5	22.8	1.6	67	0	2000
	CC+CD	77.5	21.2	0.5	1	0.9	520
	CC+GM	83.1	21.8	1.5	45	0	2000
2/1/2020	CC	59.5	4.1	4.5	11	32	150
	CC+CD+GM	84.3	20.9	1.9	61	0	2000
	CC+CD	91.9	10.3	0.5	0	0	345
	CC+GM	74.4	21.6	2.6	34	1.4	2000
7/1/2020	CC	42	4.2	6.5	0	47.3	100
	CC+CD+GM	74.6	14.2	5.5	3	5.7	600
	CC+CD	55.4	4.4	8.9	0	34.3	210
	CC+G	76.7	12	5.1	5	6.2	650

Normalized	%CH_4	%CO_2	%O_2	%$H2_S$	Balance	Yield
CC	**17.9%**	**71%**	**4%**	**0%**	**0**	**0.71962**
CC+CD+GM	**17.7%**	**31%**	**3%**	**0%**	**0**	**0.882353**
CC+CD	**28.9%**	**67%**	**1%**	**17%**	**0**	**0.709975**
CC+GM	**20.9%**	**76%**	**0%**	**16%**	**0**	**0.7479**
CC	**39.8%**	**50%**	**2%**	**25%**	**0**	**0.604961**
CC+CD+GM	**51.5%**	**45%**	**0%**	**8%**	**0**	**0.861**
CC+CD	**54.6%**	**44%**	**0%**	**4%**	**0**	**0.931185**
CC+GM	**54.1%**	**44%**	**0%**	**8%**	**0**	**0.880055**
CC	**63.0%**	**29%**	**1%**	**17%**	**0**	**0.762612**
CC+CD+GM	**61.6%**	**36%**	**0%**	**5%**	**0**	**0.911435**
CC+GM	**60.5%**	**33%**	**0%**	**0%**	**0**	**0.937031**
CC+GM	**62.2%**	**36%**	**0%**	**7%**	**0**	**0.896298**
CC	**75.6%**	**13%**	**1%**	**7%**	**8**	**0.898383**
CC+CD+GM	**77.0%**	**22%**	**0%**	**3%**	**0**	**0.956297**
CC+CD	**77.4%**	**21%**	**0%**	**0%**	**1**	**0.958172**
CC+GM	**78.1%**	**20%**	**0%**	**2%**	**0**	**0.966978**
CC	**59.4%**	**4%**	**3%**	**6%**	**0**	**0.884434**
CC+CD+GM	**78.7%**	**20%**	**0%**	**3%**	**0**	**0.959785**
CC+CD	**89.5%**	**10%**	**0%**	**0%**	**0**	**0.969646**
CC+GM	**74.4%**	**22%**	**0%**	**2%**	**1**	**0.971723**
CC	**42.0%**	**4%**	**6%**	**0%**	**0**	**0.903342**
CC+CD+GM	**74.6%**	**14%**	**1%**	**0%**	**6**	**0.963546**
CC+CD	**53.8%**	**4%**	**4%**	**0%**	**0**	**0.940439**
CC+G	**76.7%**	**12%**	**1%**	**1%**	**6**	**0.967118**

Appendix C: Experimental anaerobic digester results

T_1=Cactus Cladodes

Day	CH_4	CO_2	O_2	H_2S	BAL	Volume
1	11.5	45.8	6.8	0	0	135
3	32.8	41.5	8.1	81	0	200
5	62.9	28.7	3.7	89	4.6	390
7	75.7	13.4	3.5	34	7.5	450
9	59.5	4.1	4.5	11	32	150
14	42	4.2	6.5	0	47.3	100

T_2=CC+CD+GM

Day	CH_4	CO_2	O_2	H_2S	BAL	volume
1	18.3	31.5	8.5	0	44.8	300
3	45.6	39.5	3.4	58	0	625
5	63.6	36.8	2.8	60	0	1025
7	81.5	22.8	1.6	67	0	2000
9	84.3	20.9	1.9	61	0	2000
14	74.6	14.2	5.5	3	5.7	600

T_3=CD+CC

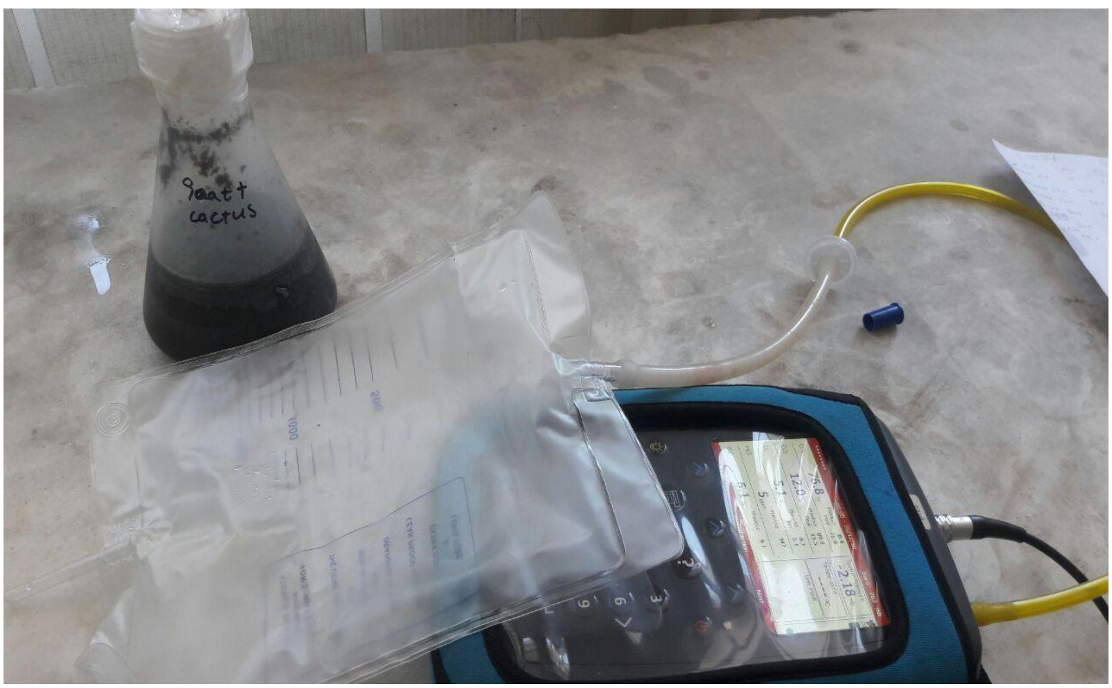
Day	CH_4	CO_2	O_2	H_2S	BAL	volume
1	28.6	66.6	3.8	93	0	400
3	43.2	35	0.9	38	0	1000

5	**60.5**	**33.4**	**0.2**	**0**	**5.9**	**500**
7	**77.5**	**21.2**	**0.5**	**1**	**0.9**	**520**
9	**91.9**	**10.3**	**0.5**	**0**	**0**	**345**
14	**55.4**	**4.4**	**8.9**	**0**	**34.3**	**210**

T_4=CC+Gm

Day	**CH_4**	**CO_2**	**O_2**	**H_2S**	**BAL**	**volume**
1	**20.9**	**76**	**3.1**	**140**	**0**	**650**
3	**48.6**	**39.5**	**1.8**	**80**	**0**	**890**
5	**66.9**	**38.7**	**2**	**75**	**0**	**1000**
7	**83.1**	**21.8**	**1.5**	**45**	**0**	**2000**
9	**74.4**	**21.6**	**2.6**	**34**	**1.4**	**2000**
14	**76.7**	**12**	**5.1**	**5**	**6.2**	**650**

Biogas Volume measurement set up



Methane quality Measurement set up

Appendix D: Anova analysis for daily biogas production

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
cactus	6	1425	237.5	21377.5
CC+CD+GM	6	6550	1091.667	548166.7
CD+Cd	6	2975	495.8333	73684.17
CC+GM	6	7190	1198.333	404216.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3869158.3	3	1289719	4.925202	0.010106	3.098391
Within Groups	5237225	20	261861.3			
Total	9106383.3	23				

Appendix E: ANOVA analysis for daily methane production

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CC	6	798.335	133.0558333	16854.0476
CC:CD:GM	6	4755.4	792.5666667	488016.5707
CC:CD	6	1685.295	280.8825	18863.12116
CC:GM	6	4885.94	814.3233333	379862.4459

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2201678.746	3	733892.9154	3.248765001	0.043452	3.098391224
Within Groups	4517980.926	20	225899.0463			
Total	6719659.673	23				

8. ANNEXES

Cactus cladodes **Cow dung**

Goat manure **Cow dung inoculum**

Adjusting the pH value of the mixtures

Measuring biogas production by gas syringe in the gas holder

Measuring methane quality by Gas analyzer